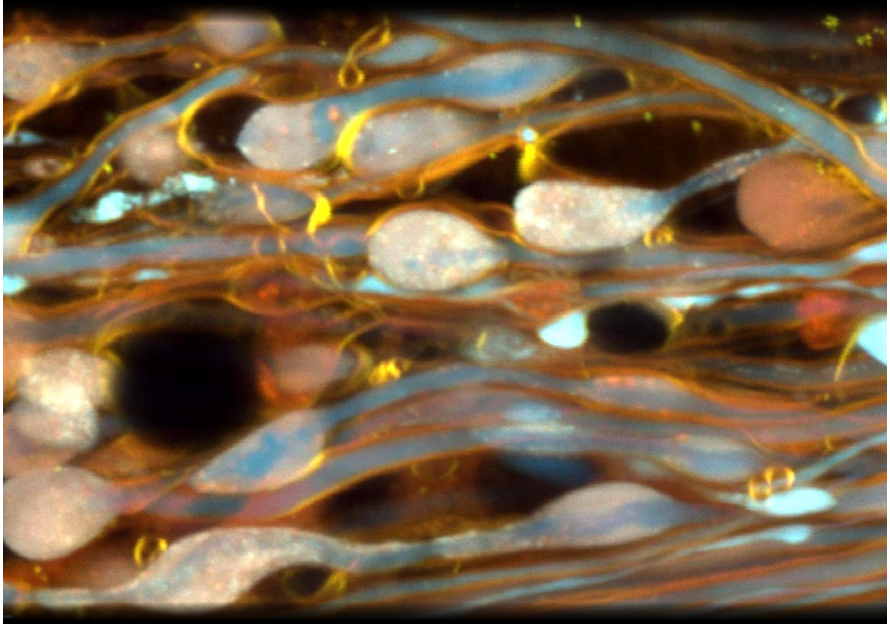
29th Annual

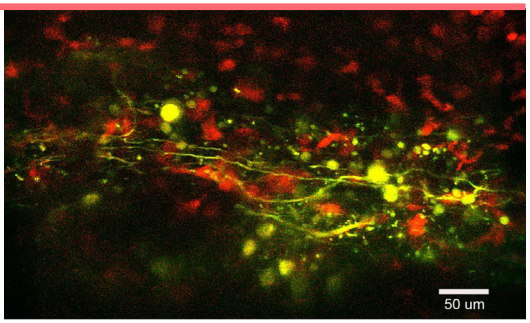
Kentucky Spinal Cord and Head Injury Research Trust Symposium



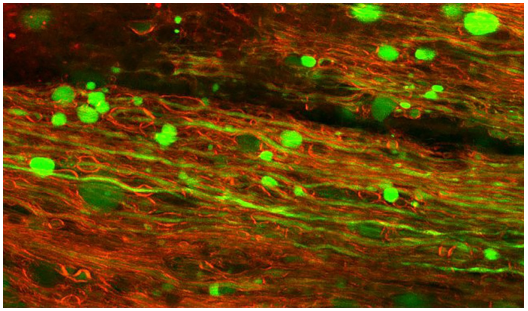
May 8 - 9, 2024

University of Louisville Shelby Campus
Founders Union Building
Louisville, Kentucky

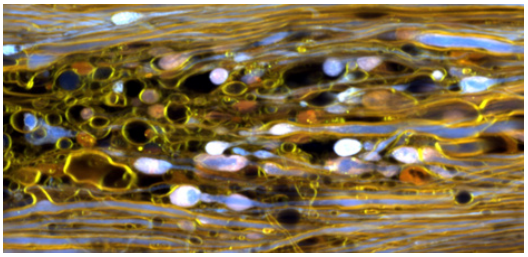
Intravital 2-photon excitation microscopic image of regenerating dorsal column axons (yellow) interacting with microglia (red) 7 days following SCI.



In vivo 2-photon excitation microscopic image of contused spinal cord showing periaxonal swelling of myelin (red) separating away from their axons.



Ex vivo 2-photon excitation microscopic image of transected spinal cord myelinated axons (blue) forming retraction bulbs (pink) and spheroids (pink) within myelin sheaths (orange) following SCI.



All Photo Credits: D. Stirling

Cover Photo: *Ex vivo 2-photon excitation microscopic image of transected spinal cord myelinated axons (blue) forming retraction bulbs (pink) and spheroids (pink) within myelin sheaths (orange) following SCI.*

Welcome to the

Kentucky Spinal Cord and Head Injury Research Trust Symposium

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Wednesday, May 8th

8:00 – 8:45 am	**Registration • Breakfast**
8:45 – 8:55 am	**Opening Remarks** Max Boakye, MD, MPH, MBA • Co-Director KSCIRC • University of Louisville Charles H. Hubscher, PhD • Co-Director KSCIRC • University of Louisville
8:55 – 9:00 am	**Keynote Speaker Introduction** Chair: Carlos de Almeida • University of Louisville Chair: Benjamin Rood • University of Louisville Chair: Natasha Wilkins • University of Louisville
9:00 – 10:00 am	**Keynote Address** *A Lesson in Humility: Underestimating the Complexities of Bladder Dysfunction After Spinal Cord Injury* Linda J. Noble-Haeusslein, PhD • University of Texas Austin Pages 6 – 7
SESSION ONE	**Plasticity Along The Neuraxis** Dena R. Howland, PhD • University of Louisville David S.K. Magnuson, PhD • University of Louisville Jeffery C. Petruska, PhD • University of Louisville
10:00 – 10:30 am	*From Neural Control of Movement to Treatments for Spinal Cord Injury* Monica Perez, PT, PhD • Northwestern University Page 8
10:30 – 11:00 am	*Novel Role for EphB2 Signaling in Spinal Cord Injury-induced Neuropathic Pain* Angelo C. Lepore, PhD • Thomas Jefferson University Page 9
11:00 – 11:30 am	**Coffee Break • Poster Viewing** Pages 33 – 82
11:30 am – 12:00 pm	*Central Plasticity in Premotor Spinal Circuits Modulated by Local Neuroinflammation After Injury in Peripheral Nerves* Francisco Alvarez, PhD • Emory University Page 10 – 11
12:00 – 12:30 pm	*Spinal Cord Cell Types for Motor Control* Ariel Levine, MD, PhD • National Institutes of Health NINDS Page 12 – 13

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12:30 – 1:30 pm	Lunch • Poster Viewing Pages 33 – 82
SESSION TWO	Autonomic Plasticity Chair: Alexander Ovechkin, MD, PhD • University of Louisville Chair: Yury Gerasimenko, PhD, D Sci • University of Louisville
1:30 – 2:00 pm	*A Closer Look on Autonomic Dysfunctions and Maladaptive Plasticity Following Spinal Cord Injury* Andrei Krassioukov, MD, PhD, FRCPC • University of British Columbia, ICORD Page 14 – 15
2:00 – 2:30 pm	*Decentralized Autonomic Cardiovascular Control Following Spinal Cord Injury: What We Know and What We Don't* Jill Wecht, EdD • Icahn School of Medicine at Mount Sinai Page 16
SESSION THREE	Kentucky Spinal Cord Injury Research Center Alumni Session Chair: Charles H. Hubscher, PhD • University of Louisville
2:30 – 3:00 pm	*Advances in Spine Imaging for Trauma: Promises and Reality* Jason Talbott, MD, PhD • University of California San Francisco Page 17
3:00 – 3:30 pm	*Skeletal Muscle Health Following Spinal Cord Injury* Jill Ward, PhD • Emory University Page 18
3:30 – 3:45 pm	Coffee Break • Poster Viewing Pages 33 – 82
3:45 – 4:45 pm	Pre-Poster Data Blitz 15 Selected Posters Presentations Max Boakye, MD, MPH, MBA • University of Louisville
4:45 – 6:30 pm	Cocktail and Hors D'oeuvres Reception Poster Presentations Pages 33 – 82
6:30 pm	Dinner • Independent Networking

Thursday, May 9th

8:00 – 8:45 am	Registration and Breakfast
8:45 – 9:00 am	Opening Remarks Charles H. Hubscher, PhD • Co-Director KSCIRC • University of Louisville
SESSION FOUR	Emerging Topics: Bluegrass Trainee Neuroscientists Chair: David S.K. Magnuson, PhD • University of Louisville Chair: Warren Alilain, PhD • University of Kentucky Chair: Susan Harkema, PhD • University of Louisville Chair: Meifan (Amy) Chen, PhD • University of Kentucky
9:00 – 10:00 am	*Investigating Peripheral Plasticity of Nociceptive Afferents Following Spinal Cord Injury* Morgan Sharp Forston, BS • University of Louisville, Graduate Student Interdisciplinary Program in Translational Neuroscience *Mentor: David S.K. Magnuson, PhD* Pages 19 – 20
	Strategies for Protecting Lung Health After Spinal Cord Injury and Gastric Reflux Michael Sunshine, PhD • University of Kentucky, Postdoctoral Associate *Mentor: Warren Alilain, PhD* Pages 21 – 22
	Translating Findings from Lumbosacral Epidural Stimulation to Design Non-invasive Neuromodulation Strategies to Enable Hand Function in Spinal Cord Injury Pawan Sharma, PhD • University of Louisville, Postdoctoral Associate *Mentor: Susan Harkema, PhD* Page 23
	Regulation of Scar-Forming Astrocytes by Leucine Zipper-Bearing Kinase: Impacts on Cytoskeletal Dynamics and Migration Matin Hemati, PhD • University of Kentucky, Postdoctoral Associate *Mentor: Meifan (Amy) Chen, PhD* Page 24 – 25
10:00 – 10:30 am	Coffee Break • Poster Viewing Pages 33 – 82
SESSION FIVE	Mental Health Spinal Cord Injury Research – Addiction/Depression Chair: Michal Hetman, MD, PhD • University of Louisville Chair: Beatrice Ugiliweneza, PhD, MSPH • University of Louisville

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10:30 – 11:00 am	*Understanding the Psychological Morbidity Faced by Individuals with Chronic Spinal Cord Injury* Mark Peterson, PhD, MS, FACSM • University of Michigan Page 26
11:00 – 11:30 am	*Neuroinflammation: Parallels in Alcohol Use Disorder and Alzheimer's Disease* Gyongyi Szabo, MD, PhD • Harvard University Page 27
SESSION SIX	Sports Performance Chair: David S.K. Magnuson, PhD • University of Louisville
11:30 am – 12:00 pm	*Manipulating the Cardiorespiratory System to Improve Exercise Performance in the Setting of Cervical Spinal Cord Injury* Christopher West, PhD • University of British Columbia, ICORD Page 28
12:00 – 12:30 pm	*Early Acute Activity-based Therapy After Traumatic Spinal Cord Injury* Jean-Marc Mac-Thiong, MD, PhD • University of Montreal Page 29
12:30 – 1:30 pm	Lunch • Poster Viewing Pages 33 – 82
SESSION SEVEN	Developmental Impact: Perinatal Spinal Cord Injury and Pediatric Spinal Cord Imaging Chair: Andrea Behrman, PhD, PT, FAPTA • University of Louisville
1:30 – 2:00 pm	*Have You Checked To See That It Is Plugged-in? Quantitative Structural and Functional MRI Evaluation of Human Spinal Cord In Development and Disease.* Seth Smith, PhD • Vanderbilt University Page 30
2:00 – 2:30 pm	*Functional Recovery After Severe Neonatal and Adult Spinal Cord Injuries: Plasticity, Neuroregeneration and Neurorehabilitation.* Ronaldo M. Ichiyama, PhD • University of Leeds Page 31 – 32
2:30 – 3:15 pm	*Initiatives and Final Closing Remarks* John Gensel, PhD • University of Kentucky Max Boakye, MD, MPH, MBA • University of Louisville Charles H. Hubscher, PhD • University of Louisville

A Lesson in Humility: Underestimating the Complexities of Bladder Dysfunction After Spinal Cord Injury

Linda J. Noble-Haeusslein, PhD

Professor
Department of Psychology, College of Liberal Arts, Department of Neurology, Dell Medical School, The University of Texas at Austin

Bladder dysfunction profoundly effects quality of life for those who have spinal cord injuries. We have approached this key clinical issue through several different pathways that collectively speak to the value of collaborations. And, while there is optimism for the future resolution of bladder dysfunction after spinal cord injury, I will also highlight the unanticipated challenges we have faced in achieving this objective. Our early collaborative studies with a stem cell biologist involved the transplantation of medial ganglionic-like cells derived from human embryonic stem cells (hESC-MGEs) into the injured rodent spinal cord that resulted in an improvement in bladder function as well as (surprisingly), indications of a reduction in neuropathic pain. In collaboration with an academic veterinarian, we conducted comparative studies in dogs that sustained naturally occurring spinal cord injuries and in mice subjected to a moderate spinal cord injury. In both studies where bladder function was improved when treated systemically treated with a metalloproteinase inhibitor within the first several days post injury. Lastly, I will share the stumbling blocks that we have encountered as we focus on improving bladder compliance, a clinically relevant issue that we have mistakenly considered "low hanging fruit".

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About The Speaker The Noble-Haeusslein Lab has studied cord injuries for well over 3 decades with funding provided by NINDS, the Craig H Neilsen Foundation and the Department of Defense. A major focus has been on members of the family of matrix metalloproteinases (MMPs), and notably MMP-9 and MMP-2. Our early studies demonstrated that MMP-9 is markedly upregulated in the acutely injured spinal cord. Subsequent studies of mice deficient in MMP-9 revealed that this protease, primarily conveyed by infiltrating neutrophils, contributed to early disruption of the barrier and degradation of myelin basic protein and was a key determinant of long-term white matter sparing and neurological recovery. Comparative studies of mice deficient in either MMP-9 or MMP-2 during wound healing after spinal cord injury, confirmed that MMP-9 supported astrocyte migration and formation of an inhibitory glial scar. These findings, together with a long-standing interest in translation to the clinical arena, led to the evaluation of a general MMP inhibitor in supporting bladder function in spinal cord injured mice and a clinical trial in dogs. This research demonstrated an improvement in bladder compliance and voiding in dogs and mice, respectively. Lastly, in concert with out our interest in bladder function, our lab was directly involved in transplantation of human stem cell-derived interneuron precursors into the mouse lumbar spinal cord two weeks after spinal cord injury in mice. We found that these cells assumed an inhibitory phenotype and resulted in an improvement in bladder function at 6 months post injury. Collectively, our efforts to restore function after spinal cord injury in mice and dogs have revealed promising findings based upon both pharmacologic and stem-cell based approaches to restore locomotor and bladder function.

Monica Perez, PT, PhD

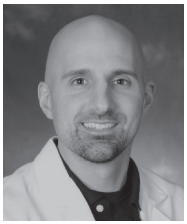
Professor, Physical Medicine and Rehabilitation and Physical Therapy and Human Movement Sciences, Northwestern University

From Neural Control of Movement to Treatments for Spinal Cord Injury

Spinal cord injury (SCI) disconnects ascending and descending axons from their neuronal targets, resulting in partial or complete paralysis. Over the years, our laboratory has focused on understanding the neural mechanisms that contribute to the control of movement in humans with and without SCI, with the long-term goal of maximizing the activity of spared pathways and so functional restoration. During this lecture, I will discuss key findings in the reorganization of the central nervous system after SCI and its applications to rehabilitation. First, we showed that concomitant patterns of residual corticospinal connectivity and spasticity exist in muscles below the injury in humans with a diagnosis of a motor complete SCI, highlighting that a clinical exam of spasticity could be a good predictor of residual corticospinal connectivity and recovery after severe paralysis (Sangari et al., 2019, 2022a,b, 2023). Second, we pioneered the use of spike-timing- dependent plasticity in the corticospinal pathway in people with SCI, from basic proof-of-principle studies on Hebbian plasticity to randomized clinical trials, showing that Hebbian plasticity at residual corticospinal-motoneuronal synapses represents an avenue for functional restoration after SCI (Jo and Perez, 2020; Jo et al., 2023). On our scientific journey, we benefitted from our discoveries in the neural control of movement to develop effective treatments and rehabilitation strategies for people with SCI.

About The Speaker Dr. Monica Perez is the Scientific Chair of the Arms + Hands Lab at the Shirley Ryan AbilityLab, a Professor in the Department of Physical Medicine and Rehabilitation at Northwestern University, and a Research Scientist at the Edward Jr. Hines VA Hospital. She has studied neural mechanisms contributing to the control of voluntary movement in healthy humans and in people with spinal cord injuries for over 15 years. Her research aims to understand how the brain and spinal cord contribute to the control of movement with the ultimate goal of using this mechanistic information to develop more effective rehabilitation therapies for people with spinal cord injuries. This theme is mainly investigated from a neurophysiological point of view, using a combination of transcranial magnetic stimulation, magnetic resonance imaging, electrical stimulation, and behavioral techniques.

Angelo C. Lepore, PhD



Professor, Department of Neuroscience, Director, Neuroscience Graduate Program, Director, Jefferson Synaptic Biology Center, Vickie and Jack Farber Institute for Neuroscience, Sidney Kimmel Medical College, Thomas Jefferson University

Novel Role for EphB2 Signaling in Spinal Cord Injury - Induced Neuropathic Pain

A major portion of individuals with spinal cord injury (SCI) suffer from debilitating chronic neuropathic pain. Importantly, currently available pharmacological approaches are often ineffective and can have severe adverse effects, highlighting the critical need to identify novel therapeutic target mechanisms. Central sensitization – the hyperexcitability of CNS pain circuitry – is a major substrate for SCI-induced neuropathic pain. EphB (erythropoietin-producing hepatocellular carcinoma B) receptor signaling has been shown to impact neuronal excitability via mechanisms such as regulating the subcellular localization of NMDA receptors to excitatory synapses. Using a multi-disciplinary set of approaches that include antibody microarray analysis, super-resolution microscopy imaging of excitatory synapses, chemogenetic EphB inactivation, inducible lineage-specific EphB2 knockout mice, and an array of sensory behavioral assays, we find that enhanced EphB2 expression and signaling plays a central role in hyperexcitability of dorsal horn pain circuitry and persistent neuropathic pain following cervical contusion SCI.

