XIX

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Abstracts

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Need for speed: the impact of spinal cord injury on overground high-speed gait expression in the adult rat

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Abstract

Spontaneous recovery of locomotion in rodents after incomplete spinal cord injury is poorly understood, and few previous studies have assessed locomotor gaits beyond walk and trot. Here, we aim to improve our understanding of the effect of incomplete spinal cord injury on the neural control of locomotion by quantifying biomechanical characteristics of overground locomotion in uninjured and spinal cord injured rats. To assess these biomechanical features, six adult female Sprague-Dawley rats were trained using food rewards to rapidly locomote the length of a 10’ slip-resistant tank, allowing for a full examination of speed dependent gaits ranging from walk to bound. A custom 6-camera set up was used to evaluate both inter- and intralimb coordination at baseline and after mild or moderate T10 contusion injuries, aided by DeepLabCut (DLC), a deep neural network program that allows for markerless pose estimation. DLC enabled us to improve our throughput while eliminating physical markers on the animals and proved to be as accurate as manual digitization. Our data reveals that uninjured animals trained to traverse this 10’ long tank expressed bound as their preferred gait, where the hindlimb and forelimb pairs move synchronously, in phase. After injury, the average speed is reduced, speed-dependent gait transitions are altered, and the forelimbs are limited to alternation at all speeds. Our results emphasize the importance of examining the full repertoire of gaits while highlighting the utility of machine learning as an aid for assessing locomotor deficits following spinal cord injury.

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PAK1 inhibition with romidepsin attenuates H-reflex hyperexcitability after spinal cord injury (SCI)

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Abstract

A large majority of SCI patients develop spasticity, a clinical symptom of H-reflex hyperexcitability. Spasticity is a debilitating condition that disrupts rehabilitation and negatively impacts quality of life. We have identified common structural motif of motor neuron dendritic spine dysgenesis to be associated with H-reflex hyperexcitability following SCI. Previously, we have found that conditional Rac1 knockout in motor neurons (i.e., cre-flox system) restores H-reflex rate-dependent depression (RDD) after SCI. Pak1 is a downstream effector of Rac1 and is a promising druggable target for neurological disease. Here, we administered the Pak1 inhibitor romidespin (a clinically available drug) one month following contusive SCI in Thy1-YFP mice. In longitudinal studies, electromyograms (EMGs) studies were performed before and after injury, with or without romidepsin treatment. This study design permitted us to assess pathological changes and the effect of drug intervention within the same animal over time.

Our results show a significant reduction of RDD, i.e., or reduced H-reflex excitability, in comparisons across subjects treated with romidepsin or vehicle (“between” cohort analysis). A comparison within the same animals with SCI, before vs after treatment, demonstrated a significant reduction in hyperreflexia after romidepsin treatment. As expected, SCI animals with hyperreflexia produced abnormal motor neuron dendritic spine morphologies associated with hyperexcitability, including an increase in mushroom dendritic spine density as well morphometric changes in dendritic spine shape. Romidepsin reversed SCI-induced observations of dendritic spine dysgenesis Taken together, our research suggests that Romidepsin has the potential to be a successful treatment for spasticity after spinal cord injury.

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The Asilomar Declaration: What is really going on during passive (motorized) cycling?

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Abstract

During the “Exercise as a therapy for SCI” panel discussion (2017 ISNR meeting at Asilomar), the concept of passive (motorized) cycling as an “exercise” was hotly debated. The Magnuson lab proposed to somehow address this issue. 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. 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 using cycle cadences (speeds) ranging from 5 to 60rpm beginning 7 days post-injury. We observed both “spastic” (S) and “non-spastic” (NS) EMG responses (identified based on primary freq. 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 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 suggesting 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.

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Neuromodulation of sensory cortical-locomotor pathway promotes locomotor recovery after spinal cord injury

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Abstract

Spinal cord injury (SCI) often leaves patients with significant impairment in their locomoting ability. Despite an increasing need to develop ways to restore function below the level of injury, treatment options remain limited. The primary somatosensory cortex (SI) can have direct neural control on the locomotor central pattern generator relayed through cervical excitatory interneurons (SI locomotor pathway) independent of the motor cortex. Here, we use in vivo calcium imaging to decode that SI pyramidal neurons of the locomotor pathway participate in the onset of movement and play a role in the speed programming of movement. Furthermore, detailed mapping of cervical projecting sensory pyramidal neurons (SI-cervical pyramidal) demonstrates that this pathway consists of collateral projections to other subthalamic and brainstem regions involved in locomotor control. Optogenetic activation SI-cervical pyramidal neurons are sufficient to initiate movement. Part of this SI- locomotor circuitry is preserved after thoracic SCI, and selective optogenetic and chemogenetic stimulate the SI pyramidal neurons that project to the cervical spinal cord (SI-cervical pyramidal neurons) after SCI promote locomotor recovery. Long-term chemogenetic activation of the SI-cervical pyramidal neurons after moderate contusion injury restores locomotor function without any adverse effects on movement, such as loss of balance or pain. Importantly, we demonstrate that the recovered locomotor function after SCI is attributed to the neuromodulation of this pathway. Thus, modulating the novel SI locomotor pathway enhances movement in health and represents a promising therapeutic approach for functional locomotor recovery after SCI.

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Antioxidant nanoparticles promote endothelial cell survival after spinal cord injury.

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Abstract

Spinal cord injury (SCI) results in the immediate severing of ascending and descending axons along with the disruption of the vascular network. The primary trauma results in the generation of an ischemic environment along with oxidative damage, cytotoxicity and edema triggering a cascade of secondary wave injury. The study aims to determine the acute treatment efficacy of poly (ethylene glycolation) (PEG) of oxidized activated charcoal (OAC) administered after SCI. Experimentally, adult male Sprague-Dawley (n=80) rats received a moderate to serve spinal contusion (150 kDynes with a 1 second dwell time) at thoracic vertebrae (T10) using an Infinite Horizon Impactor (Precision Systems and Instrumentation, Lexington, Kentucky). Animals were then randomized into groups treated with PEG-OAC, PEG alone, or saline. The treatments were administered intravenously 3 hours after injury then daily for a 48-hour period. At the 72-hour time point, animals were then processed to assess spinal cord barrier permeability using Evan's Blue extravasation, or histology. BSCB permeability assessment determined that PEG-OAC treated animals significantly reduced vascular permeability ($p=0.0033$) compared to vehicle and PEG alone treated animals. There was no significant difference between saline and PEG alone treated groups. Acute lesion volume analysis by ex vivo magnetic resonance imaging indicated a significant reduction in lesion volume in PEG-OAC treated animals compared to saline and PEG alone treated groups. Histological analysis indicated a greater number of endothelial cells in the PEG-OAC and reduced activated microglia. The current data illustrates the efficacy of PEG-OAC antioxidant nanoparticles in reducing BSCB permeability and inflammation.

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A pilot study demonstrating that intrathecal LRP1 antagonism promotes respiratory recovery following cervical contusion injury in adult rats

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Abstract

Respiratory impairments and complications are the leading cause of death following high cervical spinal cord injury (SCI). In rodent models of SCI modest recovery occurs over the first month post injury, but long-tract regeneration is largely absent. One factor that impairs regeneration is RhoA, which is highly expressed following injury. RhoA is important in many cellular processes and broad RhoA inhibition can lead to toxic drug side effects. Here we utilize a novel compound, NOVO-118, to reduce RhoA activation through inhibition of its activator, low-density lipoprotein receptor regulated protein 1 (LRP1). We hypothesize that through inhibition of RhoA via LRP1, we can promote regeneration and functional recovery following cervical contusion SCI. As LRP1 is broadly expressed throughout many organ systems, we delivered NOVO-118 (or vehicle) to the intrathecal space above the lesion over a 30-day period after injury to mitigate off target effects. We measured the effect of NOVO-118 on ventilation and locomotor function using whole body plethysmography and open-field activity chambers. Behavioral assessments were performed pre-injury, 3, 7-, 14-, 21-, and 28-days post injury. Minute ventilation under normoxia was reduced in both groups at days 3 and 7 post injury which recovered towards pre-injury levels by day 14. The vehicle treated animals also had reduced minute ventilation during hypoxic challenge at days 3 and 14 post injury. However, the NOVO-118 treated rats were able to maintain pre-injury minute ventilation under hypoxic challenge at all time points. This suggests LRP1 as a new therapeutic target to promote recovery after SCI.

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An investigation into the perturbed peripheral organ metabolome following acute spinal cord injury and subsequent identification of phospho-mTOR as a treatment target

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Abstract

The majority of spinal cord injury (SCI) research focuses on restoring lost motor or sensory function. Studies have demonstrated peripheral organs are also impaired after injury. It is thought that impairments in the peripheral organs leads to negative feedback to the central nervous system (CNS). In the SCI setting we hypothesis this can lead to system-wide dysfunction. To test this hypothesis, we examined the spleen, liver, and lung following C2Hx 3- and 7-days post injury. Additionally, we collected spinal cord and cerebrum to quantify changes within the CNS. We investigated an array of individual metabolites using gas chromatography mass spectrometry (GCMS) in all collected organs. We performed a supervised clustering analysis to assess overall metabolic profiles within each organ. Metabolites were up and downregulated in an organ and injury specific manner. The GCMS analyses revealed general organ and group difference in glycolytic intermediates and more profoundly, amino acid pools among different organs. Furthermore, based on significant changes in amino acid pools, we probed the tissue collected from the liver and spleen with an ELISA targeting mTOR and phospho-mTOR that are known to regulate amino-acid metabolism. Within the liver, there were higher levels of phospho-mTOR three days post injury. Overall, the liver appeared to have the largest changes in metabolic profile suggesting metabolites could serve as early biomarkers and potential targets for therapeutic intervention. This work has important implications for the SCI community as improving peripheral organ health may be a key to improving recovery and quality of life following SCI.