About The Speaker Major goals of Dr. Lepore's lab work include investigating and therapeutically targeting chronic neuropathic pain, as well as degeneration and plasticity of respiratory neural circuitry, after spinal cord injury (SCI). We also study the contribution of both astrocyte dysfunction and synaptopathy to the pathogenesis of SCI and amyotrophic lateral sclerosis. My laboratory has expertise in the fields of SCI, neuropathic pain, rodent behavioral testing, astrocyte and motor neuron biology, axon regeneration/plasticity, respiratory neurobiology, glutamate transporters, neurodegenerative disease, stem cell biology, viral vector technology, and cell transplantation.

Francisco Alvarez, PhD

Professor,
Department of
Cell Biology,
Emory University
School of Medicine

Central Plasticity in Premotor Spinal Circuits Modulated by Local Neuroinflammation After Injury in Peripheral Nerves

Recovery of motor function after nerve injury is usually disappointing with only 10% of patients that undergo nerve repair surgery after full transections regaining normal fine motor control even after successful regeneration of axons in the periphery, muscle reinnervation and recovery of muscle strength and proprioception. Errors in muscle targeting are well known to result in motor control deficits, but in addition we have been pursuing the idea that synaptic plasticity in spinal cord motor circuits also prevents recovery of fine motor control. Specifically, we have been investigating the changes in Ia afferent mediated stretch reflexes as an indication in deficits in proprioceptive feedback reaching the ventral horn during motor action. In this talk I will review how neuroinflammation and specifically ventral horn microglia activity induced by injuries in peripheral nerves is a key mechanism that removes Ia afferent axon collaterals and their synapses from the ventral horn and how this is modulated by injury severity.

About The Speaker During my PhD and early postdoctoral period (1985-1992) I was trained in electron microscopy immunocytochemistry (ICC) to study the synaptic interactions of various types of nociceptors in the spinal cord dorsal horn and trigeminal nucleus caudalis. One point of emphasis was GABA axo-axonic presynaptic control of different types of nociceptors. During this time, I became interested in the molecular structure of synapses and its correlation with synaptic biophysics and found that the ventral horn of the spinal cord with its well-defined spinal motor circuits and neurons was an excellent model. It was also a timely interest because the early 90's coincided with the period of cloning of most postsynaptic receptors and the early studies on receptor clustering and postsynaptic architecture.

After my postdoctoral training I accepted a staff scientist position at Wright State University (Dayton, OH) but this quickly transformed into a faculty position after securing my first funding. I became an independent investigator in 1995 and developed a research program that studied the organization of inhibitory synapses on key neurons of the spinal motor circuitry. We combined confocal

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and electron microscopy ICC with in vivo sharp electrode or in vitro patch-clamp electrophysiology, tract tracing, single cell labeling and 3D neuron reconstructions. From 1995 to 2005 we worked mainly on structure-function relations of inhibitory synapses; how synapse location, structure, receptor number and subunit composition control features of postsynaptic inhibitory currents. From here my interests evolved in the early 2000's towards motor circuit developmental questions. This was prompted by the discovery by developmental biologists of the cardinal genetic classes of spinal neurons. In 2002 we became a mouse transgenics lab to investigate genetically-defined premotor inhibitory interneurons. For this project I acquired experience in genetic targeting to deliver genes to specific cell groups for altering activity or labeling. We completed several projects describing the diversification, development, synaptic connections and electrophysiological properties of inhibitory interneurons from one genetic subclass named V1. Recently, we moved towards testing behavioral and physiological motor outcomes both in adult and during postnatal development after silencing specific interneurons. This project has now been continuously funded by NIH since 2004.

In addition, in 2005, I started a collaboration with Dr. Timothy Cope (Georgia Institute of Technology) aimed at deciphering changes in these same circuits after peripheral nerve injuries. We characterized in detail the anatomical and functional changes that happen in spinal motor circuits after remote nerve injuries and how this synaptic plasticity affects recovery of motor function after nerve regeneration in the periphery is completed. In 2011, I was recruited by Emory and gained many great new colleagues that heavily influenced my work. Specifically, our project on synaptic plasticity after nerve injuries evolved into the realm of microglia biology and neuroinflammation and this now constitutes the major second project in my lab. During this time and established a very meaningful collaboration with my colleague Dr. Arthur English and we developed a new project to investigate the role of inhibitory interneurons and synaptic circuits in axon regeneration.

In summary, I am and spinal cord researcher that centered his career on inhibitory synaptic circuits and how they control sensory gating in the dorsal horn and motor circuit function in the ventral horn in normal animals and after injury or neurodegeneration.

Ariel Levine, MD, PhD

Investigator, Chief of Spinal Circuits and Plasticity Unit, National Institutes of Health NINDS

Spinal Cord Cell Types for Motor Control

The mammalian spinal cord and peripheral nervous system forge the links between the brain and the body to enable sensorimotor control. These functions are mediated by a diverse array of spinal cord cell types, each with their own molecular repertoires, morphology, activity, and contribution to behavior. Spinal neurons can also display differential vulnerabilities to disease or specific potential for plasticity, suggesting that cell type is a critical factor for understanding spinal cord pathophysiology. To address the molecular basis of neuronal diversity in the spinal cord, our lab uses single nucleus RNA sequencing to profile the mouse and human adult spinal cord. Within the mouse spinal cord, we profiled the spared tissue distal to an injury and discovered rare populations of spinal neurons that express regeneration-associated genes and undergo sprouting outgrowth and remodeling after injury. Ultimately, we hope that our work will provide fundamental knowledge and enhanced therapeutic targets in spinal cord injury and disease.

About The Speaker I am a MD-PhD basic scientist whose research focuses on how the mammalian nervous system mediates simple movements. After graduate work in molecular pathways of vertebrate development and postdoctoral work in spinal cord circuits, I joined NINDS as an Earl Stadtman Investigator in 2015 and lead the Spinal Circuits and Plasticity Unit. I am driven to identify and understand the cell types of the spinal cord, how they contribute to behavior, and how we may use this knowledge to help patients with spinal cord injury or disease.

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Ariel Levine, MD, PhD

In the past several years, my group has emerged as a leader in spinal cord cell type studies. We have done foundational work in creating single cell atlases of the mammalian spinal cord and have used this information to make important discoveries in spinal cord biology. We highlighted the importance of spatial organization in defining spinal cord cell types, identified rare spinal neurons whose axons show outgrowth and remodeling after injury, discovered a molecular link between human motoneuron gene expression and the vulnerability of these cells to degeneration, and studied how the spinal cord is integrated with the brain to control movement and motor learning. We are now poised to apply our deep knowledge of spinal cord cell types to unravel the cellular basis of simple movements.

As part of this work, I have mentored a fantastic and diverse team of scientists, including one undergraduate student, six postbaccalaureate students, one graduate student (Johns Hopkins-NIH graduate program partnership), one data scientist, and four postdoctoral fellows. Lab trainees have won numerous awards and fellowships, and the first postdoctoral fellow in our group left in 2020 to begin her own lab at IIST Bangalore. I was honored to receive the NINDS Director's Award for Mentorship in 2021 and again in 2023. I have also contributed to the scientific community by mentoring trainees through programs for under-represented scientists, co-organizing multiple conferences, developing a popular publicly available web resource to share our data, and presenting my group's work at over thirty seminars and conferences.

Andrei Krassioukov, MD, PhD, FRCPC

Professor and Endowed Chair, Associate Director, Scientist, International Collaboration on Repair Discoveries (ICORD), Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

A Closer Look on Autonomic Dysfunctions and Maladaptive Plasticity Following Spinal Cord Injury

Two features of spinal cord injury (SCI) ultimately produce maladaptive responses within sensory and autonomic circuits, resulting in various autonomic dysfunctions. As an immediate consequence of SCI, projection neurons (including sympathetic preganglionic neurons [SPNs]) and interneurons within the spinal cord lose normal supraspinal excitatory and inhibitory control. Over time following SCI, plasticity within sensory and autonomic circuits alters their connectivity and functions.

When the spinal cord is completely transected, amplified afferent input contributes to dysfunction by means of exaugurated spinal reflexes. The best-characterized of these phenomena are lower urinary tract dysfunction (LUTD) and autonomic dysreflexia (AD), episodic hypertension elicited by sensory stimulation below the level of SCI. Both conditions are associated with injury-induced plasticity within central and peripheral sensory-sympathetic circuits; also, both represent life-altering complications for people with SCI.

LUTD after SCI (including abnormal sensation associated with bladder filling, an inability to completely void urine and to store urine until voluntary release) is dependent, in part, on plasticity of bladder afferent pathways as well as reorganization of synaptic connections within the spinal cord. AD typically develops after SCI that occurs at or above T6. In AD, visceral or somatic sensory input below the level of SCI stimulates sympathetic outflow to the vasculature to increase vascular resistance (and thus blood pressure). Baroreflex-mediated bradycardia often, and thus not solely, is a consequence of the loss of descending control over spinal sensory-sympathetic circuits. Clinically, AD, is most commonly triggered by distention/irritation of the urinary bladder.

Reflex plasticity is associated with changes in the properties of ion channels and electrical excitability of afferent neurons and appears to be mediated in part by neurotrophic factors released in the spinal cord and/or the peripheral target organs, accompanied by changes in relevant afferents. The emergence of AD is accompanied by central

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Andrei Krassioukov, MD, PhD, FRCPC

sprouting of nociceptive terminals in the spinal dorsal horn. The terminal arbour of nociceptive neurons expands over time after injury, and primary afferents sprout to occupy broad regions of the dorsal horn and the intermediolateral cell column (site of he majority of SPNs).

This presentation, will describe the current evidence on potential courses and consequences of aberrant sensory and autonomic nervous system plasticity. We also will discuss evidence of perturbed neurotrophic factor signaling that involved in the pathogenesis of autonomic dysfunctions following SCI.

About The Speaker Professor of Medicine and Chair of Rehabilitation Medicine at the University of British Columbia and a staff physician at the spinal cord injury (SCI) program at the GF Strong Rehabilitation Centre in Vancouver, where I helped establish an autonomic dysfunction clinic. I am head of the Autonomic Research Laboratory, Co-Director at the International Collaboration on Repair Discoveries (ICORD), and I was the former President of the American Spinal Injury Association (ASIA). My basic and clinical research on SCI for the past 30 years has seen: >280 peer-reviewed publications, >13,000 citations, and an H-index of 55. As a clinician–scientist and a boardcertified specialist in Physical Medicine & Rehabilitation, I have a strong background in 1) clinical and basic experimental research regarding autonomic control and 2) medical management and rehabilitation of individuals with SCI who suffer from autonomic impairments including blood pressure instability and bladder/bowel dysfunction. My scientific background and clinical expertise uniquely position me to conduct high-quality, clinically relevant translational research. For instance, my work includes a recent publication (*Nature Communications*) that combines experimental animal models and prospective clinical studies to investigate the cardiac consequences of SCI injury on the acute-to-chronic continuum. In addition, my 2021 publication with Dr. Rahul Sachdeva (Neurotherapeutics) on the efficacy of transcutaneous spinal cord stimulation (TCS) to regulate blood pressure in animals and a clinical case has laid the framework for expanded translational research in TCS for improving autonomic function after SCI.

Jill Wecht, EdD

Research Health Scientist at the James J. Peters VA Medical Center Professor in the Department of Rehabilitation Medicine and Human Performance Icahn School of Medicine at Mount Sinai

Decentralized Autonomic Cardiovascular Control Following Spinal Cord Injury: What We Know and What We Don't

Neural output from the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) are integrated to appropriately control cardiovascular responses during routine activities of daily living. Following spinal cord injury (SCI), sympathetic cardiovascular innervation, which arises from vertebral levels T1 to L2, is impacted leading to a number of cardiovascular impairments. Although the vasculature is not directly innervated by the parasympathetic nervous system, the SA node is innervated by post-ganglionic vagal nerve fibers via cranial nerve X, which are not anatomically impacted by SCI. Due to impaired sympathetic cardiovascular control, many individuals with SCI, particularly those with lesions above T6, are prone to blood pressure (BP) disorders including hypotension, orthostatic hypotension (OH) and autonomic dysreflexia (AD). Most, individuals with SCI who experience these BP disorders do not report symptoms and therefore may not be clinically managed. Moreover, there are no interventions available that are FDA approved and are safe, and effective at restoring ANS cardiovascular control following SCI. Up-to-date evidence will be presented to discuss cardiovascular ANS impairments post-SCI along with current and emerging treatment strategies.

About The Speaker Dr. Wecht is a Research Health Scientist at the James J. Peters VA Medical Center and is a professor in the departments of Rehabilitation Medicine and Human Performance and Medicine at the Icahn School of Medicine at Mount Sinai. Her research involves gaining insight into associations between decentralized autonomic nervous system control of cardiovascular and cerebrovascular function and how these alterations impact cognitive function, mood, and quality of life following spinal cord injury.

Jason Talbott, MD, PhD

Associate Professor of Radiology and Biomedical Imaging, Associate Program Director, Diagnostic Radiology Residency, University of California at San Francisco and San Francisco General Hospital

Advances in Spine Imaging for Trauma: Promises and Reality

Advances in quantitative, functional and molecular imaging techniques hold great potential for enhancing diagnosis, prognosis, and elucidating important pathyphysiologic mechanisms for patients with traumatic spinal cord injury. A brief review of these advanced tools and their applications, along with their limitations when attempting to implement them in routine clinical practice will be covered. Conventional MR imaging techniques, including T2-weighted imaging, still play an invaluable role in guiding patient management. The emerging role for advanced image processing tools, such as atlas-based image analysis and AI-based segmentation and injury classification algorithms combined with conventional MRI techniques will also be highlighted.

About The Speaker Jason Talbott, MD, PhD, is an Associate Professor in Clinical Radiology in the Emergency Radiology and Neuroradiology sections in the Department of Radiology and Biomedical Imaging at the University of California, San Francisco and Zuckerberg San Francisco General Hospital. He is chief of the Emergency Radiology section and also serves as Associate Residency Program Director. He earned his MD and PhD degrees from the University of Louisville, Kentucky, in 2007. He completed a one-year internship in Internal Medicine from the California Pacific Medical Center in San Francisco. From 2008-2012, Dr. Talbott completed a four-year Diagnostic Radiology residency at UCSF, followed by a one-year fellowship in Neuroradiology from UCSF, in 2013.

In 2012, as a resident, Dr. Talbott received the Alexander Margulis Outstanding Resident Research Award and also served as a Chief Resident in the Department of Radiology at UCSF. Dr. Talbott currently serves as the Associate Program Director for the Radiology residency program and member of the resident selection committee.

Research interests include emergency imaging with specific interests in traumatic and non-traumatic spine emergencies and advanced spinal cord imaging techniques. As part of a multi-disciplinary clinical team at Zuckerberg San Francisco General Hospital, Dr. Talbott is active in multiple SCI trials as part of the Transforming Research and Clinical Knowledge in Spinal Cord Injury (TRACK-SCI) initiative. Dr. Talbott has authored or co-authored more than 85 peer-reviewed articles in addition to numerous book chapters and review articles and serves as Associate Editor for Emergency Neuroradiology section for the *American Journal of Neuroradiology*.

Jill Ward, PhD

Assistant Professor
Department of
Cell Biology
Emory School
of Medicine

Skeletal Muscle Health Following Spinal Cord Injury

Skeletal muscle comprises an enormous source of the body's metabolism. Supporting healthy muscle following spinal cord injury (SCI) is necessary for both rehabilitation efforts as well as for the prevention of secondary complications, co-morbidities (diabetes), and reduced mortality after SCI. Following SCI, rapid sub-lesional muscle atrophy occurs along with some evidence of supra-lesional atrophy (muscle wasting in non-paralyzed muscle). Work in our lab indicates that sympathetic innervation to skeletal muscle modulates muscle performance and mitochondrial metabolism. After SCI, we see rapid onset of mitochondrial dysfunction in skeletal muscle – even in non-paralyzed muscle. We are working to understand how sympathetic nervous system plasticity may underlie these rapid changes in skeletal muscle health following SCI.

About The Speaker After graduating summa cum laude from Auburn University (2007), Dr. Jill Ward completed her graduate studies at the University of Louisville with Dr. Charles Hubscher where she studied the role of locomotor training on recovery of non-locomotor functions, such as bladder function and allodynia after spinal cord injury. She earned her PhD in Anatomical Sciences and Neurobiology in 2012. She then worked as a post-doctoral fellow at Emory University with Dr. Arthur English (2013 - 2017) studying mechanisms of neuronal activity-dependent therapies for axon regeneration. She completed further training in Translational Research through the Georgia Clinical and Translational Science Alliance (2017 - 2019). Dr. Ward began her independent lab in 2019 as an Assistant Professor in the Department of Cell Biology where she runs an NIH-funded lab and teaches gross anatomy in the School of Medicine at Emory University. She is the 2022 recipient of the Bensley Award in Cell Biology from the American Association of Anatomy. The Ward lab's current interests are focused on the plasticity and regenerative ability of sympathetic neurons and the role of the sympathetic nervous system in skeletal muscle health.

Morgan Sharp Forston, BS

University of Louisville, Graduate Student Interdisciplinary Program in Translational Neuroscience

Mentor: David S.K. Magnuson, PhD

Investigating Peripheral Plasticity of Nociceptive Afferents Following Spinal Cord Injury

Following spinal cord injury (SCI), CGRP + nociceptors (C-fibers) sprout into dorsal horn laminae across multiple spinal segments, contributing to secondary complications such as neuropathic pain. While such plasticity is well-defined intraspinally, numerous studies have demonstrated that inflammation and atrophy can cause C-fibers to hyperinnervate peripheral tissues in addition to the spinal cord. However, it is unknown if C-fibers sprout peripherally after SCI, a condition of chronic inflammation and significant atrophy. Therefore, we sought to investigate the anatomical plasticity of C-fibers in peripheral tissues post-SCI. Female Sprague Dawleys were divided into four groups: Naïve, Uninjured AAV, Transection, and Transection AAV. AAV groups received bilateral sciatic nerve injections of AAV PHP.S-hSyn1tdTomatof and recovered for 4 weeks. At 4 weeks post-injections, SCI groups received a T10 spinal transection. Following SCI, BBB locomotor recovery scores were assessed weekly and tail flick was performed terminally at 6 weeks post-SCI. Animals were perfused to harvest spinal cord, muscle, skin, fascia, and L1-L6 dorsal root ganglia for iDISCO and RNAseq. Transection resulted intraspinal sprouting of C-fibers and reduced CGRP + motoneuron cell bodies in the lumbar ventral horn. These changes correlated with thermal and mechanical hypersensitivity as well as limited locomotor recovery. Transection also resulted in significant muscle atrophy in addition to reduced motor endplate size and expression of CGRP. SCI animals also displayed an increase in volume and/or length of CGRP + axons localized particularly around blood vessels in peripheral tissues. These findings will provide fundamental information regarding mechanisms that may contribute to sensory-dependent pathologies.

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Emerging Topics: Bluegrass Trainee Neuroscientists

SESSION FOUR

Morgan Sharp Forston, BS

About The Speaker Morgan Sharp Forston is a 6th year PhD Candidate at the University of Louisville performing her doctoral studies under the mentorship of Dr. David Magnuson at the Kentucky Spinal Cord Injury Research Center. Morgan completed her Bachelor of Science in Biomedical Engineering at the University of Louisville in 2018 and has since been pursuing her PhD in the Interdisciplinary Program in Translational Neuroscience. Morgan has been an active trainee in the scientific community, including serving as a moderator for the International-Online Spinal Cord Injury Research seminar series, participating in the Pain Research Forum Correspondents Program, and engaging in numerous scientific outreach and mentorship programs for young students. In addition to pursuing research, Morgan is also a passionate educator. Morgan has received fellowships through the Schools of Medicine and Dentistry for teaching medical and dental gross anatomy during her PhD Morgan has also been invited to speak on her research at conferences such as the American Spinal Injury Association Scientific Meeting and National Neurotrauma Symposium and has received multiple trainee awards, including the Kentucky Spinal Cord and Head Injury Research Trust Trainee Award. Through funding support from the Department of Defense and under the mentorship of Dr. David Magnuson, Morgan currently studies the anatomical plasticity of nociceptive afferents in peripheral tissues after spinal cord injury and aspires to develop novel pharmacological and rehabilitative interventions to target sensory-dependent pathologies.

Michael Sunshine, PhD

University of Kentucky, Postdoctoral Associate

Mentor: Warren Alilain, PhD

Strategies for Protecting Lung Health After Spinal Cord Injury and Gastric Reflux

Spinal cord injuries have a significant and far-reaching impact, affecting more than 300,000 people in the United States alone. Among these injuries, cervical-level injuries are the most common, accounting for approximately 60% of all spinal cord injuries. Pulmonary complications, specifically aspiration-related pneumonia, rank as the primary cause of death in individuals with these injuries. Notably, those with cervical spinal cord injuries have an elevated risk of experiencing acute lung injury and acute respiratory distress syndrome. Moreover, spinal cord injuries, regardless of their location, often lead to gastric dysmotility, a condition frequently linked with gastroesophageal reflux disease. This research aims to untangle the intricate relationship between these interconnected health issues and their collective impact on pulmonary function. Here we tested the hypotheses 1.) that cervical spinal cord injury increases the susceptibility for developing acute lung injury associated with gastric juice aspiration and 2.) that TRPV4inhibitors will mitigate gastric fluid/SCI-induced lung injury. These studies employed a rat model of spinal cord injury, C2 hemisection (C2Hx). This model mimics the reduced inspiratory tidal volume observed in individuals with cervical spinal cord injuries and has more recently been shown to recapitulate certain aspects of lung injury associated with spinal cord injuries. Additionally, to produce the effects of gastric reflux, gastric juices were introduced into the trachea in a subset of animals following spinal cord injury. Furthermore, TRPV4 inhibitors were administered to assess their potential to mitigate lung damage after spinal injury, gastric juice instillation or at both times. Preliminary data suggest that C2Hx and low levels of gastric juice increase inflammatory markers in the lung and circulation above even high levels of gastric instillation. Further, preliminary data supports the hypothesis that TRVP4 inhibition may protect the lung. The outcomes of these investigations will provide valuable insights into how spinal cord injuries might predispose individuals to lung injury. Additionally, they will evaluate the efficacy of a promising therapeutic approach using TRPV4 inhibitors to restore lung barrier function, potentially reversing this susceptibility.

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Michael Sunshine, PhD

About The Speaker Michael Sunshine is an electrophysiologist focused on restoring motor function following spinal cord injury and neurodegenerative diseases. He worked as a research scientist at the University of Washington developing brain-machine spinal interfaces focused on restoring forelimb function in rats after spinal cord injury. During his PhD, Michael, was a predoctoral fellow on a Neuromuscular Plasticity T32 training grant prior to receiving an F31 fellowship focusing on developing novel methods of spinal stimulation to restore breathing after opioid overdose and spinal cord injury. His initial post-doctoral training focused on the utilization of oxygen therapy following spinal cord injury to promote respiratory function. His current post-doctoral work at the University of Kentucky focuses on lung health and secondary complications related to spinal cord injury.

Pawan Sharma, PhD

University of Louisville, Postdoctoral Associate

Mentor: Susan Harkema, PhD

Translating Findings from Lumbosacral Epidural Stimulation to Design Non-invasive Neuromodulation Strategies to Enable Hand Function in Spinal Cord Injury

Spinal cord injury (SCI) results in sensorimotor deficits at and below the injury level, including upper extremities, lower extremities, and trunk. Recent discoveries on the adaptive plasticity within the sensorimotor system following an injury have led to the development of novel spinal cord stimulation strategies to augment natural motor recovery after SCI. Over two decades of research at the Kentucky Spinal Cord Injury Research Center provide evidence that lumbosacral epidural stimulation improves spinal cord excitability and potentially neuromodulates the spinal network into physiological states to recover lost lower limb functions. The plethora of information gained from these studies can help design spinal cord stimulation strategies targeting other motor functions, such as hand function in individuals with cervical SCI.

Approximately 60% of injuries occur at the cervical spinal cord level, affecting hand function. We will discuss how the findings from lumbosacral epidural stimulation have shaped our research strategy to utilize non-invasive transcutaneous spinal cord stimulation to enable hand function in individuals with cervical SCI. More specifically, we will discuss the presence of preserved motor capabilities in individuals with clinically complete cervical SCI, present methodology for stimulation site selection, voluntary motor control mapping strategies to select stimulation parameters for training, and the association between neuroimaging markers with the initial clinical and functional presentation and recovery post-training. We will also present the initial findings of our ongoing research demonstrating the effectiveness of multi-site transcutaneous spinal cord stimulation in facilitating the recovery of upper extremity function in cervical SCI. Small functional gains in upper extremity function can significantly enhance individuals' quality of life and independence with tetraplegia.

About The Speaker Dr. Pawan Sharma is a postdoctoral associate at the Kentucky Spinal Cord Injury Research Center, Louisville. Under the guidance and mentorship of Dr. Susan Harkema, he is researching invasive and non-invasive spinal cord stimulation to improve motor functions in individuals with SCI. Dr. Sharma obtained his PhD from Stony Brook University, New York, where he investigated the effectiveness of closed-loop cervical epidural stimulation to improve hand following cervical spinal cord injury in rats. Dr. Sharma is a physical therapist by training and his research interest lies at the intersection of neurorehabilitation and neuromodulation. He hopes to contribute significantly to spinal cord injury and scientific community by undertaking high impact research.

Matin Hemati, PhD

University of Kentucky, Postdoctoral Associate

Mentor: Meifan (Amy) Chen, PhD

Regulation of Scar-Forming Astrocytes by Leucine Zipper-Bearing Kinase: Impacts on Cytoskeletal Dynamics and Migration

Background: Following spinal cord injury (SCI), reactive astrocytes migrate toward the injury site to form the astrocytic scar, aiming to prevent inflammatory cell infiltration and further damage to the other sites of spinal cord. Prior to migration, astrocytes undergo cytoskeletal rearrangement to form the polarized structure. Our laboratory identified leucine zipper-bearing kinase (LZK) as a major positive regulator of astrocyte reactivity after SCI. Considering the significance of cytoskeleton remodeling and the formation of polarized structures preceding cell migration, this study investigates role of LZK in regulating cytoskeletal dynamics and the migration of scar-forming astrocytes.

Material and Methods: Astrocytes were isolated from injury sites of tamoxifen-inducible, astrocyte-specific LZK knockout (KO), and LZK overexpressed (OE) mice for in vivo analysis. Additionally, cortical astrocytes were isolated from tamoxifen-inducible, astrocyte specific LZK-KO or LZK-OE mice, and gene deletion was induced in vitro using 4-Hydroxytamoxifen. Changes in cytoskeleton dynamics and cell migration were assessed through the analysis of the filamentous actin-to-globular actin ratio, microtubule acetylation, scratch assays, and lamellipodia characterization. Changes in expression of cytoskeleton-associated genes were studied by performing RT-qPCR. To provide more insights into the mechanism through which LZK modulates cytoskeleton dynamicity and cell migration, primary astrocytes were treated with Akt and Stat3 inhibitors. To assess motor recovery in LZK-KO and LZK-OE mice, Horizontal Ladder test and Basso mouse scale (BMS) were conducted.

Results: LZK-KO astrocytes exhibited significant reduction in differentially expressed cytoskeletal-associated genes in primary astrocytes cultures and astrocytes isolated from injury sites. Astrocytes lacking LZK showed reduced levels of polymerized actin, and acetylated tubulin, leading to attenuated cell polarization and migration. Conversely, astrocytes overexpressing LZK showed elevated levels of genes involved in cytoskeleton dynamics, the filamentous-to-globular actin ratio, and acetylated tubulin. Our results showed that

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Matin Hemati, PhD

aforementioned changes in cytoskeletal dynamics promoted formation of polarized structures and astrocytic cell migration. To provide the mechanistic insight, we demonstrated that LZK deletion resulted in reduced levels of p-Akt and p-S6, master regulators of cytoskeleton remodeling, while in LZK-OE astrocytes, level of p-Akt and p-S6 were significantly increased, confirmed in both in vivo and in vitro analyses. Our results also showed that inhibition of Akt resulted in reduction of cytoskeleton-associated gene expression levels in LZK overexpressing astrocytes. Furthermore, our behavioral analysis revealed that although significant differences were observed at the molecular level, there were no statistically significant differences in motor recovery as assessed by BMS and Horizontal ladder tests.

Conclusion: Our findings indicate that LZK is a critical regulator of cytoskeleton dynamics in astrocytes following SCI. LZK regulates cytoskeleton dynamics, cell polarity, and astrocytic cell migration by modulating actin polymerization and tubulin acetylation. Moreover, our results shed light on the mechanism through which LZK modulates cytoskeleton remodeling and astrocytic migration. We demonstrate that LZK deletion inhibits the activation of Akt signaling pathway, while overexpression of LZK activates Akt signaling pathway through activation of p-Akt and p-S6, promoting cytoskeleton reorganization. Therefore, modulation of LZK function at different stages after SCI could either promote the cell polarization and astrocytic migration, favoring the proper formation of the astrocytic scar, or reduce glial scarring and promote axonal regrowth.

About The Speaker I, Matin Hemati, am a postdoctoral researcher in the Spinal Cord and Brain Injury Research Center (SCOBIRC) at the University of Kentucky, currently affiliated with Dr. Amy Chen's Lab. Prior to this position, I earned my PhD in Neuroscience from the University of Sussex, United Kingdom, where I conducted research on neurodegenerative disease in Dr. Majid Hafezparast's Lab. Upon completing my doctoral studies, I transitioned to the University of Kentucky. My current research focuses on the better understanding of molecular mechanisms that regulates cytoskeleton remodeling and migration in scar-forming astrocytes following spinal cord injury. I am passionate about understanding mechanisms that modulates astrocytes to promote the functional recovery after spinal cord injury.

Mental Health Spinal Cord Injury Research – Addiction / Depressions

Mark D. Peterson, PhD, MS, FACSM

SESSION FIVE

Charles E. Lytle, Jr. Research Professor, Professor University of Michigan-Medicine Department of Physical Medicine and Rehabilitation

Understanding the Psychological Morbidity Faced by Individuals with Chronic Spinal Cord Injury

People living with spinal cord injury (SCI) experience motor, sensory and autonomic impairments that may in turn result in the development of numerous secondary and comorbid conditions in the years and decades after SCI onset. This session will be devoted to discussing the physical and mental health issues facing individuals with chronic spinal cord injury. Recent findings from large cohort studies will be presented from US population representative samples of adults with spinal cord injury.

About The Speaker Dr. Mark Peterson is the Charles E. Lytle, Jr. Endowed Professor at the University of Michigan-Medicine, Department of Physical Medicine and Rehabilitation. His work focuses on understanding factors that influence health and life expectancy among individuals with disabilities across the lifespan. This includes efforts directed at identifying precision strategies to prevent cardiometabolic dysregulation and secondary physical and psychological morbidity among children and adults with cerebral palsy and spinal cord injury, as well as a variety of frailty syndromes, and to better understand health disparities among individuals with disabilities from the context of access to preventive care and community wellness. Dr. Peterson has published more than 185 peer reviewed scientific articles in the fields of rehabilitation research, physical activity epidemiology, healthcare disparities, and preventive medicine. He is a PI and Co-I on numerous current and previously funded NIH, NIDILRR, and National Foundation grants.

Gyongyi Szabo, MD, PhD

Professor of Medicine; and Faculty Dean, Harvard Medical School, Chief Academic Officer, Beth Israel Deaconess Medical Center, Chief Academic Officer, Beth Israel Lahey Health

Neuroinflammation: Parallels in Alcohol Use Disorder and Alzheimer's Disease

Alcohol use disorder is associated with systemic inflammation and organ dysfunction especially in the liver and the brain. For more than a decade, studies have highlighted alcohol abuse-mediated impairment of brain function and acceleration of neurodegeneration through inflammatory mechanisms that directly involve innate immune cells. Furthermore, recent studies indicate overlapping genetic risk factors between alcohol use and neurodegenerative disorders, specifically regarding the role of innate immunity in the pathomechanisms of both areas. Considering the pressing need for a better understanding of the relevance of alcohol abuse in dementia progression, here we summarize the molecular mechanisms of neuroinflammation observed in alcohol abuse and Alzheimer's disease, the most common cause of dementia. In addition, we highlight mechanisms that are already established in the field of Alzheimer's disease that may be relevant to explore in alcoholism to better understand alcohol mediated neurodegeneration and dementia, including the relevance of the liver brain axis.

About The Speaker Gyongyi Szabo, MD, PhD a physician scientist and an internationally known expert in liver immunology and inflammation. Her laboratory studies the cellular and molecular mechanisms of inflammation and innate immunity in liver injury to identify therapeutic targets focused on non-alcoholic liver disease, NASH and alcoholic liver disease. Her investigations recently revealed the importance of micro-RNAs and extracellular vesicles in liver diseases. She showed that exosomes can not only be biomarkers but also vehicles of inter-cellular and inter-organ communication. Her studies identified microRNA-122 as a central player in steatohepatitis and showed that miR-155 regulates exosome release. Dr. Szabo's group made the novel discovery that NLRP3 activation and the IL-1ß pathway are potential therapeutic targets in alcoholic hepatitis and NASH. Her translational studies with the use of IL-1 receptor antagonist in a preclinical model of alcoholic hepatitis provided basis for a subsequent first-time clinical trial in alcoholic hepatitis with IL-1 inhibition. She is elected member of the Hungarian Academy of Sciences, and fellow of the AASLD, AGA and the American College of Physicians (ACP). Dr. Szabo serves on advisory boards of several federal agencies, leading academic institutions and pharmaceutical companies. Dr. Szabo served on the Governing Board and as President of the American Association for the Study of Liver Diseases (AASLD) in 2015 and she the inaugural Editor-in-Chief of Hepatology Communications. She is recipient of the 2020 Distinguished Scientific Achievement Award from the American Liver Foundation.

Christopher West, PhD

Associate Professor
Department of Cell and Physiological Sciences
University of British Columbia

Manipulating the Cardiorespiratory System to Improve Exercise Performance in the Setting of Cervical Spinal Cord Injury

In this lecture, I will present the view that the cardiorespiratory system is the limiting factor governing exercise performance in athletes with cervical spinal cord injury (SCI). I will then present data from our recent studies in athletes with cervical SCI that manipulating the respiratory system via either task specific training or altering breathing patterns improves function and the cardiovascular system and subsequently exercise capacity. I will supplement the data presented in athletes with SCI with some data collected in animal models to demonstrate underlying mechanisms. Finally, I will discuss future endeavors that will seek to combine these approaches with emerging technologies to further enhance exercise capacity in this population.

About The Speaker Dr. West is an Associate Professor in the Department of Cell and Physiological Sciences, Faculty of Medicine, at the University of British Columbia. Dr. West is also a Principal Investigator at both the International Collaboration on Repair Discoveries (ICORD) and the Centre for Chronic Disease Prevention and Management (CCDPM). He sits on the Board of Directors for the American Spinal Injuries Association and regularly collaborates with both Industry and Professional Bodies across North America. The goal of his research program is to advance our understanding of the cardio-autonomic disturbances that occur with traumatic spinal cord injury, with a view to prevent/delay the development of cardiovascular disease. A subsequent goal is to leverage this circuit level understanding of physiological function(s) post-SCI to develop restorative approaches to activate the sub-lesional spinal sympathetic circuits that control the heart and blood vessels.

Jean-Marc Mac-Thiong, MD, PhD

Professor
Department of Surgery, Orthopedist
University of Montreal

Early Acute Activity-based Therapy After Traumatic Spinal Cord Injury

Activity-based therapy (ABT) can be used to improve the neurofunctional outcomes after a traumatic spinal cord injury (SCI). It is initiated only weeks or months after the injury because clinicians remain fearful that earlier initiation of ABT may be harmful to patients, and could lead to neurological deterioration. Based on compelling preclinical evidence showing that ABT initiated within days of a SCI is effective for promoting recovery, our group from Université de Montréal and University of Louisville was the first to apply early acute ABT in patients within 72 hours of a severe SCI. The results of our PROMT-SCI (Protocol for Rapid Onset of Mobilization in patients with Traumatic SCI) study suggest that early acute ABT in the form of in-bed leg cycling is safe, feasible and has the potential to harness plasticity.