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Surgical decompression fails to restore adaptive ventilatory ability in hypercapnia

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Abstract

Degenerative cervical myelopathy (DCM) is a debilitating neurological condition characterized by chronic, progressive compression of the cervical spinal cord leading to impaired upper and lower limb function. Despite damage to areas of the cervical spinal cord that house the respiratory network, respiratory dysfunction is not a common manifestation of DCM; thus, changes in respiratory function are not monitored. Nevertheless, with surgical decompression being the primary treatment for DCM, it is critical to assess ventilatory response to respiratory challenge, as anesthesia causes respiratory depression. Although surgical decompression leads to improved sensorimotor function, its impact on ventilation has not been investigated. Here, using a clinically relevant model of DCM, we chronicle subclinical respiratory deficits over time and asses hypercapnic ventilatory ability before and after surgical intervention.

Using a mouse model of DCM, we show that despite significant and progressive forelimb and locomotor deficits, there was no significant decline in eupneic ventilation from the early to late phases of compression. Additionally, for the first time, we demonstrate that despite normal ventilation under resting conditions, DCM impairs acute adaptive ventilatory ability, particularly in response to hypercapnia. Moreover, despite improved motor function following surgical decompression, animals' hypercapnic ventilatory ability failed to improve. These findings emphasize the effect of chronic spinal cord compression on respiratory function and highlight the impact of DCM on the ability to maintain ventilation through strenuous exercise or respiratory illness. Further, lack of improvement in adaptive breathing following decompression surgery indicates a need for subsequent development of strategies to improve patient outcomes.

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A brainstem-spinal circuit critical for adaptive breathing

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Abstract

Cervical excitatory interneurons (eINs) are emerging as a significant component in respiratory motor control and plasticity. Investigations of cervical eINs have demonstrated their involvement in diaphragm function and ventilatory recovery following cervical spinal cord injury (cSCI); however, the mechanism and extent to which they integrate into the supraspinal respiratory network and their functional involvement in breathing during health, is unknown. While chemoreflex ventilation, a form of adaptive breathing, has been shown to be modulated by brainstem raphe-serotonin (5HT) neurons that innervate the cervical spinal cord, yet anatomical connections between these neurons and cervical eINs have never been examined. As such, we set out to determine if there is a direct brainstem-spinal cord circuit between raphe-5HT neurons and cervical eINs that modulates adaptive breathing in health and cSCI.

Using retrograde monosynaptic tracing, we have identified direct connections between raphe-5HT neurons and cervical eINs which are spared following cSCI. We also observed injury induced alterations in 5HT receptor expression on cervical eINs. Furthermore, cellular specific in vivo calcium imaging demonstrated that cervical eINs are activated by hypercapnia, meanwhile chemogenetic silencing of these cells established their necessity for the hypercapnic ventilatory response in health. Interestingly, our results indicate eINs are not necessary for this function in cSCI, implicating injury-induced plasticity to the raphe-5HT-eIN network. Together, these findings demonstrate, for the first time, a supraspinal-eIN network that is essential for acute adaptive breathing in health which is subsequently altered by cSCI to maintain basal respiration

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Neuropathic pain in chronic SCI is mediated by CST-targeted spinal interneurons

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Abstract

Chronic neuropathic pain is one of the most 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. Using intersectional viral transduction, we show that CST-SINs activation within laminae III-V by either chemogenetic or MAP kinase pathway activation induces tactile allodynia similar to chronic pyramidotomy. Allodynia depends on afferent input from the paw as it is temporally attenuated by injection of the local anesthetic bupivacaine into the hindpaw. 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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Antagonizing Endothelin B receptor in satellite glial cells improves nerve regeneration and aging-dependent neuronal regenerative decline

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Abstract

Peripheral sensory neurons regenerate their axon after nerve injury to enable functional recovery. The ability of injured neurons to regenerate their axons declines with age, but the mechanisms are incompletely understood. While excessive production of endothelin 1 (ET-1), a potent vasoconstrictor, is linked to many diseases that are increased with age, the role of ET-1 and its receptor on axon regeneration is unknown. Using the dorsal root ganglia (DRG) model, we found that Satellite glial cells (SGC), which surround sensory neuron soma, express the endothelin B receptor (ETBR), while ET-1 is expressed by vascular endothelial cells (ECs). Blocking ETBR with an antagonist increased axon outgrowth in DRG cultures *in vitro*. Blocking ETBR *in vivo* with the FDA-approved compound Bosentan promoted axon outgrowth in cultured DRG neurons and improved axon regeneration after sciatic nerve injury and dorsal root injury. Mechanistically, antagonizing ETBR enhanced gap junctions, labeled by connexin 43, in SGCs. Bosentan treatment reversed the age-dependent axon regeneration decline and the decrease in SGC gap junctions. These data revealed that ETBR antagonism can enhance axon regeneration and revert age-dependent axon regenerative decline, suggesting a new avenue that could be exploited in future therapies.

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Therapeutic implications of the gut-CNS-axis in promoting recovery after cervical spinal cord injury

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Abstract

Spinal cord injury (SCI) is a devastating condition with limited motor and sensory recovery, as well as profound secondary internal organ pathology and dysfunction. Potentially influencing the recovery and repair process is the gut microbiome which impacts a variety of central nervous system (CNS) functions. Gut dysbiosis, or the imbalance of beneficial and pathogenic bacteria, exacerbates a number and variety of neurological disorders. Here we examine the impact of cervical SCI on the gut microbiome and find that immediately after injury there is a transient gut dysbiosis but persistent pathology. However, treatment with probiotic interventions leads to better gut health, as well as improved respiratory motor function measured through whole body plethysmography. Alongside these improvements was a systemic decrease in the cytokine tumor necrosis factor alpha, as well as an increase in neurite sprouting and regeneration potential of neurons. Collectively, the results of these experiments present evidence that the gut microbiome is a valid and important therapeutic target to improve visceral organ health and motor recovery after SCI.

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IL-1 receptor on astrocytes is crucial for locomotor recovery and glial scar formation after SCI

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Abstract

Spinal cord injury (SCI) afflicts hundreds of thousands of people in the United States. SCI causes a robust systemic inflammatory response which can lead to organ pathology. In the liver, this inflammation and associated lipid accumulation is known as non-alcoholic steatohepatitis (NASH). NASH is characterized by elevated levels of inflammatory cytokines such as TNF and IL-1b within the liver. We hypothesized that knockout of IL-1 receptor (IL-1R1) would improve intraspinal tissue sparing and reduce liver pathology. Results demonstrate that IL-1R1 knockout is hepatoprotective, reducing incidence of both steatosis and inflammation in the liver after mid-thoracic SCI. This positive effect was not replicated in the spinal cord, as IL-1R1 knockout (Il1r1r/r) mice had impaired locomotor recovery compared to wild-type mice. Worse behavior correlated with reduced white matter sparing and decreased GFAP+ astrocyte reactivity in the glial scar. Since astrocytes are known to express IL-1R1, we hypothesized that astrocytic IL-1R1 expression is crucial to regulating glial scar formation and white matter sparing after injury. We crossed Il1r1r/r mice with Aldh1l1-CreERT mice to generate astrocyte IL-1R1 restoration mice, maintaining IL-1R1 knockout in non-astrocyte cells. Notably, in mice with restored astrocyte IL-1R1 expression, locomotor recovery after SCI matched that of wild-type mice. Investigations are underway to determine if these mice maintain the improvements to hepatic pathology seen in the IL-1R1 knockout mice. Taken together, these data indicate that IL-1R1 functions to propagate hepatic inflammation and contributes to NASH development, but is required for glial scar formation, intraspinal tissue sparing, and locomotor recovery after SCI.

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Development and Recovery of Sensorimotor Reflexes in Neonatal Spinal-Transected Rats

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Abstract

Sensorimotor function is often impaired following neural injury. However, plasticity in early development allows for substantial recovery of function. After a complete lower thoracic spinal cord transection, rats still exhibit hindlimb reflexes, but the timing of this recovery remains unclear. The purpose of this study was to examine the trajectory of reflex recovery following a neonatal spinal injury. On postnatal day 1 (P1), rats received either a complete spinal cord transection at T8/T9 or a sham surgery. Sensorimotor reflexes were then tested on P7, P14, or P21. Results from reflex testing showed that spinal-transected subjects showed slower responses compared to sham control subjects in some reflexes (surface righting and hindlimb placing), but not in others (hindlimb crossed-extensor response). Additionally, some animals did not exhibit the hindlimb placing reflex, but did exhibit the crossed-extensor reflex. These differences may be explained by the difference in the type and strength of stimulus used to elicit each reflex (i.e. light touch vs. pinch). Generally, older rats showed faster responses than younger rats, though the results varied by type of reflex. These findings show that the hindlimbs are capable of responding to sensory input, but the type and intensity of this input are important factors. This study indicates that the lumbar spinal cord can respond to environmental stimuli and exhibits neural plasticity independently from the brain.