About The Speaker Dr. Jean-Marc Mac-Thiong is a clinician-scientist specialized in the surgical management of spinal disorders, and with a particular interest in spinal trauma and deformity. He is a Professor of Surgery and Chairholder of Medtronic Research Chair in Spinal Trauma at Université de Montréal. His clinical practice in spine surgery is based at Hôpital du Sacré-Coeur de Montréal, Hôpital Sainte-Justine and Shriners Hospital for children – Canada. After a bachelor's degree in mechanical engineering from École Polytechnique de Montréal, he completed an MD – M Sc at Université de Montréal. He then completed a PhD in biomedical sciences during his residency in orthopaedic surgery at Université de Montréal. He performed a fellowship in spine surgery at Twin Cities Spine Center. He is particularly recognized for his research on outcome predictors and activity-based therapy after spinal cord injuries. He is internationally known for his contributions in the study of sagittal spino-pelvic balance and spondylolisthesis. He has published over 200 articles in peer-reviewed journals and filed more than 20 patents for spine-related inventions.

Developmental Impact: Perinatal Spinal Cord Injury and Pediatric Spinal Cord Imaging

Seth Smith, PhD

Professor
Institute of Imaging Science,
Department of Radiology and Radiological Sciences,
Department of Biomedical Engineering

Have You Checked To See That It Is Plugged-in? Quantitative Structural and Functional MRI Evaluation of Human Spinal Cord In Development and Disease.

MRI has been a mainstay imaging modality for the diagnosis and evaluation of CNS injury and recovery due to its unprecedented ability to view lesions and evaluate pathology. However, in the CNS, the bulk of MRI research focuses on the brain, while clinically, the brain and spinal cord are often assessed together. As such, clinical spinal cord MRI image quality has lagged behind brain MRI, and advanced quantitative MRI, has even more significantly lagged behind its brain counterparts. However, there is much that can be learned from MRI using these advanced methods, but they need to be tailored to the study, population, disease, and patient rather than translating similar sequences from the brain to the spinal cord and hoping for the best. In this presentation, we will discuss the opportunity that advanced quantitative MRI can have for the evaluation of disease, the leave behinds that can improve clinical MRI, show applications in the pediatric and adult cord, and wrap up with a Magic 8 Ball view of future opportunities.

About The Speaker Dr. Smith hails originally from the foothills of the Appalachians in West Virginia. He graduated with dual BS degrees in Physics and Math from Virginia Tech and received his PhD from The Johns Hopkins University under the supervision of Dr. Peter van Zijl. His PhD focused on developing novel spinal cord magnetization transfer (MT) imaging with application to adrenomyeloneuropathy and multiple sclerosis. He was appointed junior faculty at Johns Hopkins and leveraged spinal cord work to develop and apply advanced imaging to the human optic nerve. Returning to his roots in the Spinal Cord, Dr. Smith joined the Vanderbilt Faculty in 2009 and was promoted to Professor of Radiology in 2019. Dr. Smith is the director of the Center for Human Imaging at the Vanderbilt University Institute of Imaging Science (VUIIS) as well as the inaugural Associate Director of the VUIIS under the direction of Dr. John Gore. His current research focus is developing and applying advanced MRI methods to study the small, somewhat underrepresented, neurological structures in the human with a constant eye towards improving clinical opportunities for spinal cord imaging.

Developmental Impact: Perinatal Spinal Cord Injury and Pediatric Spinal Cord Imaging

Ronaldo M. Ichiyama, PhD

Professor of Neural Control of Movement, School of Biomedical Science, University of Leeds

Functional Recovery After Severe Neonatal and Adult Spinal Cord Injuries: Plasticity, Neuroregeneration and Neurorehabilitation

Locomotor and exercise training following spinal cord injuries (SCI) induces changes in the spinal networks controlling stepping, which correlate with improved stepping ability and function. The underlying mechanisms associated with such functional improvements are only incompletely known. Understanding such changes will lead to better and more efficient interventions to maximize the benefits of training for rehabilitative purposes. Precisely how spinal locomotor circuitry is altered by rehabilitation, however, has yet to be fully elucidated. We have previously demonstrated that extensive synaptic remodelling occurs after injury in lumbar motor circuits caudal to the lesion. Locomotor training completely reversed those changes back to intact control levels following neonatal SCI.

The combination of plasticity enhancing strategies with locomotor training after adult SCI to potentiate functional recovery will also be discussed. For example, we have combined administration of an antibody against the myelin-associated axonal growth inhibitor, Nogo-A, with locomotor training, which surprisingly produced negative functional outcomes. Either treatment alone was beneficial for functional recovery but the combination produced the lowest functional scores. Our most recent evidence demonstrates that timing of delivery of each intervention is critical to overcome such negative outcome. We have also been investigating how modulation of chondroitin sulfate proteoglycans after injury and rehabilitation interact. Our recent results demonstrate that manipulation of perineuronal nets with the enzyme chondroitinase ABC is only beneficial when combined with rehabilitation driven by epidural electrical stimulation. We will discuss recent evidence of how enhanced plasticity must be guided by rehabilitation to result in functional recovery.

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Developmental Impact: Perinatal Spinal Cord Injury and Pediatric Spinal Cord Imaging

Ronaldo M. Ichiyama, PhD

About The Speaker Ronaldo Ichiyama is Professor of Neural Control of Movement in the School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom. He completed his PhD at the University of Illinois at Urbana-Champaign, USA under the supervision of Prof. Gary Iwamoto. He then moved to the University of California Los Angeles (UCLA), USA where he studied the effects of locomotor training following spinal cord injuries (SCI) under the supervision of Prof. V. Reggie Edgerton. While at UCLA, he was also an Associate of the Christopher and Dana Reeve Foundation International Research Consortium. He demonstrated that epidural electrical stimulation of the spinal cord was able to elicit weight bearing and coordinated stepping in rats with a complete spinal transection. In his laboratory at the University of Leeds he continues to investigate the processes underlying the recovery of motor function after SCI in both animal and clinical models. His main interests in SCI research are currently on how activity and plasticity-enhancing interventions can be used to promote functional recovery of both sensorimotor and autonomic circuits.

Luis R. Alvarado, PhD[1,2,4]; Molly E. King BA[2,4]; Brendan E. Depue, PhD[1,3]; Andrea L. Behrman, PhD, PT [1,2,4]

[1]Departments of Anatomical Sciences and Neurobiology, [2]Neurological Surgery, and [3]Psychological and Brain Sciences, [4]Kentucky Spinal Cord Injury Research Center, University of Louisville , Louisville, KY

Exploring the Mechanisms of Step Initiation Post-SCI in Children: A Neuroimaging and Electrophysiological Study

This study builds on prior research sponsored by KSCIRT, demonstrating the efficacy of combining transcutaneous spinal stimulation (scTS) with activity-based locomotor training (ABLT) to initiate stepping in children with chronic motor complete spinal cord injury (SCI). We employed neuroimaging and electrophysiological assessments to investigate the underlying neural mechanisms.

Six children aged 6-12 years, at least one-year post-SCI, underwent multimodal neuroimaging and electrophysiological assessments before and after the ABLT + scTS intervention. Our goal was to assess changes in brain functional connectivity and the integrity of spinal pathways including the corticospinal tract (CST), reticulospinal tract (RST), and dorsal column medial lemniscus (DCML).

Post-intervention, all participants acquired the ability to initiate the swing phase of stepping, highlighting the potential of such integrative approaches to facilitate key aspects of motor recovery. Despite these functional gains, no significant changes in motor output were observed below the injury level using current assessment techniques, suggesting the need for more sensitive methods. However, assessments of acoustic startle reflex unveiled significant changes in RST excitability above the injury level, suggesting supralesional neural remodeling and increased plasticity. MRI analyses corroborated these findings, demonstrating increased functional connectivity in brain regions associated with motor control, correlating with reported improvements in motor and sensory functions.

Overall, ABLT + scTS promote appears to promote significant neural changes in children with SCI, particularly within supralesional spinal pathways and brain networks involved in sensorimotor functions. These adaptations may underpin the observed improvements in stepping ability, highlighting the importance of integrated rehabilitation approaches that enhance neural connectivity and plasticity.

Goutam Singh[1], Kathryn Lucas[2], Nicole Stepp[2], Parth Parikh[2], Molly King[2], Mackenzie Good Roberts[3], Mathew Davis[3], Beatrice Ugiliweneza[2], Yury Gerasimenko[2], Andrea Behrman[2]

[1]Kosair for Kids School of Physical Therapy, Spalding University
[2]Department of Neurological Surgery, University of Louisville
[3]University of Louisville Hospital, Louisville, KY

Noninvasive Spinal Stimulation With Activity-based Training to Improve Trunk Control in Children With Spinal Cord Injury

Objective: The aim of this ongoing study is to test the efficacy of a multi-modal approach combining sensorimotor, activity-based-locomotor training (ABLT), and transcutaneous spinal cord stimulation (scTS) to improve sitting posture and trunk control in children with chronic spinal cord injury (SCI). We hypothesized that children undergoing 60 sessions of ABLT + scTS will demonstrate significantly improved sitting posture and trunk control.

Material and Methods: Twelve participants with acquired SCI and trunk control deficits, aged 3-15 years, will be recruited. Currently, 5/12 participants (mean age of 6 years, 2M and 3F) enrolled and completed the study. A 5-channel scTS was used to deliver mono/biphasic rectangular waveform current with 1-ms pulse width, 15-30Hz frequency with 10kHz modulated carrier frequency. ABLT + scTS was provided 5 days a week for a 1.5-hour session. Clinical outcomes, including the Segmental Assessment of Trunk control (SATCo) and Modified functional Reach (MFR) were measured at baseline (pre-training) and post 60 sessions of training. Three trials of upright sitting posture without using arms for support and trunk control during perturbations (anterior and posterior) were assessed at pre- and post-60 training sessions. Trunk kinematics and center of pressure (COP) displacement were collected and compared pre and post-training.

Results: All five participants, P207, P235, P239, P240, and P241 demonstrated improved upright sitting posture and trunk control as measured via trunk segmental angles and trunk segmental angles, MFR, and SATCo scores, respectively. 3/5 participants showed decrease in COP displacement during the anterior and posterior perturbation attempts.

Conclusions: Preliminary findings suggest that multi-modal training combining ABLT + scTS improves upright sitting posture and trunk control in children with chronic SCI.

Liubov Amirova[1], Anastasia Keller[1,2,3], Goutam Singh[1,2], Molly King[1,2], Parth Parikh[1], Yury Gerasimenko[1,4,5], Andrea L. Behrman[1,2,6]

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Cumulative Effects of Transcutaneous Spinal Cord Stimulation with Activity-based Locomotor Training to Augment Trunk Control in Children with Spinal Cord Injury

Chronic spinal cord injury (SCI) in children significantly impacts their trunk control, hindering daily activities. Spinal cord stimulation (scTs) has emerged as a potential therapeutic tool to address this challenge. Our previous work demonstrated the safety and feasibility of acute scTs application in improving trunk posture in children with SCI. However, the long-term effects of repeated scTs applications remain unclear. This pilot study aimed to investigate the cumulative effects of scTs combined with activity-based training (AB-LT) on trunk control in children with SCI.

Three male children (10 ± 4yo) with chronic SCI and trunk deficits underwent ~40 sessions of activity-based locomotor training in combination with scTs at T11 and L1 sites (15, 30, or 60Hz; 10kHz). Adverse events were monitored and reported during each training session. Primary outcomes included blood pressure, heart rate, and pain every 10-15 minutes.

ScTs exposure time averaged 44.0 ± 2.3 min with an average amplitude of 98.2 ± 24.2 mA. No spasms, numbness, or strong pain from stimulation were detected/reported. There were 9 cases (10%) of skin redness observed which were resolved the following day and 3 observed cases (3.3%) of autonomic dysreflexia with scTs. The episodes were managed by rest and resolved within 6 minutes. Blood pressure and heart rate varied during training, which could be a response to both AB-LT and scTs, but did not extend beyond normal values.

Cumulative use of scTs with AB-LT was safe and well-tolerated in 85.6% of cases. The adverse effects were resolved and had no delayed effects.

Funding: The National Center of Neuromodulation for Rehabilitation

Mattingly G[1], Willhite A[1], Sharma P[1], Siu R[2], Stanis N[1], Ovechkin A[1,3], Gerasimenko Y[1,4,5]

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Integrative Effects of Multi-site Transcutaneous Spinal Cord and Plantar Stimulations on Stepping Patterns in Individuals with SCI

We previously reported that spinal cord transcutaneous stimulation (scTS) or direct pressure of the plantar surfaces of the feet can elicit rhythmic, involuntary stepping-like movements in non-injured individuals in a gravity-neutral device (GND). Based on these results, we suggested that scTS and plantar pressure stimulation (PPS) would have excitatory effects on locomotor activity and that the neuromodulatory effects of these stimulation modalities would be complementary.

The neurophysiological implications of these findings in non-injured individuals support the hypothesis that these modalities can be used together as effective neuromodulatory interventions for enhancing the recovery of sensory-motor function following neurological injuries/disorders, including spinal cord injury (SCI).

Two individuals with chronic motor-incomplete SCI (AIS C) underwent an assessment of voluntary oscillatory movement in a GND. Participants were instructed to move their legs in a stepping-like pattern in the GND without scTS, with scTS, and following conditioning stimuli including PPS. Lower extremity (LE) joint kinematics and EMG were analyzed to assess differences.

Maximum LE joint angle excursions and area of hip-knee cyclograms increased in both participants with scTS compared to without. A marked impact of PPS conditioning was evident in joint kinematics, integrated EMG, and total power of EMG after conditioning compared to before. Kinematic outcomes from attempts with scTS after conditioning were additionally increased compared to before. The differences between attempts without and with scTS was lesser after PPS conditioning.

These results indicate that a non-invasive multimodal approach to rehabilitation of locomotor behaviors can be very effective in SCI, even upon first therapeutic use.

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Neuroimaging Features of Cervical Spinal Cord Injury and Recovery of Upper Extremity Function with Spinal Cord Transcutaneous Stimulation

Introduction: In Spinal cord injury (SCI), motor improvements are related to the extent of spinal cord damage. Such relationship has been explored for walking or lower limb function with or without spinal cord stimulation. Approximately 60% of SCI occurs at the cervical cord region and recovering arm and hand function is one of the top rehabilitation goals. Establishing the relationship between extent of spinal cord damage and upper extremity recovery with scTS can serve as an additional measure explaining motor recovery post cervical SCI.

Methods: The presented preliminary data is from two cervical SCI participants enrolled in the study (C2-C4, AIS A). MRI images of the cervical spinal cord were obtained using 3 Tesla scanner. Participants underwent sixty sessions of activity-based recovery training combined transcutaneous spinal cord stimulation (scTS). For MRI, measurements such as cord crosssectional area, diameter, and overall architecture were obtained using the open-source Spinal Cord Toolbox. Pre and post training assessments included Neuromuscular recovery scale (NRS), graded assessment of strength, sensibility and prehension (GRASSP) and grip strength.

Results: We observed reduction in the cross-sectional area, right-left diameter, and distortion of overall shape of the injured spinal cord compared to uninjured cord. Participant with greater damage to the cervical spinal cord demonstrates greater upper extremity clinical and functional deficits. Both participants with motor complete cervical SCI demonstrate improvements in upper extremity outcomes post training. This is an ongoing work that will allow investigating relationship between extent of cervical spinal cord damage and upper extremity recovery with scTS.

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Serum Biomarkers of Functional Recovery Following Treatment of Degenerative Cervical Myelopathy

Degenerative cervical myelopathy (DCM) is the largest cause of spinal cord injury in adults. Decompression surgery is the standard treatment but surgery does not always result in a patient improvement. Here, we have screened for markers of spinal damage in serum from DCM patients before receiving decompression surgery and 6 months after surgery. These markers include Ubiquitin C-terminal hydrolase L1 (UCH-L1), Glial fibrillary acidic protein (GFAP), Apolipoprotein E (ApoE), Neuron-Specific Enolase (NSE), Neurofilament Light Chain (Nf-L) and Amyloid Beta Peptide (AB40, AB42) and others. The goal is to identify serum based biomarkers that associate with the quality of functional recovery following surgery to appropriately recommend future patients as candidates for surgery treatment. Functional recovery is measured here by modified Japanese Orthopaedic Association scale (mJOA) score. We hypothesize that an increase in markers for neuronal damage before surgery will correlate to lower recovery rate. Utilizing a biomarker panel to predict functional recovery will help identify patients for surgical treatment. Eventually, these biomarkers may be utilized as a diagnostic tool to identify early signs of spinal cord damage in DCM as well as other spinal cord injuries. Serum based diagnostics will be more accessible to patients, allow earlier identification of disease so patients can get treatment as soon as possible, and aid physicians in recommending the correct treatment following spinal cord injury. Our goal is to eventually provide a non-invasive, accessible and cost-effective diagnostic tool that will facilitate earlier and more accurate prognosis of disease progression and treatment outcomes.

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Acute Effects of Spinal Cord Epidural Stimulation on Respiratory Performance in Patients with Chronic Spinal Cord Injury

Physiologically inter-related motor and autonomic deficits associated with respiratory-cardiovascular complications are the leading causes of morbidity and mortality in patients with spinal cord injury (SCI). Our previous work demonstrates that respiratory and cardiovascular function in these patients can be improved by using our original respiratory training rehabilitative approach. However, the effectiveness of this intervention is limited by the levels of functional capacity preserved below the neurological level of injury.

Our group has pioneered the use of lumbar spinal cord Epidural Stimulation (scES) to activate motor and autonomic networks below the level of spinal cord lesion. We hypothesized that specific scES configurations targeting voluntary trunk motor (Vol-Trunk-scES) or autonomic cardiovascular (CV-scES) controls alone and the combination of both (Resp-scES) increase spinal network excitability due to enhanced activation of respiratory neural circuitry.

Nine individuals with chronic motor-complete SCI above T1 underwent mapping sessions to investigate if CV-scES, Vol-Trunk-scES, or Resp-scES lead to the amplified respiratory functional responses as measured by changes in maximum expiratory pressure (PEmax) assessed without and in the presence of scES configurations. Overall, we observed that the respiratory performance was increased in the presence of the scES configurations. These results indicate that scES-induced enhancement of the spinal excitability below the level of SCI could be used as a novel rehabilitative approach to provide amplified therapeutic effects when it is combined with respiratory training.

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Preliminary Outcomes of 24-hour Ambulatory Blood Pressure Monitoring in Children with Spinal Cord Injury

Introduction: Spinal cord injury (SCI) is known to disrupt the autonomic nervous pathways leading individuals with SCI at risk of cardiovascular dysfunction and other. Literature shows that adults with SCI have huge fluctuation in blood pressure (BP) throughout a 24-hour period. In children with SCI, there is no such protocol or data to identify these fluctuations. Thus, the objective of this ongoing study is to report the 24-hour ambulatory blood pressure monitoring (ABPM) data in children with chronic spinal cord injury.

Methodology: TM-2441 device from A&D Medical was used to collect the BP. Currently, 8 SCI participants (Age 13 ± 3) with injury ranging from C3-L5, and 5 healthy participants (Age 10 ± 2) have completed the study. The device was programmed to measure BP at 10-minute intervals during the time participant would be awake and 30-minute when participant would be asleep. Each participant was fitted with adjustable arm cuff with device to record ABPM. Each participant and their caregiver were familiarized with the device and provided a diary to log their activities throughout the 24-hour period.

Results: n healthy participants, the average awake BP (115 ± 32 / 77 ± 27) was greater than the sleep BP (89 ± 13 / 55 ± 13). Similarly, in SCI participants, the awake BP (106 ± 21 / 64 ± 18) was also greater than sleep BP (89 ± 13 / 49 ± 10). SCI participants demonstrated decreased blood pressure compared to healthy participants.

Conclusion: In conclusion, preliminary findings suggest that SCI participants demonstrate a decreased BP compared to healthy participants. SCI participants also demonstrate greater BP fluctuation throughout the 24-hour period.

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Lumbosacral Spinal Cord Epidural Stimulation Modulates Autonomic Function and Normalizes High and Low Blood Pressure

Introduction: Cardiovascular dysfunction is common after cervicothoracic spinal cord injury (SCI), manifesting as episodic life-threatening hypertension (autonomic dysreflexia), chronic low blood pressure (BP), and orthostatic hypotension (OH). The BP abnormalities reflect disrupted supraspinal sympathetic reflexes and maladaptation in the spinal circuitry caudal to the injury. We reported that lumbosacral spinal cord epidural stimulation (scES) optimized for cardiovascular function (CV-scES) mitigates OH in individuals with chronic, severe SCI. Here we report the ability of CV-scES to normalize both high and low BP in daily life and improve orthostatic tolerance.

Methods: Thirty-two participants with chronic cervical SCI and OH underwent implantation of a 16-electrode array over dorsal lumbosacral spinal segments. Electrical stimulation parameters were optimized to maintain systolic BP (SBP) between 110mmHg-120mmHg without evoking lower extremity muscle activity. Daily-life brachial BP was monitored with stim OFF and ON. During lab visit, participants underwent 70° head-up tilt lasting up to 30 minutes, with Stim OFF and ON.

Results: Comparing to Stim OFF, CV-scES decreased BP instability during 6-hour daily life, by decreasing standard deviation of SBP (21 ± 9 vs.10 ± 2mmHg, mean±SD, Stim OFF vs. CV-scES, $p = 0.0003$) and SBP range above 115mmHg anchor (36 ± 25 vs. 13 ± 5mmHg, $p = 0.0033$). CV-scES also improved orthostatic tolerance, increasing tolerated tilt time (12 ± 11 vs. 28 ± 7 minutes, $p < 0.0001$) and BP during tilt (lowest SBP: 68 ± 11 vs. 93 ± 11 mmHg, $p < 0.0001$).

Conclusions: In individuals with SCI, cardiovascular function targeted lumbosacral scES was able to modulate autonomic function to normalize high and low blood pressure in daily life and improve orthostatic tolerance.

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Patterns of Opioid and Cannabis Use Disorders in Spinal Cord Injury Patients Treated in the United States Emergency Departments

Background: Chronic pain affects more than 60% of individuals with Spinal Cord Injury (SCI). Despite the prevalence, no effective management is available, leaving them susceptible to substance misuse. Current literature describes increased use in other populations, but trends for SCI-individuals remain understudied.

Objective: To evaluate the trend and epidemiology of opioid use disorder (OUD) and cannabis use disorder (CUD) over the years in SCI-population treated in Emergency Departments (ED) in the United States.

Methods: Nationwide Emergency Department Sample from 2006 to 2020 was used to extract adults with SCI.

Results: There were 1,664 OUD, 2,687 CUD, 272 OUD + CUD, and 90,185 No-use (NU) ED visits. From 2006 to 2020, substance-use ED visits have increased: OUD ED from 1171 to 2775 (2-fold), CUD from 490 to 2390 (5-fold), and OUD + CUD from 98 to 215 (2-fold). Those in CUD were significantly younger (median age 36) with higher rates of males (86%), in the lowest income quartile (42%), and with Medicaid insurance (41%) compared to NU (Median age 53, 71% males, 31% lowest income quartile, 19% Medicaid all $p < 0.0001$). The same trend was observed in OUD with median age 45, 75% males, 36% in the lowest income quartile, and 40% with Medicaid, all significantly different from OUD ($p < .0001$). These patterns remained consistent throughout the study period.

Conclusion: Despite healthcare and legal efforts to reduce substance misuse, emergency visits related to opioid and cannabis disorders continue to increase. A comprehensive understanding of demographic profiles for each substance is essential to inform future prevention strategies.

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Review of the Supraspinal Mechanisms of SCS on Chronic Pain and Cognition

Chronic pain is one of the leading causes of disability worldwide, with a myriad of debilitating factors, including physical and cognitive burden. Spinal cord stimulation (SCS) is a minimally invasive treatment option for drug-refractory chronic pain. Although SCS can improve pain perception and related physical well-being, it is not well understood how SCS affects cognitive function in pain patients. Cognitive impairments arising from chronic pain were analyzed and compared with cognitive effects measured after SCS treatment, and likewise the activation in supraspinal areas resulting from chronic pain were compared with altered activity in those areas following SCS treatment. Increased activity was found in the anterior cingulate cortex, whereas stronger connectivity was found between the thalamus and the insular cortex, as well as between the primary somatosensory cortex and emotional-processing areas. All findings point to SCS influencing and modulating the cognitive-emotional aspect of pain perception. Future research should focus on the effects of SCS on cognition, as very limited studies are currently available on the topic.

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Assessment of Radiological Parameters Associated with Traumatic Cervical Spinal Cord Injury

Objective: Understanding the impact of cervical traumatic spinal cord injury using baseline radiological assessments on patient outcomes.

Methods: We evaluated baseline magnetic resonance imaging (MRI) parameters for 169 patients admitted with tSCI across two institutions. Various imaging parameters including maximal canal compromise (MCC), maximal spinal cord compression (MSCC), extent of cord compression (ECC), maximal cord swelling (MCS) and maximum cord diameter above the injury level (dmax) were independently measured by two individuals. Patients were stratified into intradural, and combined compression groups based on their MSCC (< 5% and > 5% respectively) and the predictive values of these parameters on American Spinal Injury Association Impairment Scale (AIS) conversion was further assessed.

Results: 37 and 132 patients were classified into intradural and combined compression groups respectively. The MCC, MSCC, MCS and dmax values were significantly different between these two groups (p-value = 0.0396, < 0.0001, 0.009 and 0.0009 respectively). Binary ordinal logistic regression analysis revealed an overall decrease in AIS conversion probability at 12 months with each a mm increase in ECC (p = 0.012) or with 1 % increase in MCS (p = 0.012). Moreover, receiver operating curve analysis revealed ECC and MCS as good and excellent predictors of AIS conversion at 12 months in the intradural compression group (Area Under Curve = 0.72 and 0.94 respectively).

Conclusion: Admission cervical MRI findings indicative of intradural cord compression and maximal swelling are predictive in situ biomarkers for the likelihood of AIS conversion at one-year follow-up.

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Comparative Outcomes of Dual Traumatic Spinal Cord Injury and Traumatic Brain injury versus Isolated Injuries in United States Emergency Departments

Background: Dual injuries, TSCI + TBI, are often classified as either TBI or TSCI. Consequently, they have not been sufficiently studied. This study aimed to compare dual injuries with TBI and TSCI treated in United States (US) Emergency Departments (ED).

Methods: NEDS 2006-2020 data were used to extract ED visits of adults with TSCI or TBI or TSCI + TBI.

Results: There were 68,578 with TSCI + TBI, 356,562 with TSCI, and 23,579,060 with TBI. Dual injuries were cervical TSCI with mild/moderate TBI (66.9%), thoracic TSCI with mild/moderate TBI (19.37%), lumbar/sacral/cauda-equina TSCI with mild/moderate TBI (7.03%), and severe TBI with any TSCI (6.69%). The majority were males (78%) with median age 50. Demographics and socioeconomics for TSCI + TBI were statistically different from TSCI and TBI. From ED, 85% of those with dual injuries were admitted to the hospital compared to 73% of TSCI and 20% of TBI ($p < .0001$). Their median length of hospital stay was 9 days vs 7 for TSCI and 4 for TBI ($p < .0001$). Median charges were $181,985 vs $92,327 for TSCI vs $46,696 for TBI ($p < .0001$). Hospital mortality was 12% compared to 5% TSCI and 6% TBI ($p < .0001$).

Conclusion: Dual injuries have distinct profiles different from TBI and TSCI regarding demographics and socioeconomic factors. They have higher charges in ED, with a majority necessitating subsequent hospital admission, where they have more extended stays, higher charges, and higher mortality risk. Understanding their unique needs is essential to deliver tailored care to optimize their health outcomes and overall quality of life.

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3D In-Vitro Model of Human Neurovascular Unit to Study Traumatic Brain Injury

Introduction: Studies demonstrated a link between mild repetitive traumatic brain injuries (mrTBI) and the progression of neurodegenerative tauopathy called chronic traumatic encephalopathy (CTE). The human neurovascular unit (NVU) plays a crucial role in CTE onset and progression; however, the molecular mechanisms that drive it remain unknown due to the lack of a physiologically relevant model that can replicate the CTE phenotype. Thus, here, we developed a human 3D in vitro model of the neurovascular unit to study the molecular mechanisms of mrTBI-induced neurodegeneration.

Methods: Our NVU comprises 3 main cell types: green fluorescent protein-expressing human brain microvascular endothelial cells (GFP-hBMEC) (E), astrocytes (A), and pericytes (P) seeded into porous silk scaffolds at a ratio of 1:0.5:0.3 million cells and enveloped into collagen type I gel. Tested conditions (1.) Extracellular matrix (ECM): laminin, fibronectin, collagen type 4, and Matrigel; (2.) Cellular compositions: EAP, EP, and EA. All procedures were completed following the manufacturer's specifications.

Results: We optimized the 3D in-vitro NVU model along 3 main factors: cell density, ECM supplementation, and cell composition. We demonstrated that high cell density (1-2 mln), Matrigel, and co-culture with both astrocytes and pericytes are required for vascular-like network formation within 2 months post-seeding. Homeostatic growth of all cells was validated through Western Blot analyses of tight junction (ZO1, claudin-5), BMEC functional (CD31), and glial (Aquaporin-4, PDGF) markers.

Discussion: Our results demonstrated endothelial cells' sensitivity to their environment for long-term stability. We will further test whether our NVU model with/without neurons demonstrates p-tau pathology after mrTBIs.

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Human Triculture 3D in Vitro Brain Tissue Model to Study Chronic Traumatic Encephalopathy Phenotype With Mild Repetitive Injury

Introduction: Chronic traumatic encephalopathy (CTE) is a progressive, neurodegenerative disease associated with mild repetitive head trauma. CTE's hallmark is p-tau aggregates, demonstrated in post-mortem brains (the only model to study the final stage of this disease). Thus, developing a system that allows interrogation of the molecular mechanisms and biomarkers associated with CTE disease is of utmost importance. Using an earlier developed human 3D in vitro triculture model, we determined the criteria for a mild injury that ensures neuronal network stability and allows for repetitive impacts. Next, we narrowed down the required injury conditions that lead to CTE-like p-tau phenotype.

Methods: Human neurons, primary astrocytes, and a microglial cell line were seeded in silk scaffolds (d = 6mm) and embedded in a collagen gel at 2:0.5:0.1 million cells, respectively. The injury was inflicted using a controlled cortical impactor (CCI) with different conditions – tip size, speed, number of hits, time interval, and time point.

Results: Our results demonstrated that 1mm and 3mm tips with a 1m/s injury preserved neuronal network integrity, thus allowing us to perform repetitive injuries. Next, we demonstrated that 7D and 14D intervals between injuries resulted in a p-tau aggregates increase 7D, 14D, and 4W after the last injury. At last, we demonstrated significant changes to energy production mechanisms in response to injury.

Conclusions: Our results demonstrate the feasibility of using our model to study the onset and progression of mild repetitive injury-induced neurodegenerative disease. Based on our preliminary data, we will evaluate the vascular contribution to the disease progression.

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A Comparison of Sleep Quality Between Women With and Without Head Injuries Due to Intimate Partner Violence

Intimate partner violence (IPV) results in adverse outcomes for women, such as repetitive head injuries, poor mental health, and decreased sleep quality; however, limited research has examined the sequelae of head injuries in this population. The current study examined the relationship between head injury and sleep quality among women survivors of IPV. Participants were recruited from Kentucky court jurisdictions after receiving a protective order against an intimate partner. The sample included women with no head injury (n = 260) and women with IPV-related head injury (n = 244) who completed an in-person interview, including the Pittsburgh Sleep Quality Index (PSQI), Conflicts Tactics Scale, and a posttraumatic stress disorder (PTSD) diagnostic interview. A t-test indicated a significant difference in global PSQI score between head injury groups ($t = 7.10$, $p < .001$, $d = .63$), with women who had IPV-related head injuries experiencing worse sleep quality. A series of Mann-Whitney U tests indicated significant differences between groups on all but one PSQI component, indicating that women with IPV-related head injuries had worse subjective sleep quality, longer sleep latency, shorter sleep duration, more sleep disturbances, greater use of sleep medication, and more daytime dysfunction. An ANCOVA indicated the relationship between head injury and sleep remained significant ($p = .001$), even after controlling for sociodemographics, IPV severity, and PTSD. Clinicians treating IPV survivors may consider head injury as a potential cause of sleep quality complaints, and also proactively recognize poor sleep as a possible indicator of an untreated head injury.

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Explainable AI for Prediction of Functional Connectome Self-Similarity Changes Arising from Head Acceleration Event Exposure

Introduction: Repetitive exposure to head acceleration events (HAEs) has been demonstrated to alter functional brain networks in high school American football athletes. Quantitative evaluation of the causal relationships underlying these HAE-induced changes in brain health is critical for advancing injury rehabilitation and prevention in collision sports athletes.

Methods: Using explainable artificial intelligence methods, a model has been developed for predicting the longitudinal alterations in brain resting-state fMRI (rs-fMRI) functional connectomes (FCs) as a function of both HAE exposure and athlete demographics. 58 high school American football athletes (FBA) and 14 peer non-collision sport controls underwent serial rs-fMRI around a competition season. FBA were also monitored for HAEs in all practices and games. Following quality assurance, FCs were generated for 42 FBA and converted to a tangent space (defined from controls) to enhance the unique "fingerprint" associated with each subject. Fingerprint self-similarity (Iself) was evaluated for FBA across sessions during (In1, In2) and after (Post) the season, relative to the pre-season (Pre). Iself was observed to decrease with HAE exposure (especially at In2), and to recover after the end of HAE exposure. ElasticNet was used to select features for decision tree modeling, predicting Iself change from In2 to Post. Key predictive variables were explained by Shapley values.

Discussion: Key variables predicting post-season I, self recovery were related to late-season participation (positive), exposure to HAEs exceeding 40g and 50g (negative), and history of concussion (positive). These variables may be monitored for use in prevention of, and recovery from, HAE-induced functional connectivity changes.

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Social Determinants of Health Influence on Mild Traumatic Brain Injury Symptom Burden

Intimate partner violence (IPV) results in adverse outcomes for women, such as repetitive head injuries, poor mental health, and decreased sleep quality; however, limited research has examined the sequelae of head injuries in this population. The current study examined the relationship between head injury and sleep quality among women survivors of IPV. Participants were recruited from Kentucky court jurisdictions after receiving a protective order against an intimate partner. The sample included women with no head injury (n = 260) and women with IPV-related head injury (n = 244) who completed an in-person interview, including the Pittsburgh Sleep Quality Index (PSQI), Conflicts Tactics Scale, and a posttraumatic stress disorder (PTSD) diagnostic interview. A t-test indicated a significant difference in global PSQI score between head injury groups ($t = 7.10$, $p < .001$, $d = .63$), with women who had IPV-related head injuries experiencing worse sleep quality. A series of Mann-Whitney U tests indicated significant differences between groups on all but one PSQI component, indicating that women with IPV-related head injuries had worse subjective sleep quality, longer sleep latency, shorter sleep duration, more sleep disturbances, greater use of sleep medication, and more daytime dysfunction. An ANCOVA indicated the relationship between head injury and sleep remained significant ($p = .001$), even after controlling for sociodemographics, IPV severity, and PTSD. Clinicians treating IPV survivors may consider head injury as a potential cause of sleep quality complaints, and also proactively recognize poor sleep as a possible indicator of an untreated head injury.

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Increased Hemorrhagic Stroke and Impaired Hemostasis in Mice Deficient of the Integrated Stress Response Kinase HRI

After injury, the integrated stress response (ISR) reduces general protein synthesis and upregulates downstream ISR genes to promote cell survival or initiate cell death. In addition, ISR regulates various physiological processes, including immunity and synaptic plasticity. However, role of ISR in brain damage/functional recovery after hemorrhagic stroke has not been directly addressed. We report upregulation of multiple ISR genes in the peri-hematoma region one day after collagenase-induced intracerebral hemorrhage (ICH) in mice. Moreover, worsened functional impairment post-ICH was observed in knockout mice for the protein kinase HRI/EIF2AK1 that activates ISR in response to oxidative- or mitochondrial stress. In the hemisphere ipsilateral to the collagenase injection site, increased lesion area, elevated number of degenerating neurons, and greater staining area for the neuroinflammatory marker CD36 were also present on day 7 post ICH induction. However, such an enhancement of tissue damage was preceded by increased hemoglobin levels in the ipsilateral hemisphere one day after collagenase injection. Therefore, increased ICH severity in Hri-/- mice may be driven primarily by increased bleeding. In support of this notion, prolonged tail bleeding time was found in those mice together with a 50% reduction in blood platelet number. Our findings demonstrate the unexpected role of HRI in hemostasis and open an avenue for future studies into significance of pro-hemostatic ISR in pathogenesis of cerebrovascular disease.