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Spinal cord injury causes visceral adipose tissue inflammation and liploysis

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Abstract

Spinal cord injury (SCI) is a significant neurological impairment with ~18,000 new cases each year. Individuals with SCI are disproportionately affected by metabolic syndrome (MetS), a collection of co-morbidities including abdominal obesity and insulin resistance. A possible driver of MetS after SCI is the accumulation of visceral white adipose tissue (WAT). Increased WAT causes infiltration of macrophages, which form crown-like structures (CLS) around dying adipocytes. CLS also produce pro-inflammatory adipokines that stimulate adipocyte lipolysis via phosphorylation of hormone sensitive lipase (pHSL). Currently, no experimental studies directly assess WAT pathology after SCI. Thus, we hypothesize that SCI increases adipose tissue inflammation and lipolysis. To test this, SCI rats were compared to rats fed a high fat diet. Rats given diet-induced obesity (DIO) or a control diet were fed for 8w prior to injury and then given a moderate T8 contusion SCI. Animals were sacrificed at 56d post-injury (dpi) by intracardiac perfusion. Epididymal (eWAT) and retroperitoneal (rWAT) adipose tissues were collected. In support of our hypothesis, adipose histology revealed SCI and DIO+SCI animals had significantly increased CLS formation in eWAT and rWAT. In addition, pHSL was significantly elevated in eWAT and rWAT of SCI and DIO+SCI animals compared to lean and DIO naïve controls. Notably, pHSL levels in SCI were comparable to those in the obese rats, revealing that SCI causes adipose pathology to the same extent as diet-induced obesity. Future studies will investigate mechanisms driving post-SCI WAT dysregulation in order to reduce cardiometabolic disease and improve overall health.

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Mapping regenerative stem cells during innate spinal cord repair

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Abstract

Adult zebrafish exhibit full recovery within weeks following a complete spinal cord transection. In the zebrafish spinal cord, *sox2*$^{+}$ progenitor cells are thought to comprise the stem cell populations that underlie regeneration. Here, we examine the heterogeneity, stem cell properties, and contributions of *sox2*$^{+}$ progenitors after spinal cord injury (SCI). Cellularly, we traced the contributions of progenitor-derived cells using Cre-loxP-based genetic lineage tracing. We found that zebrafish spinal progenitors, while quiescent in uninjured tissue, proliferate and give rise to the majority of regenerative neurons and glia after injury. Molecularly, single-cell sequencing and HCR in situ hybridization identified 19 lineage-restricted clusters with biases towards different cell fates including neurons and glia. Several clusters expand following injury with three clusters emerging only at 1- and 2-weeks post-injury. Cross species comparisons between zebrafish and mouse progenitors revealed 2 zebrafish specific clusters that correlate with increased regenerative capacity. We are currently using functional genomics to dissect the regenerative functions of these regenerative clusters. Our studies highlight the potency and diversity of spinal progenitor cells during innate spinal cord repair.

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Harnessing the immune system to drive spinal cord regeneration

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Abstract

Adult zebrafish naturally regenerate a fully severed spinal cord (SC), and thus provide a system for the discovery of factors necessary to promote regeneration. Following SC injury (SCI), the immune system is necessary to provide a permissive environment for tissue repair; however, chronic immune activation is detrimental to neural regeneration. Here, we use the evolutionarily conserved zebrafish immune system to tease apart the immune balance required for SC regeneration. We found the immune response to SCI in adult zebrafish is distinct from that of mammals in cell composition, timing, and clearance. Following leukocyte depletion, we show microglia and macrophages are necessary for SC regeneration, specifically in the acute phase of injury. Combining transcriptional datasets of the regenerating SC with CRISPR/Cas9 neurobehavioral screening, we identified *transcription and immune response modulator* (*tcim*) as a previously unknown microglia/macrophage-enriched gene that regulates the clearance of post-injury inflammation. In *tcim* mutants, the SC does not regenerate, an excess of blood-derived leukocytes amass in the lesion site, and the phagocytic capacity of microglia and macrophages is reduced. Additionally, overexpression of human *TCIM* is sufficient to enhance regeneration after SCI in zebrafish in a microglia/macrophage-dependent manner. Together, our data indicate an intricate choreography of post-injury inflammation comprised of leukocyte identity, activation state, and gene expression that is necessary for spontaneous SC regeneration.

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EphB kinase inhibition reverses already-established neuropathic pain following spinal cord injury.

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Abstract

A major portion of individuals with spinal cord injury (SCI) suffer from debilitating neuropathic pain (NP). Central sensitization, or the hyperexcitability of CNS pain circuitry, is a major substrate for SCI-induced NP and can include alterations to NMDA receptor (NMDAR) signaling in dorsal horn (DH). In a mouse model of cervical contusion-type SCI that produces both evoked and spontaneous NP-related behavior, we found increased EphB2 gene and protein expression in DH, as well as enhanced colocalization of EphB2 and GluN1 (the obligate NMDAR subunit) at vGlut-positive sites in superficial DH neurons, suggesting enhanced EphB2-NMDAR interaction at excitatory synapses that may alter DH neuron excitability. Furthermore, in situ hybridization analysis revealed that upregulated EphB2 expression occurred specifically in the NK1R/tacr1-expressing population of DH projection neurons. Targeted inducible inhibition of intracellular tyrosine kinase activity of EphB1, EphB2 and EphB3 using a chemogenetic approach reversed already-established NP-related behavior in cervical SCI mice. Furthermore, this phenotypic effect of EphB inhibition was sensory modality-specific, affecting mechanical allodynia but not thermal hyperalgesia. In addition, there was no impact of EphB inhibition on sensory behavior in uninjured mice, suggesting a differential role for EphB in pathological pain versus normal pain physiology. Lastly, using an antibody microarray assay, chemogenetic EphB inhibition in SCI mice resulted in significant down-regulation in DH of EphB phosphorylation and signaling and, importantly, of activation of downstream pathways associated with regulating neuronal excitability. Collectively, these findings suggest that enhanced EphB function underlies alterations in excitatory synaptic transmission in DH and consequent NP following SCI.

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Single-cell transcriptomics reveal a pro-regenerative neuron population in adult zebrafish.

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Abstract

Unlike mammals, **zebrafish** have the innate ability to regenerate **spinal cord** (SC) tissues within 6-8 weeks of **injury**. To achieve a comprehensive understanding of the pro-regenerative cell identities that direct SC repair, we performed **single nuclear RNA sequencing** at 0, 1, 3 and 6 weeks post-injury (wpi). Our analysis revealed dynamic changes in cell populations and signaling pathways across time points. By analyzing neurotransmitter gene expression, both *in silico* and *in vivo*, we characterized the excitatory and inhibitory landscape during successful SC regeneration. Analysis of neuronal subclusters identified one injury-induced population of neurons that is exclusively present at 1 wpi and that we refer to as **iNeurons**. HCR *in situ* hybridization confirmed the expression of iNeurons genes *in vivo* and high-efficiency CRISPR/Cas9 mutagenesis elucidated their importance during SC repair. Interestingly, cross-species transcriptomic comparisons identified an analogous cell population after mouse SC injury, indicating conservation of iNeurons in mammals. Our study provides a comprehensive resource of cell populations that direct innate SC regeneration, cross-species comparisons of SC injury responses in zebrafish and mammals, and novel therapeutic targets for **neural repair**.

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Hepatocyte growth factor protects respiratory neural circuitry and preserves diaphragm function following cervical spinal cord injury

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Abstract

A major portion of spinal cord injury (SCI) cases occur in the cervical region where essential components of respiratory neural circuitry are located. Phrenic motor neurons (PhMNs) housed in the C3-C5 spinal cord directly innervate the diaphragm, and SCI-induced damage to these cells severely impairs respiratory function. In this study, we tested a biomaterial-based approach towards preserving this critical phrenic motor circuitry after cervical SCI by locally delivering hepatocyte growth factor (HGF). HGF been found to possess a range of therapeutic capabilities relevant to nervous system repair, including anti-inflammatory, anti-fibrotic, and anti-apoptotic effects. We developed a hydrogel-based HGF delivery system that can be injected into the intrathecal space for sustained, local delivery of high levels of HGF without damaging the spinal cord. Implantation of HGF hydrogel after unilateral C5 contusion-type SCI in rats preserved both diaphragm function (as assessed by in vivo recordings of compound muscle action potentials and inspiratory electromyography amplitudes) and PhMN innervation of the diaphragm (as assessed by detailed neuromuscular junction morphological analysis and retrograde PhMN tracing). Furthermore, HGF hydrogel significantly decreased lesion size and degeneration of cervical motor neuron cell bodies, as well as reduced the levels of two scar-associated molecules surrounding the injury site: chondroitin sulfate proteoglycan, an inhibitor of axon growth capacity; and collagen type III, a marker of fibrotic scar formation. Our findings demonstrate that local biomaterial-based delivery of HGF hydrogel to the injured cervical spinal cord is a robustly effective strategy for preserving respiratory circuitry and diaphragm function.

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Role of heme binding proteins after spinal cord injury

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Abstract

Spinal cord injury (SCI) is a severe condition with a significant impact on the life of affected individuals. The functional outcome is strongly influenced by location and extent of the tissue damage, which is determined by both primary and secondary damage mechanisms, the latter being mediated by a range of factors including inflammation and hemorrhage.

Hemorrhage contributes to secondary damage via direct reactivity and toxicity of hemoglobin breakdown products and indirectly via an increased inflammatory response.

Hemoglobin and heme binding proteins can limit tissue damage and improve recovery after SCI. Alpha-1 anti-trypsin (A1AT) and alpha-1 microglobulin (A1M) are heme binding, radical scavengers and have shown promise in models of CNS hemorrhage.

In this study, we are investigating cell specificity and expression levels of A1M and A1AT, in vitro effects in controlling heme toxicity and after SCI.

Both A1AT and A1M are significantly upregulated in the spinal cord parenchyma after SCI but differentially expressed. While A1AT is predominantly expressed by astrocytes, A1M can be detected on neurons, microglia and oligodendrocytes.

We used bone marrow derived macrophages and astrocyte cultures to demonstrate the reduction of heme mediated ROS production and cell death in A1AT treated sells.

To assess the therapeutic potential of A1AT and A1M after SCI, we are applying a proof-of-concept approach: A1M or A1AT are overexpressed by viral vector injection into the cervical spinal cord, followed by C5 contusion injury. Preliminary data indicate improved functional recovery after A1M overexpression. Confirmation studies and parallel investigations of A1AT overexpression are ongoing.

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Spinal cord injury in mice amplifies anxiety: a novel light-heat conflict test exposes increased salience of anxiety over heat

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Abstract

Anxiety is a common secondary condition associated with spinal cord injury (SCI). In a recent meta-analysis, 15-32% of individuals with SCI reported experiencing anxiety at the time of assessment. Anxiety-like behavior is modeled in mice using validated tests; however, these often test a single variable and might underestimate differences in anxiety-like behavior that would occur in more complex natural environments. Here, we aim to use a new conflict test to unmask previously underappreciated differences in anxiety-like behavior. This test uses a novel application of place preference by placing thermal preference (heated vs. isothermic plate; thermal sensitivity) in conflict with light-dark preference (anxiety-like behavior). The Thermal Increments Dark-Light (TIDAL) conflict test consists of two plates connected by a walkway; one plate remains illuminated and at an isothermic temperature, whereas the other plate is dark but is heated incrementally from 31 to 44°C. Using the TIDAL conflict assay, our results show that female mice prefer the dark plate more than male mice under various conditions, indicating increased anxiety-like behavior. Additionally, mice with SCI exhibit increased dark-heated plate preference on the TIDAL assay relative to uninjured mice. Here, we reveal the TIDAL conflict test as a new behavioral strategy in mice that is optimized to unmask latent anxiety-like behaviors. Our data suggest that SCI in female and male mice exacerbates anxiety-like behaviors; accordingly, anxiety-like behavior and related neural circuitry could represent an underappreciated, therapeutically relevant target for post-SCI interventions.

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The phagocytic receptor MerTK is required for typical repair and locomotor recovery after spinal cord injury

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Abstract

Spinal cord injury (SCI) causes neuroinflammation that persists indefinitely, worsening neurologic recovery and promoting chronic neuropathic pain. SCI-elicited immune responses lead to cascading inflammation, and expansion of the lesion site. Therefore, promoting inflammatory resolution may support neuroprotection and recovery after SCI. Phagocytosis aids inflammatory resolution through engulfment of extracellular debris following injury to promote wound healing. Engulfment of apoptotic cells and cellular debris is facilitated by the phagocytic receptor MerTK. Here, we hypothesize that deletion of MerTK in male and female mice will exacerbate secondary damage and locomotor recovery after T9 contusion SCI. Compared to wildtype mice, MerTK knockout mice show impaired locomotor recovery after SCI. In accordance, MerTK knockout spinal cord epicenters had a larger volume and cross-sectional area. qPCR analysis shows MerTK knockout spinal cords to have increased expression of cellular and molecular inflammatory mediators. Our results support our prediction that MerTK is required for typical healing after SCI and reveal consideration for future exploration of MerTK and development of novel targeted neuroimmune therapies to aid in recovery after SCI.

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L-selectin Shedding Regulates Neutrophil Function, Accumulation and Neurological Recovery after Spinal Cord Injury in a Sex-dependent Manner

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Abstract

Inflammation after spinal cord injury (SCI) contributes to secondary tissue damage and loss of function. Neutrophils are the first immune cell type to enter the injury site in large numbers, however, the mechanisms underlying damaging neutrophil properties are not fully understood. We have previously shown that augmenting the cleavage or shedding of L-selectin, an adhesion and signaling receptor on neutrophils, can improve neurological recovery after SCI. To determine the effect of L-selectin shedding on neutrophils, we utilized L(E) mice, which express an uncleavable version of L-selectin that cannot be shed. Using in vitroneutrophil stimulation assays, we observed a decrease in the levels of lactoferrin in the culture supernatant, indicative of reduced degranulation, from stimulated bone marrow neutrophils from female, but not male, L(E) mice compared to wild types (WTs). To determine if L-selectin shedding affects neutrophil responses in vivo, we quantified neutrophil accumulation in the injured spinal cord at 1 and 3 days post-SCI in adult male and female L(E) and WT mice. Interestingly, we observed a sex and genotype-dependent difference in neutrophil accumulation at 1 day post-SCI. At 3 days post-SCI, we observed greater neutrophil accumulation in L(E) mice compared to WTs. Coinciding with the sex-dependent differences in neutrophil responses, we observed diminished functional recovery in female L(E) mice compared to WTs, however, no difference was observed between these genotypes in male mice. Our data demonstrate sex-dependent differences in the role of L-selectin shedding in neutrophil function and long-term recovery after SCI.

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Identifying biomarkers for central neuropathic pain in rats with mechanical and thermal sensory abnormalities after spinal cord injury

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Abstract

Central neuropathic pain (CNP) commonly develops in patients after spinal cord injury (SCI), causing debilitating symptoms and sensory abnormalities to traditionally non-noxious mechanical and thermal stimuli. CNP regularly presents itself around a year after injury in humans, resulting from permanent cellular and anatomical changes. Previous scientific studies have demonstrated greater efficacy of treatments when delivered preemptively, but there is currently no biomarker to indicate which individuals are more susceptible to developing CNP. Thus, it is necessary to investigate the physiological and behavioral processes contributing to sensory changes that develop over time. Here we assess gait and inflammation as potential biomarkers of CNP, out to 8 weeks after injury. Using the tail flick and von Frey tests, we performed hierarchical clustering to determine the subpopulation of rats that developed sensory abnormalities. The tail flick test showed a subpopulation of hypersensitive rats significantly different than normosensitive SCI rats, that remained similar to sham rats at weeks 1, 3-8 post-injury ($p<.05$). The von Frey test showed a subpopulation of hyposensitive rats significantly different than normosensitive SCI rats, remaining similar to sham rats at weeks 6-8 post-injury ($p<.05$). We saw significant changes in gait with both modalities of sensory abnormalities, as well as increased levels of macrophages at the site of injury, providing novel biomarkers ($p<.05$). We conclude further investigation may reveal acute changes in inflammation through blood serum, mediating the immune response of macrophages at both the injury epicenter and regions of the cerebral cortex involved in processing of higher functions.

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Transcutaneous electrical stimulation of the cervical spinal cord enables neuroplasticity and functional recovery in chronic tetraplegia

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Abstract

Hand and arm function is central to daily tasks that allow for independent living. Therefore, loss of upper extremity (UE) function is one of the most devastating consequence of cervical spinal cord injury (SCI). Unfortunately, clinically effective neuroprotective or restorative treatment strategies for SCI are lacking. Electrical spinal cord stimulation is a promising neuromodulation strategy that enables meaningful functional recovery. Transcutaneous and epidural stimulation activates similar spinal pathways; thus have similar neuromodulatory potential. Transcutaneous spinal cord stimulation (tSCS) is non-invasive, can be applied in many settings, and is associated with reduced risk of complications and cost compared to surgery. Here we present the immediate and long-term effects of tSCS on the recovery of UE function from a randomized-cross-over study. We compared the outcomes from two intervention arms; (1) functional task practice (FTP) alone and (2) tSCS plus FTP. SCI neurological levels of 11 participants were between C3-C6, American Spinal Injury Association Impairment Scales were B, C, and D, and the average duration was 5 years (range 1.5-14 years). Following stimulation, participants improved their prehension and muscle strength as measured by Graded Redefined Assessment of Strength Sensation and Prehension and pinch force significantly more than when receiving therapy alone. Additionally, tSCS increased active participation in rehabilitation, reduced spasticity, and improved autonomic function. Many gains were maintained for 3-6 months following stimulation. Our findings demonstrate that tSCS enables effective recovery of UE function in individuals with tetraplegia. More importantly, sustained improvements in function well beyond stimulation treatment support the evidence of neuroplasticity.

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Probiotic treatment in vivo after spinal cord injury enhances in vitro outgrowth abilities of neurons

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Abstract

Within the United States, there are approximately 18,000 new cases of spinal cord injury (SCI) each year. Approximately 60% of these injuries occur at the cervical level, resulting in damage to descending bulbospinal pathways required for breathing. Fortunately, functional recovery can occur through several mechanisms including plasticity of intact pathways, sprouting of spared pathways, or regeneration of transected axons. Many factors, including the gut-brain axis, have been shown to influence these mechanisms and recovery potential. Indeed, previous studies have demonstrated that treating SCI-induced gut dysbiosis, an unhealthy misbalance of the gut microbiome, improves hindlimb functional recovery. Mechanistically, the gut can influence intrinsic and extrinsic factors mediating neuronal outgrowth and plasticity. Studies demonstrate a healthy gut produces regeneration-promoting metabolites and precursors of neuropeptides and neurotransmitters that influence neuroplasticity. We aimed to build upon these studies and investigated potential mechanisms allowing for improved breathing following treatment of cervical SCI-induced gut dysbiosis. Towards this goal, gut dysbiotic SCI animals were treated with either fecal matter transplant (FMT) from a young, healthy donor or probiotics and dorsal root ganglia (DRG) were harvested and assessed for neuronal sprouting and total neurite outgrowth. FMT treatment resulted in no significant differences. Interestingly, high-dose probiotic treatment produced a significantly increased number of projections from the DRG soma. These results suggest that probiotic treatment of the gut microbiome improves neuronal sprouting following SCI. Elucidating the influence of the gut microbiome on neuronal outgrowth could improve our knowledge of holistic recovery following SCI and improve treatment strategies.