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Temporal Dynamics of Delayed B Cell Infiltration After Traumatic Brain Injury in Mice

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity for young adults. Survivors of moderate to severe TBI often face persistent cognitive and neurobehavioral deficits. Repeated failed clinical trials targeting neuronal injury mechanisms have motivated expanded efforts to understand the roles of other cell types in the secondary injury cascade following trauma. The roles of astrocytes and microglia in driving neuroinflammation are now well established, as are contributions of systemic innate immune cells such as neutrophils and monocytes. Although clinical studies describe systemic adaptive immunity engagement, the timing and extent of B cell diapedesis into the brain after TBI remains unknown, as existing studies in experimental TBI are limited largely to a single timepoint. We hypothesize that TBI triggers delayed B cell diapedesis into the cortex following a cortical contusion injury. To test this hypothesis, tissues collected from adult mice euthanized 1, 3, 7, 14 or 28 days after receiving controlled cortical impact TBI or sham injury were immunolabeled with the B cell antibody B220. Our data demonstrates that numbers of B220+ B cells within the contused cortex are small at 1 and 3 days, peak at 7 and 14 days, and decrease by 28 days. While many B cells were found within sites of cortical hemorrhage, B cells were observed more remotely at or after 7 days post-injury, suggesting migration or delayed diapedesis. Future studies will characterize morphological and phenotypic characteristics of B cells within the injured brain to gain insight to their potential function.

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Altered Cerebral Artery Territories and Acute Microhemorrhage Following Single and Repeated Mild Traumatic Brain Injury in Mice

Mild traumatic brain injury (mTBI) is accompanied by headaches, dizziness and other neurological dysfunction, often in the absence of discernable changes in gross brain structure. Changes in cerebral blood flow may contribute to deficits in neuronal function. To better understand the effects of single and repeated concussion-like mTBI on the cerebrovasculature, we examined the major cerebral surface artery territories and labeled brain sections for intraparenchymal microhemorrhage and microglial activation. Adult male mice were subjected to either one (n = 7) or two (n = 19, 1-day inter-injury interval) mild, midline impact(s) to the closed, exposed skull using a pneumatic impactor or a sham injury (n = 3). At 1 day after the final injury, mice were transcardially perfused with ink-containing gelatin for *ex vivo* visualization and quantification of the cortical area of the territory supplied by the anterior, middle, or posterior cerebral arteries, as well as the extent of territory overlap. At one day after a single mTBI, the territory supplied by the posterior cerebral artery (PCA) increased by 17%, while that supplied by the middle cerebral artery (MCA) decreased by 10% compared to sham. These changes were amplified after double mTBI, with a 29% increase and 20% decrease, respectively, compared to sham. Mild TBI resulted in sporadic microhemorrhages in multiple brain regions, as detected by Prussian blue staining. Modest CD68-positive microgliosis was observed in the hippocampus, entorhinal cortex and optic tract, with more prominent microgliosis in lateral cerebellar regions. Diffusely distributed microhemorrhages did not appear to be associated with regionally restricted microgliosis.

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Investigating Cerebral Metabolism Following a TBI in an AD-relevant Mouse Model

Alzheimer's Disease (AD) is a neurodegenerative condition marked by metabolic dysfunction and amyloid accumulation. Glucose hypometabolism, a critical aspect of AD, manifests early in its progression and aligns with the onset of clinical symptoms. Similarly, metabolic dysfunction is associated with Traumatic Brain Injury (TBI), suggesting an intersection between TBI and AD. Understanding the interplay is essential for unraveling AD's etiology and identifying therapeutic strategies. We hypothesized TBI would exacerbate the long-term outcomes in the APP/PS1 KI model, which is predisposed to developing amyloid plaques. Utilizing this mouse model of familial AD, we examined mitochondrial bioenergetics and metabolomics following TBI. After oral administration of [U-^{13}C] glucose, higher metabolite abundance and mean enrichment of pyruvate was observed 1-month post-injury in brain tissue of KI mice but resolved by 8 months post-injury, independent of injury status. Other metabolites, such as malate, aspartate, γ-aminobutyric acid (GABA), and glutamate show higher ^{13}C enrichment in the cortex of KI mice, regardless of injury status. APP/PS1 KI mice displayed lower State IV oxygen consumption rates, suggesting differences in metabolite abundance and enrichment disrupt mitochondrial homeostasis. Injury did not lead to an increase in amyloid plaque burden in KI mice. Our findings highlight metabolic differences between KI and WT genotypes, supporting the role of metabolic dysfunction in AD. Surprisingly, TBI showed limited long-term impact on genotype. The study underscores the significance of metabolic health in AD and the potential for early interventions to mitigate metabolic dysfunction. Further research is needed to fully understand the interplay between these factors.

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Spreading Depolarizations After Lateral Fluid Percussion Alter the Hippocampal Dentate Gyrus' Transcriptomic Profile

Many traumatic brain injury (TBI) patients experience spreading depolarizations (SDs), pathophysiologic waves of depolarization that propagate across the cortical tissue and lead to a near complete loss of membrane potential. The presence of SDs after TBI are associated with poor outcomes in the patient population and preclinically, are associated with exacerbated cortical tissue damage. However, the effects of post-TBI SDs on the hippocampus, a major structure involved in learning and memory, are poorly understood. In the present study, adult male Sprague Dawley rats received a lateral fluid percussion injury (LFPI) with or without SDs induced by applying potassium chloride (KCl) to the exposed dura (n = 3/group). Three weeks post-injury, animals were euthanized, and RNA was isolated from the hippocampal dentate gyrus and sequenced (Illumina NextSeq 2000). Raw reads were processed and aligned using ExpressAnalyst and differential expression was determined using DESeq2. A total of 28 genes were differentially expressed ($q < 0.05$; $log2FC > 1.0$), all of which were downregulated in animals that received SDs after TBI. Pathway analysis suggests that SD induction after TBI reduced gene expression associated with complement activation (C4-like, C4a, C7; $q = 0.0002$) and immune system processes (Anxa1, Serping1, CD74, RT1-CE10; $q = 0.005$) compared to TBI alone. While SDs are commonly believed to be deleterious, our data suggest that SDs after injury lead to transcriptional changes associated with decreased inflammatory cascades at three weeks post-injury. This suggests a complex and potentially contradictory hippocampal response to SDs after traumatic brain injury.

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Repetitive Cortical Spreading Depolarizations Induced After Experimental Traumatic Brain Injury Are Associated With Acute Neurological dDysfunction

Cortical spreading depolarizations (CSDs), waves of mass depolarization that cause near-complete loss of ion homeostasis, occur in > 60% of patients with moderate-severe traumatic brain injuries (TBIs) and are associated with poor outcomes 6 months after injury. The effects of CSDs on behavior and cognition after TBI have not been fully elucidated. We hypothesized that repetitive CSDs would decrease acute behavior performance after experimental TBI. Adult Sprague Dawley male and female rats were subjected to either a moderate lateral fluid percussion (2.11 ± 0.09atm) or sham injury. Within 35 minutes of injury, CSDs were induced every 15 minutes for 2 hours using 1M KCl or saline (control) application to the cortex. Animals (n = 12 per group) underwent modified neurological severity score (mNSS), open field, radial arm maze (RAM), and novel object recognition (NOR) tests during the first 3 days after injury to analyze general neurologic function, locomotor activity, working memory, and short-term memory respectively. There were no differences in RAM performance between groups ($p = 0.1381$). There was also no difference between groups in time spent with the novel object ($p = 0.1301$). There were no differences in locomotor activity between groups during open field testing ($p = 0.1064$). However, TBI + CSD animals had significantly worse mNSS scores than the sham + saline, sham + CSD, and TBI + saline animals (2-way ANOVA, $p = 0.0011$). Acoustic startle and balance tasks were most affected. Our study revealed acute general neurological dysfunction, but no deficits in locomotion or memory performance, after the combination of repetitive CSDs and TBI. These findings suggest that repetitive CSDs may exacerbate neurological deficits following TBI.

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Mitochondrial Dysfunction in Caenorhabditis Elegans: An In-Vivo Model of Traumatic Brain Injury

Traumatic brain injury (TBI) affects millions of people annually, yet there is no FDA-approved treatment for this condition. Mitochondrial dysfunction propagates the defects in brain metabolism seen following TBI, making this organelle a key focus in studies on therapeutics targeting neuroprotection. Current TBI models require isolation of mitochondria to measure their function. Therefore, there is not an in vivo model of these effects. The nematode species Caenorhabditis elegans has been a successful model for other neurological conditions. Due to their small size and ability to be genetically modified, this study aimed to determine if C. elegans can be used to observe mitochondrial function following neuronal injury. C. elegans lacking the unc-70 gene, which codes for a homologue of the human protein β-spectrin, have been shown to experience axonal breakage. Thus, we hypothesized that mitochondrial function would be decreased in C. elegans lacking this gene following injury when compared to wild type worms. Adult unc-70 knockout and wild type C. elegans were injured by mixing them in solution. Control groups were not mixed. The Seahorse XFe24 measured the effects of uncouplers and inhibitors of the electron transport chain in these animals. Mitochondrial respiration was minimal in C. elegans lacking unc-70. Interestingly, decreased mitochondrial function was observed in injured wild type worms. Overall, our study highlights how C. elegans can be used to observe the mitochondrial effects of TBI in vivo. Future studies can be performed to assess the effects of pharmacotherapies, such as mitochondrial uncoupling agents, on wild type C. elegans.

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Rit2 Loss Protects Against Traumatic Brain Injury by Modulating Neuronal Bioenergetics

Traumatic brain injury (TBI) constitutes a major public health problem as a leading cause of death and disability in United States with almost 5 million people affected at an annual cost burden of more than $76 billion. Therefore, therapies that ameliorate the chronic burden of TBI by mitigating neuronal injury and improving quality of life post injury are in urgent need. We identified RIT2, a neuronally expressed small GTPase, as a novel regulator of neurodegeneration following brain injury. Preliminary data using RIT2 knockout (RIT2KO) transgenic mice found that RIT2 silencing significantly blunts in vivo hippocampal neuron death and attenuates cognitive dysfunction following contusive TBI. A TBI can compromise brain metabolism, characterized in part by impaired mitochondrial bioenergetics resulting in defective mitochondrial respiration, oxidative stress, and neuronal death. To understand whether RIT2 plays a role in neuronal metabolism, we performed targeted metabolomics analysis of RIT2KO and Wild Type (WT) mice using GC-coupled mass spectroscopy and found that RIT2 loss results in a significant alteration in brain metabolism, with a marked decrease in TCA cycle metabolites. In agreement with our metabolic analysis, seahorse bioanalysis of RIT2KO primary cortical neurons found lower levels of basal oxygen consumption (OCR) and unchanged spare respiratory capacity, with significantly higher rates of glycolysis and glycolytic reserve than WT neurons. Taken together, these data indicate that Rit2 plays a role in maintaining neuronal bioenergetics and suggests that loss of Rit2 promotes a metabolic environment with enhanced aerobic glycolysis to promote neuronal survival following TBI.

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Mitochondria Dysfunction in Moderate Traumatic Brain Injury

Mitochondria dysfunction and intercellular transfer have been demonstrated in traumatic brain injuries (TBI) and other neurodegenerative disorders; however, the role these transferred mitochondria play and the molecular mechanisms that drive such communication are yet to be elucidated. This study determined temporal alterations in mitochondria function post-moderate TBI and correlated the outcome with neuronal network integrity.

To understand the cell-specific contribution of mitochondria(mt) dysfunction to neurodegeneration, we employed a 3D triculture model consisting of astrocytes (mtBFP), microglia (mtGFP), and neurons (mtDsRed2). Immediately before injury, samples were treated with Rasarfin and B7483 drugs to inhibit mitochondria transfer and acceptance. We monitored the oxygen consumption rate (OCR) on freshly isolated mitochondria using the Seahorse method and correlated it to immunofluorescent images of mitochondrial and neuronal network damage.

Acutely, we saw no difference in OCR across all groups and significant neuronal network damage (80%, $p < 0.05$). After one week, the injury + drugs group demonstrated a significant increase in OCR ($p < 0.05$) from injury control and sham, correlated with the onset of neuronal network recovery. The trend continued to eight weeks post-injury, the sham and injury + drugs group evening out in OCR and having restored neuronal networks. Injury control never fully recovered its OCR rate but peculiarly restored its neuronal network density.

Our findings suggest inhibiting mitochondrial transfer might have a neuroprotective effect associated with improved cell ATP production capacity. These insights could pave the way for novel therapeutic strategies in the treatment of TBI and other neurodegenerative disorders.

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Cell-specific Mitochondria Alterations Post-traumatic Brain Injury in 3D In-itro Triculture Model

Introduction: Traumatic brain injury (TBI) induces significant long-term consequences with mitochondria (mt) being a potential driver, as they play a crucial role in controlling brain cells' activity and function. However, cell-specific molecular mechanisms that drive mitochondria dysfunction post-TBI remain unknown. To address this critical need, a novel approach was developed to study cell-specific mitochondria networks and functional alterations in response to injury.

Methods: A silk scaffold-based 3D model composed of 2 million mt-dsRed2-Neurons, 0.1 million mt-EGFP (green)-HMC3 microglia, and 0.5 million mt-BFP2 (blue)-astrocytes embedded in 3% collagen type I hydrogel was used for controlled cortical impact (CCI) injuries at 6m/s with a 3mm injury tip.

Results: Following FAC sorting, 6.92% of neuronal stem cells, 45.12% of astrocytes, and 8.65% of microglia were isolated and expanded for further use. The results revealed significant changes in mitochondrial function post-injury, with microglial mitochondria showing a 20% decrease in fragmentation rate ($p < 0.05$) at 48h and 1w after injury, and neuronal mitochondria showing a 15% drop ($p < 0.05$) only at 1week post-injury, while astrocytic mitochondria network remained stable. At last, a 50% increase in mitochondria's oxygen consumption rate at both time points was observed.

Discussion: A 3D in vitro model with cell-specific labeled mitochondria successfully demonstrated the differences in mitochondria dysfunction in response to injury between different cell types. Future steps will focus on dissecting cell-specific functional mitochondria changes in response to injury and the molecular mechanisms associated with it.

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A Spinal Cholinergic Pathway for the Amplification of Breathing

Breathing is a vital biological function that must be promptly adjusted to accommodate changing metabolic demands. While the generation of the breathing rhythm is well established to occur in the brainstem, increasing evidence suggests that spinal interneurons play crucial roles not only in modulating respiratory output but also in promoting recovery of breathing after spinal cord injury. Despite its significance, our understanding of the organization and functions of the spinal circuits controlling breathing remains limited.

In this study, we investigated the role of cholinergic spinal interneurons in breathing control. In the lumbar spinal cord, these interneurons are essential for facilitating the output of motoneurons that control hindlimb muscles during locomotion. Given that these cholinergic interneurons are also present in the cervical spinal cord, where respiratory motoneurons are located, we hypothesized that spinal cholinergic interneurons act as a parallel pathway to enhance breathing during states of increased metabolic demand.

Using in vitro and in vivo approaches in mouse models, we discovered that cholinergic interneurons are synaptically connected to phrenic motoneurons, receive phasic excitation from brainstem respiratory centres, and facilitate phrenic output to the diaphragm through M2 muscarinic receptors. Additionally, spinal cholinergic interneurons are activated during and contribute to increasing tidal volume during hypercapnia, which may occur during periods of heightened metabolic demand.

Collectively, these findings identify a novel spinal pathway that amplifies breathing, presenting a potential target for promoting recovery of breathing following spinal cord injury when brain inputs are compromised.

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Depletion of Peripheral Monocyte-derived Macrophages Leads to Breathing Motor and Ventilatory Deficits Following C2 Hemisection in Adult Female Rats

Spinal cord injury (SCI) most commonly occurs at the cervical level and often renders affected individuals unable to breathe. There is no current clinical treatment able to restore independent respiratory function once lost, but pre-clinical findings in animal models of caudal SCI indicate that non-respiratory motor function can be improved by acute post-injury depletion of peripheral monocyte-derived macrophages. However, it is unknown whether such immunomodulation can improve ventilatory or respiratory motor function after SCIs at the cervical level. Thus, we hypothesized that peripheral macrophage depletion would improve respiratory motor and ventilatory function following cervical SCI. To address this hypothesis, we utilized the C2 hemisection model of cervical SCI in adult female Sprague-Dawley rats and treated subjects with 2 mL of either intravenous clodronate liposomes for macrophage depletion or saline at 1, 3, and 6 days post SCI. We took ventilatory measurements via whole-body plethysmography before SCI and weekly until 4-weeks post-injury. Prior to sacrifice, we conducted terminal electromyograph recordings of the diaphragm to measure breathing motor function. Contrary to hypothesis, initial results (n = 4 per treatment) demonstrated that breathing motor recovery was evident in only 25% (3/4) of clodronate-treated subjects but was present in 75% (3/4) of control rats. Furthermore, macrophage depletion diminished minute ventilation during hypoxic hypercapnia when compared to control, though other ventilatory measures demonstrated limited statistical difference. Next steps will examine differences in locomotor function to determine whether clodronate-induced motor deficits after C2 hemisection are specific to the injury level, or specific only to breathing function.

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The Effect of APOE Genotype on Formation of Synaptic Vesicles in Respiratory Motor Control Regions Following a C2 Hemisection

Spinal cord injury (SCI) affects nearly 300,000 people in the United States, with 17,000 new cases annually. One critical aspect of SCI recovery is the role of genetic factors, particularly the apolipoprotein E (APOE) gene, known for its association with Alzheimer's disease risk (E4 allele) and encoding for the lipid membrane transport protein Apolipoprotein E. Recent findings suggest that APOE genotype has a notable impact on respiratory plasticity after cervical SCI. To further understand the mechanisms behind this effect, this study specifically focused on formation of synaptic vesicles in respiratory motor control regions following a C2 hemisection, comparing the differences in functional recovery between the APOE3 and APOE genotypes. A humanized APOE knock-in mouse model homozygous for either the APOE3 or E4 alleles was used in this study. Immunohistochemical analysis and fluorescence imaging of a synaptic vesicle marker, SV2, was quantified to assess differences in synaptic vesicle formation among the genotypes. Results indicate a genotype-specific influence on plasticity in key respiratory motor control regions in comparison to its counterpart. This demonstrates that genetic predisposition affects therapeutic success following cervical SCI. Further investigation into the genotype-influenced molecular pathways using additional markers can elucidate how these genetic variations affect other post-SCI recovery processes. Ultimately, our findings show that the diversity of genotypes in the clinical SCI population requires a tailored approach to treatment, emphasizing the notion that there is no "one-size-fits-all" strategy in the treatment of spinal cord injuries.