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Characterizing Mitochondrial Function in Astrocytes and the Potential of STAT3 to Rescue Age-Dependent Astroglial Border Dysfunction after Spinal Cord Injury

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Abstract

Spinal Cord Injury (SCI) is the second most common cause of paralysis and incurs a significant life-long burden. The average age of incidence has increased placing great importance on understanding SCI in an aging population. One hallmark of SCI is the astroglial border. Age-dependent alteration in astroglial dynamics likely impacts the efficiency of astrogliosis, diminishing ability to quickly sequester the lesion. Mitochondria play important roles in aging, and SCI progression. Dysfunctional mitochondria may have a significant impact on function of aging astrocytes. Using isolation and culture of primary astrocytes, we have analyzed mitochondrial changes in 2-, 6-, 12- and 18-month mice. We observed that astrocyte mitochondrial activity is altered with age, with an increased mitochondrial membrane potential (ΔΨm), reduced expression of OXPHOS proteins, increase in respiration, and ATP retention. STAT3 is involved in reactive astrogliosis and mitochondrial activity, and is decreased with age. We previously observed an age-dependent reduction in STAT3 expression and increase in inhibition (PIAS3). Current data demonstrates increasing STAT3 rescues mitochondrial activity in the presence of Rotenone, and STAT3 deletion exacerbates reactive oxygen species (ROS) production in astrocytes in vitro. In young adult mice in vivo, deletion of SOCS3 (a negative regulator of STAT3) in astrocytes at the site of a T8 SCI expedited astrocyte border formation and improved hind-limb recovery. We postulate that by manipulation of STAT3 in astrocytes after SCI, we can stimulate transient astrogliosis in acute injury in both young and aging mice to improve border formation and functional recovery.

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Progressive neuronal swelling in the injured mouse spinal cord

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Abstract

Neurons in the spinal cord receive descending and peripheral inputs that orchestrate essential behavioral programs in vertebrates. Spinal cord injuries (SCI) damage neurons within the spinal cord and interrupt the bidirectional flow of information, resulting in the loss of functions for SCI patients. Studies have shown that activating surviving spinal interneurons (e.g., via epidural electrical stimulation) can restore connectivity and drive voluntary functional recovery, including in SCI patients. However, the impact of SCI on spinal neurons remains largely unknown, which hinders our ability to reactivate them. To address this challenge, we developed an imaging approach that facilitates high-resolution, volumetric imaging of optically cleared, spatially preserved spinal cord samples. Here, we used this 3D reconstruction pipeline to examine the number, volume, and 3D distribution of excitatory neurons in the mouse spinal cord. Surprisingly, our results showed the temporal and spatial progression of excitatory interneurons swelling, which is accompanied by neuronal loss after SCI. Our findings also showed that administering bumetanide (a Cl- pump inhibitor and NKCC1 blocker that reduces neuronal edema) directly into the spinal cord can prevent swelling of spinal neurons. Importantly, we demonstrated that preventing neuronal swelling prevents neuronal loss, enhances neural circuits, and promotes multiple functional recoveries. This finding is important because it re-define our conventional understanding of cellular edema timeline and severity, after traumatic CNS injuries. In addition, our results highlighted that progressive neuronal edema is one of the mechanisms that cause peri-lesion intraspinal neurons to remain vulnerable to cell death even weeks after SCI.

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Targeting noradrenergic signaling to mitigate bone loss after SCI

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Abstract

At least 80% of people living with spinal cord injury (SCI) have osteoporosis or osteopenia, with loss of approximately 40% of trabecular bone volume below the level of injury (sublesional) in the first 2 years post-injury. This rapid bone loss leaves bone vulnerable to fracture. In approximately 50% of cases, post-fracture complications occur including venous thromboembolism, and respiratory and urinary tract infections. Treating or preventing SCI-induced osteoporosis is a necessary medical need. While bone loss post-SCI has been attributed to disuse, we have shown bone loss persists despite recovery of hindlimb stepping at 180 days, indicating disuse is not the primary cause.We hypothesize that increased noadrenergic signaling to bone in the acute phase of SCI underlies SCI-induced osteoporosis. To test this, bone marrow is extracted from male rats after a moderate T11-12 spinal contusion injury, as well as chemically sympathectomized rats via 6-OHDA. Bone marrow from the femur and tibia were collected 28 post-SCI. Cultures showed an increase in pre-osteoclasts, but while SCI increased osteoclast maturation, 6-OHDA blocked osteoclast maturation and increased osteoblast mineralization. Innovatively targeting the femur with an intraosseous norepinephrine (NE) administration, we found that increasing NE for 3 days post-SCI caused an increase in osteoclast formation relative to saline-treated SCI controls. Additionally, treating ex-vivo osteoclast cultures with either an α1 adrenoceptor (AR) agonist or βAR antagonist for 5 consecutive days reduced osteoclast proliferation relative to NE only. These data suggest α1- and βAR agonists and antagonists may be effective in attenuating osteoclast differentiation and bone resorption post-SCI.

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A novel intraparenchymal administration of CHASE37-AR improves recovery of function in the chronic phase of a rat spinal contusion injury

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Abstract

Pre-clinical studies have shown that Chondroitinase ABC (ChASE) can improve recovery in animal models of spinal cord injury (SCI). However, the inherent instability of the enzyme, as well as uncertainty about the best way to administer it to the lesion site, has impeded its translation into humans. To address this, we used 1) a more stable form of the enzyme (ChASE37-AR) and 2) compared the efficacy of two routes of administration: subarachnoid (SA) and intraparenchymal (IP). Young, male Sprague-Dawley rats were given a T11 moderate SCI. At 28 days post-injury, they were given a single ultrasound-guided injection of the enzyme (or vehicle) directly into the lesion site (IP), or a subarachnoid (SA) injection of ChASE37-AR or vehicle. Motor recovery (BBB) and pain (reactivity to von Frey and incremented tail shock) were monitored prior to treatment, then assessed for 84 days post-treatment. Motor recovery plateaued in all groups by 27 days post-injury and remained unchanged in the SCI-only, IP, and SA vehicle-treated SCI rats. There was no change in recovery for rats treated with SA CHASE37-AR. Excitingly, however, SCI rats that were given an IP CHASE37-AR injection had a 1.5-point increase in their BBB scores after Day 27, recovering significantly better than their vehicle-treated conspecifics. Further, irrespective of the route of administration, ChASE37-AR reduced the expression of chronic pain symptoms in comparison to the vehicle and SCI-only groups. These data suggest that ChASE37-AR promotes motor recovery and can reduce pain sensitivity, even when applied in the chronic phase of SCI.

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Daily Acute Intermittent Hypoxia Improves Hemodynamic and Cardiac Function in Rats with Incomplete Spinal Cord Injury

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Abstract

Spinal cord injury (SCI) reduces resting blood pressure (BP), orthostatic tolerance (OT) and cardiac function due to disrupted descending spinal sympathetic control. We previously found that a single-bout of acute intermittent hypoxia (AIH; exposure to brief periods of low oxygen) increases cardiac function and improves the hemodynamic response to simulated orthostatic tolerance (OT) in a rodent model of SCI. Here, we extend our findings by investigating the effect of daily AIH on cardiovascular function in an incomplete model of thoracic SCI.

Four weeks following T3 contusion SCI (300Kdyn), rats were randomized to either daily AIH (n=12) or SHAM (n=11) for 2 weeks. Six weeks post-SCI, rats were assessed for hemodynamic and cardiac function via direct arterial and cardiac catheterization. Rats also underwent lower body negative pressure (LBNP) to decrease mean arterial pressure (MAP) as a model of OT. Group differences in cardiovascular function were assessed with T-tests. A mixed-design ANOVA was performed to examine responses to LBNP.

Daily AIH rats exhibited higher MAP vs SHAM (96±10 vs. 79±5 mmHg, $p<0.001$). Daily AIH rats also exhibited a higher rate of rise of the left ventricular pressure waveform (dP/dtmax; 6860±666 vs. 5798±411 mmHg/s, $p=0.01$). There was also a significant main effect of treatment ($p=0.008$) and stage ($p<0.001$) on responses to LBNP, that demonstrated Daily-AIH rats exhibited better OT (i.e., required a stronger LBNP stimulus to reduce MAP).

Here, we demonstrate that 10 days of AIH exposure improves hemodynamic and cardiac function in chronic incomplete SCI rats.

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Correlation of Pain Outcomes with Myeloid Cell Activation State in the DRG and Dorsal Horn After C5 Spinal Cord Injury

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Abstract

Spinal cord injury (SCI) is a debilitating neurological condition that leads to the development of chronic pain. While recent studies have implicated the role of macrophages and microglia in contributing to SCI-induced pain, the specific mechanisms by which these cells mediate pain is unknown. The purpose of this work is to elucidate macrophage and microglial polarization states in the dorsal root ganglia (DRG) and dorsal horn of the spinal cord that correlate with pain development or resolution. Wildtype C57BL6 mice received a unilateral C5 contusion. Behavioral testing revealed that SCI mice exhibited paw hypersensitivity and increased escape latencies in von Frey and mechanical conflict tests compared to sham and naïve control mice ($p<.05$), indicative of SCI-induced pain. At 6 weeks post-injury, mice were sacrificed and C4-8 DRGs and C7-8 spinal cord were dissected; RNA was isolated. qPCR of macrophage/microglial and secretome markers of pro-inflammatory (CD32, CD86, iNOS, IL-1β, IL-6, IL-12, TNFα) and pro-reparative (Arg1, CCL2, CCR2, CD206, IL-10) polarization is underway. Preliminary data suggests that SCI increases macrophage and microglial concentration in the DRG and dorsal horn. Multivariate statistics will be conducted on the complete dataset to determine whether pain behavior is associated with a specific polarization state of macrophages and microglia. These data will provide important information regarding the relative proportion of pro-reparative to proinflammatory, potentially neurotoxic macrophages and microglia. Future work will explore methods to drive macrophage/microglial polarization to the reparative activation state as a means to reduce pain following SCI.