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Understanding HDL as a Risk Factor for Cardiovascular Disease After Spinal Cord Injury

Spinal cord injury (SCI) has been linked to increased risk of cardiovascular disease (CVD). Neurotrauma induces HDL dysfunction by reducing both its quantity and quality. The shift in lipoproteins after SCI is linked to increased risk of SCI. HDL profiles are distinct in the central nervous system (CNS) and the peripheral blood. The blood brain barrier isolates the cerebral spinal fluid (CSF) from the blood. Therefore, we hypothesize that spinal cord injury leads to damage in the blood brain barrier causing mixing of the blood and CSF HDL. In order to test that hypothesis, CSF was extracted from the cisterna magna and venous blood was extracted from the right atrium of six rats. Three of the rats were given a SCI whereas the other rats were healthy controls. Then, the extracted samples were run in size exclusion fast protein liquid chromatography (FPLC), which measured protein levels using UV absorption. The profiles between the healthy and injured rats were compared. The preliminary data suggests a shift towards larger lipoproteins in the plasma following SCI, which may indicate potential leakage of the CSF into the plasma as CSF lipoproteins are typically larger. As this is a pilot study, further testing is required to confirm whether CSF proteins leak into the plasma. Furthermore, understanding the mechanisms behind the decrease in HDL may help develop remedies to mitigate the development of CVD and identify biomarkers that indicate which patients are at greatest risk of developing CVD after SCI.

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Investigating Evidence of Spontaneous Intravascular Coagulation After Experimentally Induced Spinal Cord Injury in Rats

Spinal cord injuries (SCIs) are often life-threatening and can impact all areas of life. While experimental studies have produced promising treatments, many fail in clinical trials. Differences between clinical standards and experimental veterinary protocols for SCI might contribute to this phenomenon. Specifically, prophylactic anti-coagulation medication is standard for SCI patients, due to risk of deep vein thrombosis and pulmonary embolism, but no such therapy is employed for experimental SCI studies since it is unknown whether post-SCI animals experience spontaneous intravascular coagulation. Thus, this study's purpose is to investigate whether experimentally induced cervical SCI leads to coagulation in rats. Because experimental animals regain mobility sooner than humans following SCI, we hypothesize that rats will demonstrate little evidence of post-surgical thrombosis. To test this, we used an ELISA kit to analyze a marker of fibrinolysis, D-Dimer, in rat serum samples before and up to two weeks after a C2 hemisection model of SCI. We would expect to observe an initial increase in D-dimer due to the normal post-surgical healing process, but once initial evidence of surgical clotting resolves, any increase in D-dimer would likely indicate pathological coagulation. However, preliminary results demonstrate an unexpected, marked decrease in serum D-dimer at all post-injury timepoints. In summary, these data may indicate a type of post-surgical coagulative exhaustion previously unidentified in the C2 hemisection model of SCI. By better understanding the experimental rat's circulatory response to SCI, we can better enhance preclinical studies' translational value and better contribute to improving care for people with SCI.

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Neuropathic Pain in a Chronic CNS Injury Model is Mediated by CST-targeted Spinal Interneurons

Chronic neuropathic pain is a persistent and debilitating outcomes of spinal cord injury (SCI), affecting up to 80% of individuals living with SCI. Post-injury pain, especially below-level pain, is refractory to clinical treatments due to a limited understanding of the brain-spinal cord circuits that underlie pain signal processing. Increasing evidence suggests that the descending corticospinal tract (CST) plays critical roles in sensory modulation during skilled movements and tactile sensation; however, a direct role for the CST in the development of SCI-associated neuropathic pain is unclear. Here we have found that complete, selective CST transection at the medullary pyramids (bilateral pyramidotomy) leads to hindlimb allodynia in chronically injured adult mice. Furthermore, c-fos immunostaining revealed neuronal hyperexcitability within lumbar deep dorsal horn elicited by innocuous hindlimb stimulation. Transsynaptic, anterograde viral transduction allowed us to identify CST-targeted spinal interneurons (CST-SINs) throughout different spinal laminae. Chemogenetic regulation of these interneurons demonstrates that dysregulation of activity in this circuit underlies the development of tactile allodynia in chronic injury. To further elucidate the underlying circuit mechanisms of chronic neuropathic pain in SCI, we are using in vivo multi-photon microscopy to visualize activity and structural changes of CST-SINs in longitudinal studies of chronic injury. These findings shed light on an unrecognized circuit mechanism implicated in SCI-induced neuropathic pain and provide a novel target for therapeutic intervention.

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The Pudendal Plexus in the Female Yucatan Minipig

The intricate neural network governing sensory, motor, and autonomic functions within the urogenital and colorectal systems originates from nerve roots in the lower thoracic, lumbar, and sacral regions. Damage to these neural structures, whether centrally or peripherally, profoundly impacts an individual's quality of life. In recent decades, researchers have developed a series of procedures based on electrical neuromodulation, with the pudendal nerve being a prominent target of study. Animal models play a pivotal role in our understanding of the anatomical pathways associated with both normal function and dysfunction of pelvic organs. Furthermore, these models contribute to the advancement of innovative and more effective therapies.
The present study aims to provide an anatomical description of the pudendal nerve in female Yucatan Minipigs. The pudendal nerve consists of an upper cord formed by anastomotic branches S1 and S2, and a lower cord formed by S2 and S3 contributions. Cords converge to create a small plexus termed the pudendal plexus before re-entering the pelvis. Upon entering the pelvis, the pudendal plexus gives rise to the rectal nerve, a posterior perineal nerve, and a branch innervating the external anal sphincter and the puborectal muscle. Additionally, it includes the dorsal nerve of the clitoris, a nerve to the urethra-vaginal compressor, and a nerve to the external urethral sphincter and the urethra. The dorsal nerve of the clitoris further divides into three branches: one innervating the bulbospongiosus and ischiocavernosus muscles, another supplying the skin of the pubic area, and the main branch responsible for clitoral innervation.

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Locomotor Training Enhances Ejaculatory Function Triggered by Epidural Stimulation in Spinal Cord Injured Rats

Approximately 95% of men with spinal cord injuries (SCI) will exhibit sexual dysfunction with symptoms in males related to erection, ejaculation, and poor sperm quality. The current interventions focus on sperm harvest for in vitro fertilization; however, these interventions pose a risk of triggering autonomic dysreflexia and do not help individuals with physical intimacy or sexual satisfaction. Spinal cord epidural stimulation (scES) and activity-based recovery training (ABRT) are two interventions that have been found to improve bladder and sexual function in clinical and pre-clinical cases of SCI. In the current study, a clinically relevant rodent model of incomplete SCI was used to determine the efficacy of scES for ejaculatory function in animals that were non-trained (CX-NT) or received ABRT two times per week (CX-2DPWT) or five times per week (CX-5DPWT). A greater number of animals from CX-2DPWT and CX-5DPWT were responsive to scES for sexual function when compared to CX-NT, with CX-2DPWT displaying the most responses to scES. Electromyography revealed that CX-2DPWT and CX-5DPWT had significantly longer burst duration in the bulbospongiosus muscle ($P < 0.01$) and CX-NT and CX-2DPWT had significantly greater excitation in the external urethral sphincter ($P < 0.05$) during the urethrogenital reflex when compared to spinally intact rats. CX-NT and CX-5DPWT but not CX-2DPWT displayed a decreased trend in sperm counts relative to sham. ABRT twice a week has promise to improve sensitivity to scES for ejaculatory function in individuals with SCI, supporting the use of ABRT in conjunction with neuromodulation.

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Optimizing Mouse Urodynamic Techniques for Improved Accuracy

Accurate measurement of urinary parameters in awake mice is crucial for understanding lower urinary tract (LUT) dysfunction, particularly in conditions like neurogenic bladder post-traumatic spinal cord injury. However, conducting cystometry recordings in mouse presents notable challenges. When mouse is in a prone position during recording sessions, urine tends to be absorbed by the fur and skin, causing an underestimation of voided volume (VV). This study aimed to enhance cystometry and external urethral sphincter electromyography (EUS-EMG) precision in awake mice.

To achieve this, a novel method utilizing cyanoacrylate adhesive was developed to create a waterproof skin barrier around the urethral meatus and abdomen, thereby preventing urine absorption and ensuring accurate measurements. Twenty C57BL/6 mice (10 males and 10 females, 8 weeks old, 18g-20g body weight) were randomly allocated into four groups: males with/without glue and females with/without glue.

The results demonstrated consistent sums of VV, and residual volume (RV) aligned with the infused saline volume in both males and females with the glue. Moreover, there were no instances of urine soaked by fur post-experiment, indicating the successful prevention of urine absorption. Conversely, the non-glue groups exhibited inconsistent measurements of VV and RV, coupled with areas of fur soaked with urine. Additionally, the method also stabilized EUS electrodes, ensuring stable EMG signals and minimizing artifacts often resulting from mouse movement and experimenter manipulation.

The study provides crucial methodological insights into improving urodynamic techniques in preclinical research, which holds significant implications for advancing our understanding of LUT dysfunction and developing therapeutic interventions.

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Lower Urinary Tract Response to Spinal Cord Epidural Stimulation with Peripheral Neurectomy in Spinally Injured Rats

Spinal cord injury (SCI) often leads to severe impairment of multiple body systems, greatly impacting quality of life. While the urinary bladder is initially areflexic during spinal shock following SCI, reflexive voiding usually develops within 2-12 weeks in humans and 1-2 weeks in the rat. However, voiding post-SCI is often disordered and may display detrusor-sphincter dyssynergia (DSD), which is characterized by uncoordinated bladder and external urethral sphincter (EUS) contractions, causing inefficient emptying and smooth muscle hypertrophy. Likewise, the frequency of bladder contractions may increase and contribute to dangerously high intravesical pressures and storage dysfunction in a condition known as neurogenic detrusor overactivity (NDO). Spinal cord epidural stimulation (scES) is a novel therapy that has been shown to improve lower urinary tract (LUT) function in both humans and pre-clinical experimental models post-SCI. It is hypothesized that the improvements in LUT function seen with scES result from modulation of the neural networks which project to the bladder or EUS that are located within these sites of stimulation. To gain insight into the neural mechanisms behind scES-induced effects on the LUT, the Hubscher laboratory has developed a model combining thoracolumbar or lumbosacral scES with a neurectomy of either the pelvic, hypogastric, or pudendal nerves in female rats with moderate-severe SCI (215 kdyn) during urethane-anesthetized cystometry-electromyography at 28 days post-injury. Early data indicate a reduction in scES-induced LUT improvements following peripheral neurectomy, implicating their role as a functional target of neuromodulation.

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Skilled-locomotor Gait in the Yucatan Mini Pig

Background: The Yucatan Mini Pig (YMP) is a large animal model used across biomedical research. Despite its growing use in SCI research, few studies have investigated adaptive trunk and limb movements during skilled locomotion. Using knowledge from a spectrum of species, including felines, we are developing assays targeting these gait features.

Objective: Investigate performance during locomotor tasks challenging trunk posture and requiring changes in limb trajectories, hoof placements, and body weight acceptance.

Approach: Three YMPs were conditioned to perform simple overground walking and three skilled locomotor tasks: Obstacle negotiation, step up/ down, and agility ladder navigation. Spatiotemporal gait features were captured and analyzed using Vicon Nexus and custom scripts.

Results: We have developed skilled locomotor tasks adapted to the Mini Pig. Average comfortable walking speed and cadence are significantly lower on the agility ladder compared to other tasks. During obstacle clearance, vertical hoof displacement was variable with an average of 222.30mm during negotiation of a 150mm obstacle.

Conclusion: This approach begins to develop methodology for functional assessment of supraspinal inputs during gait modifications and establish baselines to understand skilled features in swine.

Acknowledgements: Hammond Endowment, KSCHIRT, KSCIRC Pig Core

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What is Really Going on During Passive (Motorized) Cycling?

The concept of passive (motorized) cycling as an "exercise" has been hotly debated. In addition, there is an apparent discrepancy between pre-clinical studies showing that passive cycling has a very positive impact on cardiovascular function (PMIDs: 24535438, 26159931) and clinical studies where the outcomes are modest or lacking (PMIDs: 8891496, 8052111, 27841091). To address this controversy, we purchased motorized bikes from Drexel University and instrumented one pedal with a tri-axial force sensor and constructed a set of short pedal cranks to supplement the standard-length cranks. Employing adult female SD rats we examined pedal forces, hindlimb kinematics, and either EMG from knee muscles or heart rate (HR)/blood pressure via two different telemetry probes. We assessed animals with T2 and T10 injuries, both full transections and severe contusions, using cycle cadences (speeds) ranging from 5rpm to 60rpm beginning 7 days post-injury. We observed both "spastic" (S) and "non-spastic" (NS) EMG responses (identified based on primary frequency components) and pedal forces with similar frequency content. The S forces could exceed 1x body weight and were accompanied by brief increases in HR and mean arterial pressure (MAP) with subsequent brief drops in MAP. The NS forces seldom exceeded 30% of body weight and were accompanied by a cadence-dependent increase in MAP along with moderate declines in HR. The NS forces/EMG responses indicate muscle activation during the lengthening phase, i.e. stretch-reflex-induced eccentric contractions. Many of these responses were greater for the standard vs the short crank showing that range-of-motion contributed to the cycle cadence-dependent observations. Overall, our results suggest that passive or motorized cycling can induce a mild exercise response highly dependent on a combination of limb range-of-motion (crank length vs. limb length) and cycle cadence.

Funding: Craig H. Neilsen Foundation.

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APOE and Sex Impacts Functional Recovery After Cervical Spinal Cord Injury

Genetic factors are key to understanding recovery following spinal cord injury (SCI). Prior work conducted in our laboratory demonstrated that APOE genotype impacts respiratory plasticity after cervical SCI. Specifically, female mice expressing the APOE4 allele had impairments in their ability to generate and maintain robust diaphragmatic activity following cervical SCI and intermittent hypoxia treatment relative to APOE3 counterparts. Subsequently we showed that different APOE alleles leads to altered neural respiratory control even without a spinal cord injury and that the effect is sex dependent. To extend these findings, we are now investigating the impact of APOE genotype and sex on locomotor and respiratory motor recovery in a humanized APOE knock-in mouse model homozygous for either the APOE3 and E4 alleles. Further, we examined how genotype and sex effected plasticity and serotonin levels below the lesion. We hypothesized that animals with the APOE4 allele will demonstrate impaired recovery following SCI across all measures. We performed a left C2 hemisection injury on male and female mice of both APOE3 and APOE4 genotype (n = 8/group). We recorded whole body plethysmography and CatWalk gait analysis weekly beginning at pre-injury through three weeks post injury. Consistent with our previous findings male APOE3 mice responded better than APOE4 counterparts during respiratory challenge even following SCI. Specifically APOE3 male mice showed greater improvements in respiratory rate during both normoxia and hypoxia challenge at early timepoints following injury. Interestingly during catwalk gait analysis APOE4 male mice recovered function faster than APOE3 counterparts in the weeks following SCI. This work is critical because it lays the foundation for understanding how diverse genotypes in the clinical SCI population may indicate the need to personalize treatment for individuals following injury.

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Inducible Promoter System Delivered by Retrogradely Transported AAVs: A New Technique for Improving Motor Recovery in Spinal Cord Injury

Spinal cord injury (SCI) poses multiple barriers to regeneration, including neuronal-intrinsic and extrinsic factors. A well-studied mechanism to promote regeneration in SCI is activating the PI3K/mTOR pathway by knocking out phosphatase and tensin homolog protein (PTEN). Early studies investigating PTEN knockout (PTEN-KO) have used adeno-associated viruses (AAVs) as vectors to induce deletion in rodents; however, these models provide significant hurdles to translation for multiple reasons. Firstly, the use of traditional AAV serotypes to induce PTEN-KO requires viral injection at the location of cell bodies to induce elongation of damaged axons within the spinal cord; this presents a challenge because of the added risk for viral spillage into other brain regions and potential for mechanical damage from the needle. We utilize a novel AAV serotype that utilizes retrograde transport mechanisms to counteract this limitation. A single spinal injection of retrogradely transported AAVs (AAVrg) targets cell bodies throughout the brain and affects nearly all axons involved in spinal functions with little-to-no off-target effects. A significant concern of gene therapy approaches is the permanent manipulation of growth programs, which may introduce deleterious effects. Temporal modulation remains a need for safe and effective gene therapy approaches. We utilize a TETon inducible promotor system to express a constitutively active AKT as a strategic approach to temporally modulate mTOR activation. We show that using an inducible promotor system to temporally modulate mTOR activation is able to improve motor recovery through behavioral testing. Our goal is to introduce a new method with better translatability for treating SCI.

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Systemic Infection After Contusion Spinal Cord Injury Further Worsens Functional Recovery

Systemic infection (sepsis) after spinal cord injury (SCI) is a predominant secondary complication that induces a severe hyperinflammatory response leading to multiple-organ damage, consecutively, resulting in poor functional recovery, or even death. To date, no experimental model is available to study post-SCI sepsis complications. We aimed to develop a novel, clinically relevant rodent model that mimics the long-term consequences of sepsis post-SCI. Adult female Sprague Dawley rats were divided in 4 groups: Sham, SCI, Sepsis and SCI + Sepsis. Rats in SCI and SCI + Sepsis groups received T10 spinal cord contusion (200 kDyn) using Infinite Horizon Impactor. To induce sepsis designated Rats were injected with 3ml cecal slurry via intraperitoneal route. All the rats received antibiotics and fluid-resuscitation starting at 8h postSCI and/or sepsis induction, which was repeated for 5 days twice daily.