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Towards a Better Understanding of Spinal Cord Injury: A TRACK-SCI Study Progress Report.

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Abstract

Traumatic spinal cord injury (SCI) is a debilitating condition that profoundly impacts the lives of patients, their families, and society as a whole. Despite extensive research and numerous clinical trials, a cure for SCI has yet to be found. The reason for this is multi-factorial and can be attributed to the complexity of the injury, the heterogeneity of the human population, and the lack of reliable biomarkers and outcome measures. In light of these challenges, there is a growing need for a better understanding of the pathophysiological events following SCI.

To address this need, the “Transforming Research And Clinical Knowledge for SCI” (TRACK-SCI) study was launched eight years ago. TRACK-SCI aims to gather detailed data from the ultra-acute stages of SCI to one year post-injury and to use advanced analytical and bioinformatics tools to analyze this data and increase our understanding of SCI.

The first eight years of TRACK-SCI will be summarized in this meeting, and the successes in various areas will be reported. These include advancements in imaging and blood biomarkers, early surgery, intraoperative blood pressure management, and long-term outcomes. The challenges the consortium faces will also be discussed, as well as the plans for expansion.

Overall, TRACK-SCI has made significant contributions to the field of SCI research and has helped to increase our understanding of this debilitating condition. The lessons from this study will be invaluable in guiding future research efforts and developing more targeted and personalized approaches to treating SCI.

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New Viral-based Approach for RNA Isolation Allows for Transcriptional Studies in NHP of Corticospinal Neurons Response to Injury

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Abstract

How adult cortical neurons respond to injury and which pathways mediate survival, regeneration, and re-establishment of connectivity after lesion remains a fundamental question in the field of spinal cord injury. We began addressing this question by using the Glt25d2-eGFP-L10a mouse line to isolate RNA specifically from Layer 5 cortical neurons. Following spinal cord injury, we showed that corticospinal tract (CST) neurons revert to an embryonic-like state upon injury and that contact with a neuroprogenitor graft extends this pro-regenerative state to 21 days (Poplawski, 2020). The extent to which this result is generalizable to other organisms, including humans, is of fundamental importance. How accurately murine transcriptional studies reflect what happens in humans is unknown. Do CST neurons of human patients show a similar window for intervention? To answer these questions, we developed a viral-based approach that allows for RNA isolation from any neurons that can be targeted by AAV injection. When applied to the CST, this sequential immunoprecipitation-based approach allows for purification of RNA from whole motor cortex that is enriched in neuronal genes, Layer 5 marker genes and depleted in glial genes. After validating this approach in rats, we applied it to non-human primates (NHP) to isolate RNA specifically from the corticospinal neurons of the hand/arm region in the primary motor cortex. We collected RNA from cynomolgus monkeys 3, 10, and 20 days after C7 hemisection-injury as well as from uninjured controls. Together, this data will show if a therapeutic window closes in the NHP and when it closes.

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Neutrophil Extracellular Traps in Acute Spinal Cord Injury

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Abstract

Neutrophils are the first peripheral immune cell to infiltrate the spinal cord in large numbers following spinal cord injury (SCI); however, their role in secondary tissue damage and locomotor recovery remains unclear. Neutrophil extracellular traps (NETs) are a neutrophil effector function wherein chromatin is decondensed, decorated with granule proteins, and expelled from the cell as a mechanism to trap and destroy pathogens. However, NETs have also been shown to be damaging to host tissues under sterile inflammation conditions. The contribution of NETs to tissue damage and functional recovery following spinal cord injury (SCI) has yet to be investigated in mice. To determine if NET formation occurs acutely in a murine model of SCI, we performed ELISAs for complexes of DNA with NET associated markers in spinal cord and peripheral blood samples acutely after SCI. We found that NET levels rapidly increased over the first 12 hours and peaked within the first 24 hours after injury. We verified NET formation in SCI via flow cytometry using neutrophils isolated from blood and spinal cord samples. Treatment with DNase I, a method used to reduce NET levels in many studies, did not significantly improve locomotor recovery in our studies. Furthermore, genetic ablation of a key enzyme in the pathway of NET formation, PADi4, did not significantly alter functional locomotor recovery in our model. Collectively, our data demonstrate the first evidence of NETs in the injured murine spinal cord, however, NETs do not appear to contribute to functional deficits after SCI.

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Periaxonal Swelling and secondary axonal degeneration following SCI

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Abstract

Ultrastructural studies of SCI in mammals have shown that the most prominent acute changes in white matter are periaxonal swelling and separation of myelin away from their axon, axonal swelling, and axonal spheroid formation. However, the underlying cellular and molecular mechanisms that cause periaxonal swelling and the functional consequences are poorly understood. We hypothesize that periaxonal swelling and loss of connectivity between the axon and myelin: i. disrupts conduction; ii. disrupts glial to axonal trophic support and iii. exposes the now vulnerable axon to cytotoxic factors within the internodal region that drives axonal swelling and axonal spheroid formation. Utilizing in vivo longitudinal imaging of *Thy1*YFP+ axons and myelin labeled with Nile red, we have confirmed that periaxonal swelling significantly (ANOVA on Ranks, $p = 0.002$; n =3-11/ timepoint) increases acutely following a contusive SCI (T13, 30 kilodyne, Infinite Horizons Impactor) versus baseline recordings (laminectomy only) and precedes axonal spheroid formation. In addition, using longitudinal imaging to determine the fate of myelinated fibers acutely after SCI, we have determined that 55.71% of myelinated fibers with periaxonal swelling at one hour post SCI transition to axonal spheroids by four hours post SCI (Binomial Proportion Test; $z=3.23$; $p < 0.05$, $n = 4$). Interestingly, the axonal spheroids form their characteristic prolate spheroid shape by occupying the myelin "mold" that forms during the enlargement of the periaxonal space. As periaxonal swelling represents a potentially reversible opportunity during secondary injury, targeting periaxonal swelling may prevent subsequent axonal spheroid formation and preserve white matter following SCI.

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Optogenetic stimulation of the rat cervical spinal cord promotes enhanced functional recovery, axonal growth and angiogenesis

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Abstract

Optogenetic stimulation is a method of stimulating the spinal cord that allows for targeted stimulation of specific cell-types-of-interest. The present work investigates the effects of optogenetic spinal stimulation on forelimb recovery, axonal growth, and vasculature after a cervical spinal cord injury (SCI) in rats.

Adult rats received a moderate cervical hemicontusion (C4) followed by the injection of an optogenetic viral vector ipsilateral and caudal to the lesion at C6. Afterwards, rats began rehabilitation on the skilled forelimb reaching task. At 4 weeks post-injury, rats received a µLED implant to illuminate the C6 spinal cord for optogenetic stimulation. Stimulation began at 6 weeks post-injury and occurred in conjunction with activities to promote use of the forelimbs. Following 6 weeks of stimulation, rats were perfused and tissue stained for GAP-43 (axonal growth), laminin (vasculature), and Cresyl violet and myelin (lesion magnitude). Location of viral transduction and transduced cell types was also assessed.

Optogenetic spinal stimulation significantly enhanced recovery of skilled forelimb reaching after SCI. As expected, lesion magnitude greatly affected the level of recovery that was achieved by optogenetic stimulation. We also found significantly greater GAP-43 labeling at the site of stimulation and at the lesioned segments following optogenetic stimulation, indicating enhanced axonal growth in those regions. Laminin staining indicated vasculature was significantly enhanced throughout the cervical spinal cord following stimulation, suggesting that optogenetic stimulation promotes angiogenesis. Viral transduction of opsins that enable optogenetic stimulation occurred within an evenly mixed population of glutamatergic and gabaergic synapses.

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Intrathecal injection of small extracellular vesicles from polarized macrophages in spinal cord injured rats reduces mechanical and thermal pain sensation

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Abstract

Neuropathic pain is a maladaptive consequence of central nervous system (CNS) damage, prevalent in ~60% of individuals with spinal cord injury (SCI). Development and maintenance of neuropathic pain after SCI has become increasingly linked to neuroimmune interactions. Exosomes are small extracellular vesicles (SEVs) that contribute to cell-cell communication in the CNS, and macrophage-derived SEVs have been implicated in several homeostatic and pathological conditions, including neuropathic pain. However, the role of macrophage derived SEVs in the SCI immune microenvironment has not been established. To explore this, we performed von Frey and Hargreaves' assessments of mechanical and thermal pain sensitivity in adult Sprague Dawley rats before and after a C5 unilateral contusion, noting decreases in paw withdrawal threshold after SCI as an indicator of neuropathic pain. 14 days post injury (dpi), rats received 10ug of SEVs derived in culture from either LPS-stimulated or unstimulated RAW 264.7 cells, or vehicle via lumbar puncture. Paw withdrawal threshold were decreased after SCI, and injection of SEVs from LPS-stimulated macrophages partially rescued behavior, reducing sensitivity of the ipsilateral forepaw. At 35dpi, and C7-8 spinal cord and dorsal root ganglia were harvested. Immunohistochemical (IHC) staining for Iba1 and CGRP, indicators of microglia/macrophage activation and nociceptor plasticity, respectively is currently underway. Preliminary IHC staining revealed an increase in peptidergic c-fiber sprouting and decreased microglial activation in the dorsal horn with LPS-stimulated SEV injection. Future directions aim to identify distinct cargoes within macrophage derived SEVs, and the effect these vesicles have on nociceptor excitability.

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Ampakine therapy improves respiratory muscle activation following cervical spinal cord injury

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Abstract

Cervical spinal cord injuries (cSCI) produce long-lasting respiratory impairments. Ampakines are positive allosteric modulators of AMPA receptors that can stimulate respiratory motor output, particularly during hypoventilation associated with drug overdose or neuromuscular disease. We hypothesized that ampakines can stimulate diaphragm activity and increase ventilation after incomplete cSCI. Two experimental models were used: C2 spinal-hemisection (C2Hx) and 150kDy C4-contusion. C2Hx severs ipsilateral glutamatergic synaptic inputs to phrenic motoneurons. C4Ct results in phrenic motoneuron loss and damage of spinal pathways. Adult male/female Sprague-Dawley rats were implanted with in-dwelling diaphragm EMG electrodes and ventilation was measured using plethysmography. Recordings were conducted at pre-injury and 4- and 14-days post-injury (dpi). Rats were given an intravenous bolus of low-impact ampakine CX717 (5 mg/kg; C2Hx: n=8; C4Ct: n=10) or vehicle (HPCD; C2Hx: n=8; C4Ct: n=10). At 4- and 14-dpi following C2Hx, infusion of CX717 increased diaphragm EMG_{peak} by 50±15% and 45±10% respectively. This was associated with 26±8% (4-dpi) and 12±7% (14-dpi) increases in tidal volume. Following C4Ct (4-dpi), CX717 increased diaphragm EMG_{peak} by 29±11% and tidal volume by 25±5%. However, there was no discernable impact of ampakines at 14-dpi, likely reflecting the robust spontaneous recovery of diaphragm EMG_{peak} that follows C4Ct in the rat. Across all experiments, vehicle infusion had little to no impact on outcome measures, and no discernable adverse effects of ampakine were detected. We conclude that low dose (5mg/kg) treatment with a low impact ampakine effectively increases diaphragm muscle activation at acute time-points following cSCI.

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Unexpected Limitations of AAV2-retro for retrograde transduction of neurons whose axons have been interrupted by spinal cord injury

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Abstract

AAV2-retro provides a unique platform to simultaneously deliver cargos to the cells of origin of multiple pathways that project to the spinal cord. Deploying this platform to introduce regeneration-enabling cargos in a therapeutically-relevant time frame after spinal cord injury requires that axons that are interrupted by SCI take up and transport AAV2-retro. Here, we explore this question using a double-injection strategy. TdT reporter mice received bilateral injections of AAV2-retro/Cre at either lumbar or thoracic levels to induce tdT expression in CST neurons projecting to these levels. Two weeks later, mice received dorsal hemisection injuries at C5, which transect essentially all CST axons passing through C5 to lower levels. Either in the same operation or 1,2, or 4 weeks post-hemisection, mice received injections of AAV-retro/GFP into the cervical spinal cord above the hemisection and were perfused 2 weeks later. If CST axons that project to lumbar or thoracic levels that were transected by the lesion take up and transport AAV2-retro/GFP, then tdT-positive CST neurons would be co-labeled with GFP. Regardless of the post-injury interval (immediate-4 weeks) or whether the initial AAV2-retro/Cre injection was at lumbar or thoracic levels, only a small proportion (less than 5%) of tdT labeled neurons co-expressed GFP. Thus, at least in the context of a C5 dorsal hemisection, there is minimal transduction of cut CST axons with AAV2-retro. These results suggest that further modifications of AAV2-retro will be needed to optimize its value for delivering regeneration-enabling gene cargos to neurons whose axons are interrupted by SCI.

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Dynamic routine blood biomarkers for spinal cord injury diagnosis and prognosis

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Abstract

Diagnosis and prognosis after acute traumatic spinal cord injury (SCI) are challenging due to pathology complexities and population heterogeneity. Routinely collected data during medical practice, such as laboratory values, can be surrogates of underlying pathophysiological processes and be used as a biomarker. We hypothesized that distinct temporal trends of blood analytes can be modeled after SCI and that those would predict patient outcomes. Using real-world data from available electronic health records (EHR), we assembled a big-data asset and modeled distinct laboratory analytes measured over time during the early hospitalization after acute spine trauma with or without SCI. We fitted longitudinal finite mixture models to determine distinct group trajectories on 20 blood analytes commonly measured in these populations over time. The probability of trajectory membership was used in a supervised learning task to predict patient outcomes. We show non-linear heterogeneous temporal trends of blood analytes after spine trauma and SCI. These trajectories are associated with different patient characteristics. In dynamic prediction experiments, the probability of specific analyte trajectories is predictive of in-hospital mortality, the patient presenting with an SCI, and SCI severity (motor complete vs. incomplete). In addition, using an external dataset to the trajectory modeling from the TRACK-SCI study, our results indicate that trajectories derived from EHR could be generalizable. Routinely real-world data can be used to model blood analytes' dynamic changes after SCI with prediction validity for patient outcomes. Our work suggests that temporal blood trends are promising early dynamic predictors for SCI.

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Impacts of epidural spatial stimulation extent on recovery of function in combined bionic and biological SCI therapies

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Abstract

A combination of therapies may be most effective in improving function after a spinal cord injury (SCI). Although each therapy modality, i.e. rehabilitation, neuroprosthetic, biological (viral), epidural stimulation (ES) improves function individually, we understand little about their synergistic interactions on function. We also have limited knowledge of the effects of combination therapies on muscle activation patterns and their underlying neuronal circuits, which may reflect different neuronal control strategies for motor coordination. Our lab has demonstrated enhanced locomotor outcomes in rats with complete thoracic 9/10 SCI after combining robot training with viral BDNF and ES. Prior work further revealed a potential critical period during the initial two weeks of training, where ES likely attenuates some spasticity development that can be an effect of the BDNF treatment on motor function. Spasticity can result in the eventual collapse of gained motor function in ~40% of rats. Over a period of 6 weeks, this collapse was prevented when broad-current spread ES centered at L2 and S1 was combined with viral BDNF and robot training. The current study aims to examine the combined actions of robot therapy, viral BDNF, and broad vs local ES on locomotion in rat models of complete SCI. We hypothesized that the combined treatment using localized ES would more selectively target the central pattern generators at L2 and S1, resulting in improvement in weight-supported stepping throughout therapy. We also hypothesized that modularity analysis of the hindlimb muscles will help reveal patterns of spinal circuit reorganization in the different rehabilitation treatments.

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Modulating Proteoglycan Synthesis for Spinal Cord Injury Regeneration

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Abstract

Spinal cord injury (SCI) is a life changing injury that can cause complete paralysis below the injury level. Following SCI a glial scar forms containing neuronal growth inhibitory proteoglycans that hinder regeneration. Manipulating proteoglycan expression after traumatic SCI improves axonal growth, regeneration, and function in animal models. Distinct glycosylation patterns have also been associated with regeneration failure and regenerative success following SCI comparing animal models that do not undergo spontaneous regeneration and those that do. We developed lentiviral vectors to deliver shRNA mediated knockdown of key enzymes involved in proteoglycan synthesis (Xylosyltransferases I and II, and Chondroitin Sulphate N-acetylgalactoasminyl-transferase-1). We hypothesise that these vectors will provide long term alteration of proteoglycan expression that aids neuronal outgrowth after SCI. We characterised the effect of the reduction of these enzymes involved in proteoglycan synthesis in an astrocytic cell line. A Lectin binding array was carried out to assess changes in the glycosylation of proteins within the membrane and excretory fractions of these cells. Cells were also stained with lectins to access changes in key glycosylation residues sialic acid associated with regenerative failure, inflammatory regulation, and chondroitin sulphate glycosaminoglycan. We hope to deliver these lentiviral vectors to an organotypic ex vivo spinal cord injury model and assess changes in glial scar make up and neuronal outgrowth.

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In vivo visualization of PTEN knockdown with inducible promoters

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Abstract

Deletion or siRNA-mediated knockdown of the gene phosphate and tensin homolog (PTEN) in cortical motoneurons (CMNs) enable regeneration accompanied by recovery of function after spinal cord injury (SCI). Previous approaches have focused on permanent PTEN knockdown, which can lead to pathophysiologies in some animals. Accordingly, we are developing strategies to enable transient knockdown using inducible expression of short hairpin RNA against PTEN (shPTEN) via the TetOn system, where transgene expression is contingent on doxycycline (Dox) delivery. Our approach to document dynamics of regulated transgene expression uses bioluminescence imaging, where Dox in drinking water activates transduction of both the transgene (shPTEN) and luciferase. When luciferase is expressed in cells, delivery of luciferin triggers production of photons which can be measured repeatedly in living animals over time to define onset and offset of luciferase expression. We examined the sensitivity of the In-vivo Imaging System (IVIS) for detecting AAV-retro-mediated luciferase expression in the brain after cervical spinal cord injections (C5) and Dox administration. Luminescence was detected 2d after AAVretro-TetON-shPTEN intraspinal injections only in mice that received Dox and luminescence persisted for at least 44d. Immunostaining for PTEN 17d post Dox revealed PTEN knockdown in CMNs. Upon Dox removal, luminescence was undetectable 14d post removal indicating cessation of expression of luciferase and presumably shPTEN. Turning PTEN deletion on and off with the administration of Dox will allow us to determine the window for PTEN knockdown to enhance axonal regeneration after SCI avoiding potential negative consequences of long-term deletion.