A decrease in survival was observed in SCI + Sepsis (~39.3%) and Sepsis (~55%) compared to SCI and Sham (100%). SCI followed by sepsis resulted in significantly impaired hindlimb locomotor recovery. Rats in SCI+Sepsis group were able to stand or walk without support (BBB~9) whereas rats in SCI group walked with occasional coordination (BBB~12) at 12-weeks post-SCI. In vivo muscle-strength test also showed significant muscle weakness in SCI + Sepsis versus SCI. The ongoing studies access histological changes and blood cytokines to corelate with BBB and skeletal muscle-strength.

In summary, this study serves as an early milestone in understanding underlying pathophysiology of sepsis after injury and to develop therapeutic strategies for sepsis survivors after SCI.

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Reduced Intraspinal Recruitment of Hematogenous Macrophage Promotes Recovery After Spinal Cord Injury: Independent Replication 25 Years Later.

Spinal cord injury (SCI) triggers an intraspinal inflammatory response that contributes to secondary injury and neurodegeneration. Here, we attempted to replicate and independently verify the therapeutic potential of hematogenous macrophage (MØ) depletion for SCI. Specifically, we selectively depleted peripheral MØ using clodronate liposomes in a rat model of SCI. 10-12-week-old female Wistar rats received T9 contusion SCI (175 Kdyn) to model clinical SCI. Rats received intravenous injections of vehicle or liposome-encapsulated clodronate (2mL of 7mg/mL anionic) at 1, 3- and 6-days post-injury (dpi). We used standardized behavioral (Basso, Beattie, and Bresnahan locomotor test, horizontal ladder walk test, Catwalk XT) and neuropathological analyses for up to 8 weeks post SCI in 4 independent cohorts. Clodronate treatment significantly reduced intraspinal macrophage infiltration at 7dpi. Clodronate treatment significantly improved locomotor function in treated animals. Concordantly, we observed significant increases in tissue sparring through the rostro caudal axis in the spinal cords of clodronate-treated animals. Interestingly, clodronate treatment improves the temporal and interlimb coordination parameters obtained during CatWalk over flat surface. Our observations implicate the crucial role of hematogenous MØ in secondary injury progression post-SCI. Furthermore, our results are consistence with previous observations made by an independent laboratory several decades before. Thus, our independent replication validates macrophage depletion as an adjunct therapy post-SCI.

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Investigating the Effects of SOCE Inhibition on Functional and Histological Recovery in a Murine Contusive Spinal Cord Injury Model

Store-operated calcium entry (SOCE) plays a crucial role in cellular processes, including cellular calcium homeostasis and signaling, with dysregulation implicated in neurological disorders and CNS trauma. To further our knowledge of the key SOCE effectors stromal interaction molecules (STIM) and Orai channels on functional and histological outcomes post-SCI, we assessed three pharmacological agents known to interfere with this signaling pathway: DPB162-AE (STIM inhibitor), YM-58483 (Orai channel inhibitor), and 2-APB (IP3R inhibitor). Following acclimation and baseline testing, 6- to 8-week-old C57Bl/6 mice were randomized into seven treatment groups and underwent a 50 kilodyne contusion at T9/10. Treatments were administered intrathecally (volume 10µL) 1 hour post-SCI. Behavioral assessments, including open-field (BMS and BMS subscore) and horizontal ladder, were conducted by blind assessors. DPB162-AE (3µm) was given BID/QD for 5-7 days, respectively, 2-APB (100µm), and a combination of DPB162-AE and 2-APB (3µm + 100µm respectively) QD for 7 days, and YM-58483 (500nM) BID for 5 days. Each treatment paradigm was compared to respective vehicle controls. DPB162-AE BID treated mice exhibited significantly (Binomial Proportion, $p < .001$) improved locomotor recovery at week 4 after SCI compared to controls, as evidenced by improved Basso Mouse Scale (BMS) subscores ($n = 10$-11/group). Mice receiving combined 2-APB/DPB162-AE exhibited enhanced functional outcomes in comparison to controls, attaining significantly (Repeated measures ANOVA, $p < .05$) higher BMS scores ($n = 10$-11/group) at weeks 4 and 6. These findings suggest usage of DPB162-AE as a therapeutic treatment for improving functional recovery following SCI, and introduces a novel target for SOCE inhibition following SCI.

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NKCC1 Inhibition Protects the Axo-myelinic Interface and Improves Neurological Outcome After Contusive SCI

Ultra-structural studies of compressive and contusive SCI have shown that the most prominent acute changes in white matter are periaxonal swelling, axonal swelling, and axonal spheroid formation. However, the mechanisms that cause periaxonal swelling and the functional consequences are poorly understood. Utilizing in vivo imaging of Thy1YFP + axons and myelin labeled with Nile red, we have shown that periaxonal swelling significantly (ANOVA on Ranks, $p < 0.001$; post hoc Dunn's method, $p < 0.05$; n = 2-11/ timepoint) increases acutely following contusive SCI (T13, 30 kdyn, Infinite Horizons Impactor) and precedes axonal spheroid formation. Additionally, we have determined that ~73% of myelinated fibers present with periaxonal swelling at one hour post SCI and ~51% of those fibers transition to axonal spheroids by four hours post SCI (Binomial proportion test, $p < 0.005$, n = 5). We hypothesized that inhibiting NKCC1 would prevent periaxonal swelling after SCI. We found that inhibition of NKCC1 using bumetanide (30mg/kg, 1h and 4h post-SCI) significantly (Mann Whitney U test, $p < 0.05$) reduced acute periaxonal swelling and increased (One-way ANOVA, $p < 0.05$; Tukey HSD post hoc t-test, $p < 0.05$) axonal survival at 24 hours after T9, 50 kdyn contusive SCI versus vehicle controls (n = 6-7/group). Bumetanide significantly improved finer aspects of locomotor recovery (Binomial proportion test, $p < 0.001$) and increased white matter sparing (One-way ANOVA, $p < 0.05$; Bonferroni post-hoc t-test, $p < 0.05$) after SCI. These data reveal a novel role for NKCC1 in protecting the axo-myelinic interface after SCI.

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Deletion of the ISR Kinase HRI Promotes OL Survival, Attenuates Neuroinflammation and Improves Recovery After Thoracic SCI

Cellular stressors inhibit general protein synthesis while upregulating stress response transcripts and/or proteins. Phosphorylation of the translation factor eIF2α by one of the several stress-activated kinases is a critical trigger for such signaling, known as the integrated stress response (ISR). The ISR is activated after acute CNS injuries and its outcomes may include cell survival, reduced plasticity, inflammation and cell death. Therefore, we investigated the role of three major ISR kinases including HRI/EIF2AK1, GCN2/EIF2AK4 and PKR//EIF2AK2 in a mouse model of moderate contusive spinal cord injury (SCI) at the thoracic T9 level. One-day after SCI, mRNAs for all those kinases were observed in both oligodendrocytes (OL) and microglia/macrophages (MG/MDM) in the spared white matter at the injury epicenter. However, reduced levels of peIF2α were found in Hri-/- and Gcn2-/-, but not in Pkr-/- mice. In addition, Hri-/- mice showed attenuated expression of the downstream ISR transcripts, Atf4 or Chop. Such differential effects on SCI-associated ISR correlated with a strong or moderate enhancement of locomotor recovery in Hri-/- or Gcn2-/- mice, respectively. Hri-/- mice also showed reduced white matter loss, increased OL content and attenuated neuroinflammation including decreased lipid accumulation in MG/MDMs. Cultured Hri-/- OL showed lower ISR cytotoxicity. Unexpectedly, cell-autonomous reduction of neuroinflammatory potential was observed in isolated MG/MDMs from adult Hri-/- mice. These data identify HRI as a major positive regulator of SCI-associated secondary injury. In addition, targeting HRI may enable multimodal neuroprotection to enhance functional recovery after SCI.

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Astrocytes Promote Acute Survival of Microglia and Motor Recovery After SCI

Spinal cord injury (SCI) results in permanent disability and affects more than 250,000 individuals in the United States. Astrocytes, together with microglia and monocyte-derived macrophages, mediate wound healing after SCI. However, the mechanisms of communication between these cells are largely unexplored. Our study found that astrocytes produce colony-stimulating factor 1 (CSF1), a trophic factor for microglia and macrophage survival and proliferation. Seven days after complete crush spinal cord injury at thoracic T8 level, mice with astrocytic CSF1 deletion (Astro-CSF1-KO) showed a reduction in astrocyte and microglia at the lesion core. Astro-CSF1-KO mice show worsening of hindlimb motor function recovery, indicating beneficial role of astrocytes and microglia in the injury site. RNA sequencing shows that deletion of astrocytic CSF1abrogated injury-dependent changes of gene expression. Our study further identifies three gene clusters significantly elevated after SCI but significantly reduced in Astro-CSF1-KO: response to interferon-beta (IFNb), response to cytokine, and wound healing. The IFNb production is colocalized with immune cells near the injury core following SCI. The administration of IFNb rescues astrocytes but not microglia in the lesion core 7 days after SCI, indicating that reduced astrocyte numbers in Astro-CSF1-KO mice are mediated by reduced IFNb signaling. These results suggest that astrocyte-derived CSF1 maintains microglia number, and IFNb production downstream of CSF1 signaling maintains astrocyte number in the lesion core.

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Expression of Cellular Senescence Markers in Glia and Macrophages from the Contused Mouse Spinal Cord

Cellular senescence is a response in proliferating cells to DNA damage that is beyond repair. Permanent inhibition of proliferation and activation of the senescence-associated secretome follow. The latter response may promote tissue repair, but also chronic inflammation. While potential role of cellular senescence in SCI is unclear, this study aimed at testing for the presence of senescent cells after a moderate contusive SCI (50kdyn, IH, T9) in C57Bl6 mice. Transcript levels for two widely used cell senescence markers (p16/Cdkn2a and p21/Cdkn1a) were increased both subacutely (3-7 days) and subchronically (6 weeks) after SCI. Moreover, SCI-associated upregulation of the p16 promoter-driven 3MR reporter gene was found in p16-3MR transgenic mice. RNAScope for p16 and p21 as well as immunofluorescence staining for p21 showed both subacute and subchronic (6 week) increases of the respective senescence marker signals in ASPA-positive oligodendrocytes, GFAP-positive astrocytes and CD68- or IBA1-positive macrophages/microglia. Senescent astrocytes were found in the glial scar region bordering the injury epicenter. Senescent microglia/macrophages were also observed in the glial scar as well as in the fibrotic scar at the injury epicenter. In those regions, senescence marker-expressing astrocytes and microglia/macrophages were relatively abundant with a positivity rate ranging from 20-50%. These observations suggest a prolonged cellular senescence response to SCI that likely follows SCI-induced proliferation of oligodendrocyte precursor cells, astrocytes and microglia/macrophages. Whether such a response is beneficial or detrimental for post-SCI recovery will be explored in future studies.

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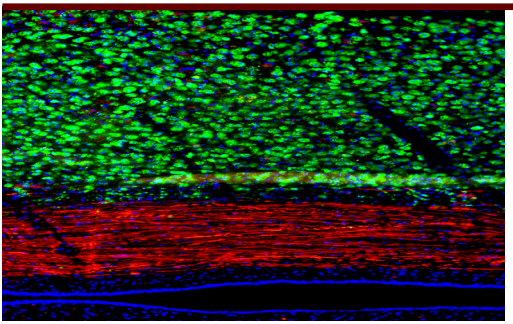
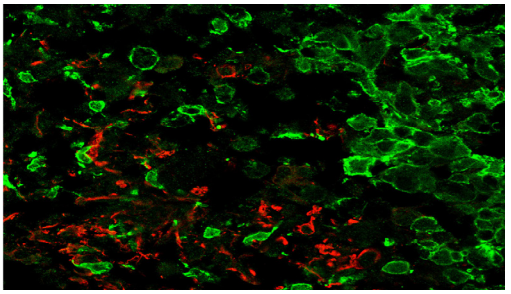
Macrophage Depletion Using Clodronate Treatment Following Severe Contusion Spinal Cord Injury

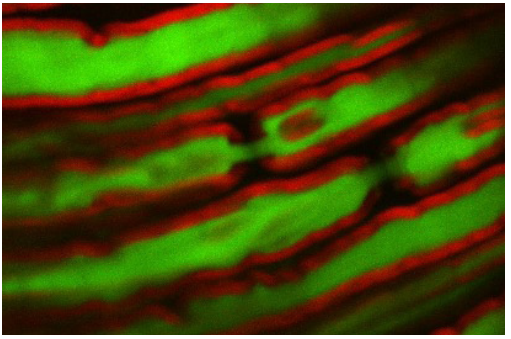
Spinal cord injury (SCI) leads to intraspinal inflammatory response including infiltrating blood leucocytes and resident glia. Some of these subsets of immune cells (monocytes) contribute to ongoing tissue degeneration after SCI. Currently, there are no FDA-approved therapies for SCI. One promising therapy, liposome-encapsulated clodronate aka clodronate liposomes, depletes monocyte-derived intraspinal macrophages and several independent laboratories have reported therapeutic effects. To date, few studies have examined the extent to which clodronate liposomes are effective across different spinal levels and severities of SCI. In this study, we investigated the effect of clodronate liposomes on macrophage depletion following severe high thoracic contusion SCI. We hypothesize that intravenous clodronate liposome, delivered after T3 contusion SCI, will reduce intraspinal macrophage activation. Adult female Wistar rats were subjected to T3 spinal contusion with two different forces (300kdyn (5s dwell time) and 400kdyn (5s dwell time). For each severity, injured rats were randomly divided into two groups, one group received 2ml Clodronate (7mg/ml) on days 1, 3, and 6 post-injury (once-a-day) through tail vein injections, and the control group received vehicle (2ml saline). At 7 days post-injury, blood was collected from heart prior to transcranial perfusion for IDEXX. Spinal cords were isolated and analysis for depletion is ongoing. This pilot run will help us understand the potency of clodronate to cause depletion in our T3 severe contusion SCI model.



Confocal image of a parasagittal section showing the microglia/ macrophage (green) response rostral to spinal cord injury. Phagocytic microglia/ macrophages are restricted to degenerating ascending fibers whereas descending CST fibers (red) remain intact.



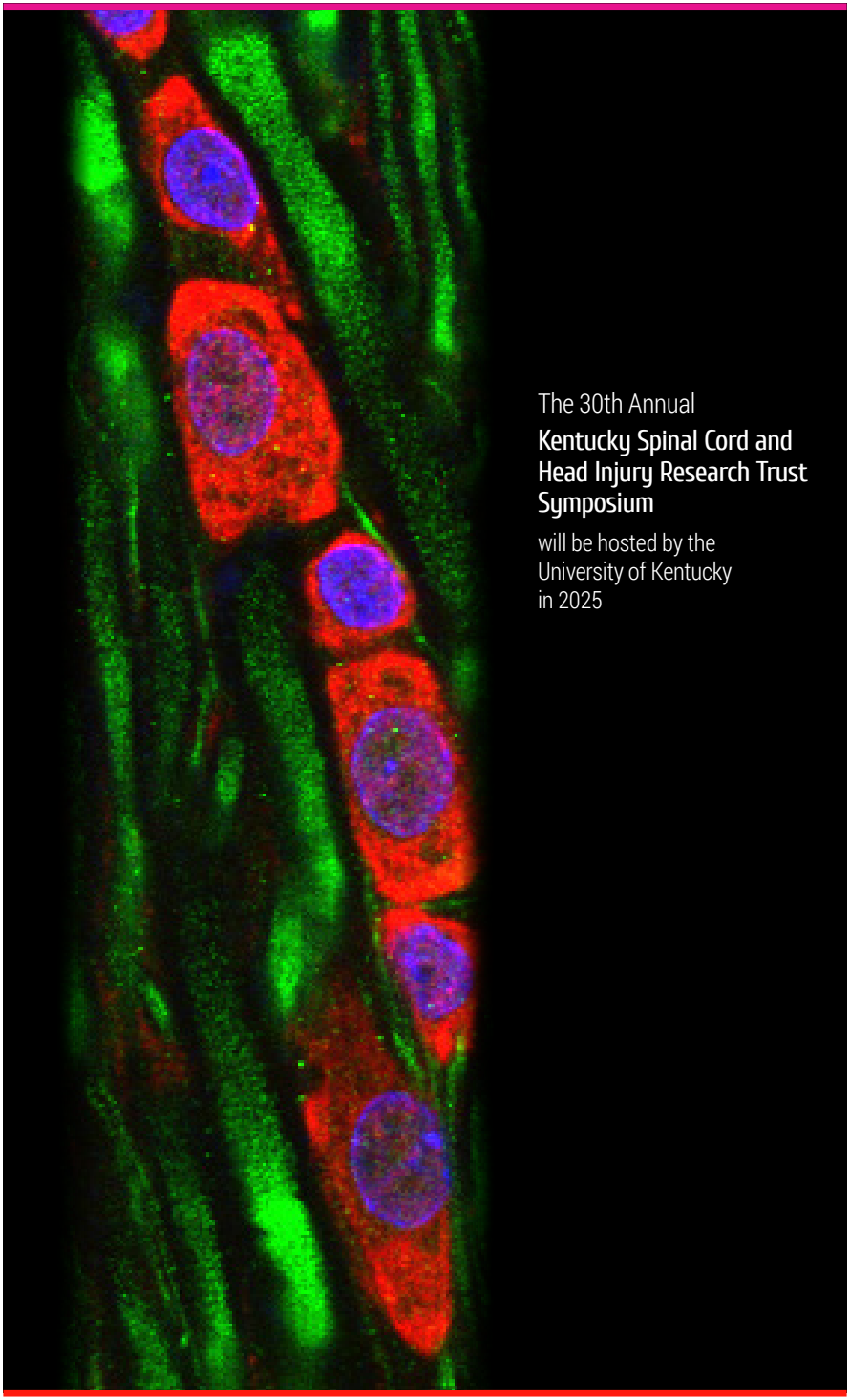
Confocal image of spinal cord microglia/macrophages (green) interacting with astrocytes (red) at the lesion border at 5 days after SCI.



2-photon image of in vivo sciatic nerve myelinated (red) axons (green).

All Photo Credits: D. Stirling

Back Cover Photo:
Confocal image of spinal cord oligodendrocytes (red) and their axons (green).



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