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Exploring two methods of selective labeling of the corticospinal tract (CST) after spinal cord injury (SCI)

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Abstract

The most commonly used approach to trace CST axons after SCI is with biotinylated dextran amine, which has limitations because tracer injections are required after SCI and there is no selectivity in labeling of neurons. Here we explore utility of two alternate approaches. The first approach uses mice with eGFP expression driven by the mu-crystallin (Crym) promoter, which selectively labels CST axons. Crym-eGFP transgenic mice received dorsal hemisection injuries at T9 and were harvested at weekly intervals. Immunostaining revealed that eGFP persisted in degenerating CST axons up to 6 weeks post-injury, which complicates the assessment of early regeneration. Surprisingly, meningeal cells near the lesion expressed eGFP and elongated GFP-positive profiles were present in the dorsal column caudal to the injury in some mice. We speculate that Crym-eGFP expression is induced in meningeal cells, and some of these migrated into the dorsal column. The second approach uses AAV/Cre-driven induction of tdTomato (tdT) expression in reporter mice. PTENff/tdT mice received AAV-Cre injections into the sensorimotor cortex to delete PTEN and induce tdT expression in cortical motoneurons. Mice received dorsal hemisection injuries at C5 three months later. Immunostaining for tdT revealed robust labeling of CST axons including fine arbors in the gray matter. Cre-dependent tdT expression offers significant advantages over AAV-driven expression of fluorescent reporters because tdT expression is turned on selectively and permanently in CST neurons, which in this case selectively marks PTEN-deleted neurons. Similar approaches could be used for any AAV-driven gene modifying cargo in tdT reporter mice.

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AAV9-mediated KCC2 upregulation restores injury-induced synaptic alterations following traumatic spinal cord injury

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Abstract

Traumatic spinal cord injury (SCI) impairs local neuronal conductance and induces a subsequent synaptic remodeling cascade in the rostro-caudal perilesional zone. K+/Cl− cotransporter 2 (KCC2) is a differentially expressed synaptic ligand-gated channel, which is pivotal for signal propagation in inhibitory spinal interneurons. Reduced KCC2 expression post-SCI disrupts the excitatory/inhibitory (E/I) ratio in the preserved spinal interneurons and blocks the relay of signals in the injured spinal cord. The recent advances in AAV9 gene therapy present a promising approach to therapeutically upregulate KCC2 upregulation and restore the functional neurotransmission in the injured spinal cord. The aim of the study is to examine the ability of KCC2 gene therapy to improve functional recovery by altering SCI-induced synaptic neuroplasticity. We demonstrate for the first time the ability of intrathecal AAV9 administration to induce KCC2 expression in the preserved neural tissue without any deleterious off-target effects. This induced KCC2 expression alters the transcriptional profile of the targeted neurons, which improves the long-term forelimb and hindlimb motor recovery. This is also accompanied by restored forelimb motor evoked potential (MEP) response and immunohistochemical alterations in the injured spinal cord. Overall, this study can have a significant impact on the neuromodulation regimen for SCI patients to improve their recovery and well-being.

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Sex-dependent differences in antibody-mediated neutrophil depletion in the context of SCI

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Abstract

Following spinal cord injury (SCI), an inflammatory cascade ensues, worsening tissue damage (secondary injury) and long-term functional recovery. Neutrophils, the most abundant leukocytes in humans, are the first immune cells to infiltrate the injured spinal cord in large numbers. Neutrophils have been shown to exacerbate secondary injury, however, recent evidence suggests diverging roles for immature and mature neutrophil subsets in SCI, as well as sex-dependent differences in neutrophil function. Antibody-mediated neutrophil depletion via anti-Ly6G (clone 1A8) is one method for depleting neutrophils from circulation to abrogate neutrophil-associated inflammatory damage post-SCI, but the effects of this depletion on neutrophil subsets with sex as a biological variable considered has not been assessed. To investigate sex-dependent effects of anti-Ly6G-mediated neutrophil depletion on neutrophil subsets in mice, we administered 2.5mg/kg of either anti-Ly6G (1A8) antibody, IgG (2A3) control antibody, or vehicle (VEH) intraperitoneally to male and female wildtype (WT) mice (age 12-20 weeks). We performed flow cytometry the following day to identify differences in depletion efficiency and neutrophil maturity in the peripheral blood and bone marrow of males and females. Antibody-mediated neutrophil depletion via anti-Ly6G administration significantly reduced mature neutrophils from circulation in female but not male mice. Our data support evidence for sex-dependent differences in neutrophil depletion, and suggest the need for a sex-specific method for robust neutrophil depletion for future studies assessing the therapeutic potential of neutrophil depletion in SCI.

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Investigating the Role of STAT3-mediated Astrogliosis on Functional Recovery and Neural Repair after DCM.

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Abstract

Introduction: Degenerative cervical myelopathy (DCM), is the most common cause of spinal cord dysfunction amongst adults over the age of 55, involving the chronic and progressive degeneration of the cervical spinal cord. The current gold standard treatment for DCM is cervical decompression surgery (DEC), which effectively delays the progression of the disease and improve functioning in most patients. Unfortunately, a significant number of patients experience postoperative complications that can be explained in part, by secondary ischemia-reperfusion injury (IRI). Recent evidence suggests that astrocyte activation, mediated by the STAT3-signalling pathway, is critical for the repair of the blood-spinal cord barrier (BSCB) and the restriction of leukocyte infiltration following spinal cord injury. **Hypothesis:** We hypothesize that if STAT3 signaling in astrocytes is critical for neuroprotection against IRI, STAT3-cKOs should demonstrate worsened functional recovery and neural repair. **Methods:** DCM was induced in 8-week-old female mice that were either; 1) STAT3-conditional knockouts, where STAT3 expression will be inactivated in astrocytes or 2) wildtype controls. At 12 weeks post-DCM, animals underwent DEC surgery and 5 weeks later, spinal cord tissue was extracted for analysis. **Results:** We predict that the knockouts will demonstrate attenuated astrogliosis, increased disruption of the BSCB and reduced axonal regeneration compared to their wildtype counterparts. **Conclusion:** This study will be the first to unveil the role of astrogliosis in the pathogenesis of DCM and IRI after decompression, as well as potential therapeutic targets to reduce post-operative IRI-related complications following surgical decompression.

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Enhanced oxidative phosphorylation, re-organization of intracellular signaling, and epigenetic de-silencing as revealed by oligodendrocyte translatome analysis after contusive spinal cord injury

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Abstract

Reducing loss of mature oligodendrocytes (OLs) is a major goal for therapeutic interventions after traumatic spinal cord injury (SCI). Therefore, OL translatome was determined in Ribotag:Plp1-CreERT2 mice at 2,10, and, 42 days after moderate contusive T9 SCI. On days 2 and 42, many mitochondrial respiration-related mRNAs were upregulated suggesting a biphasic shift of metabolism towards oxidative phosphorylation. Those changes coincided with reduced expression of actin cytoskeleton-, cell junction-, and cell adhesion-related transcripts indicating loss of myelin sheaths and morphological simplification. Various regulators of pro-survival- or cell death signaling showed peak expression on post-SCI day 2. Potential consequences included normalization of ERK1/2 and AKT activities, activation of JNK, enhanced antioxidant defenses but also oxidative stress. Many acutely upregulated OL genes are part of the repressive SUZ12/PRC2 operon suggesting that epigenetic de-silencing contributes to SCI effects on OL gene expression. Acute OL upregulation of two candidate pro-oxidant genes including iron oxidoreductase Steap3 and prenylcysteine oxidase Pcyox1l was confirmed on protein level. Finally, STEAP3 upregulation in cultured OLs that were treated with the mitochondrial uncoupler FCCP implicates it as a potential marker of mitochondrial dysfunction. Taken together, in SCI-challenged OLs, enhanced mitochondrial respiration and myelin sheath loss may coincide both acutely and sub-chronically. Such changes are likely driven by axonal loss/degeneration. Acutely, OL switch to oxidative phosphorylation may lead to oxidative stress that is further amplified by upregulation of such enzymes as STEAP3 or PCYOX1L.

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Harnessing the endogenous regenerative potential of the injured spinal cord

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Abstract

The adult spinal cord contains a population of ependymal-derived neural stem/progenitor cells (epNSPCs) that hold the potential to enhance regeneration and promote repair after injury. epNSPCs are normally quiescent but are acutely activated after spinal cord injury (SCI). Despite this, the activation of these cells is insufficient to promote robust neural regeneration and recovery. Thus, it is of therapeutic interest to harness the endogenous regenerative potential of epNSPCs. However, little is known about the underlying mechanisms that govern the biology of these cells. We thus aim to characterize the mechanisms of epNSPC activation after SCI and examine a therapeutic strategy to harness their regenerative capacity. epNSPCs isolated from the central canal region of the adult spinal cord were used for *in vitro* mechanistic analysis. *In vivo*, cervical SCI was induced in adult rodents using a clinically relevant compression-contusion model of SCI. We found that excitotoxic levels of glutamate, a hallmark in the pathogenesis of SCI, leads to calcium influx in spinal cord epNSPCs via AMPARs and this change in calcium in concert with Notch signaling drives the proliferation of epNSPCs via pCREB, and induces astrocytic differentiation through Hes1 upregulation. Positive allosteric modulation of AMPARs subacutely after SCI enhances epNSPC proliferation, astrogliogenesis, neurotrophic factor production and promotes neuronal survival and functional recovery. We uncover an important mechanism by which AMPARs regulate the growth and phenotype of epNSPCs which can be targeted therapeutically to harness the endogenous regenerative potential of the injured spinal cord.

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Evolutionarily conserved blood transcriptomic signatures as diagnostic biomarkers and pharmacological targets for spinal cord injury

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Abstract

One of the reasons for the lack of successful clinical trials for spinal cord injury (SCI) is the lack of biomarkers for patient stratification. Recently, we proposed a novel approach for discovering blood RNA biomarkers and discovered transcriptomic signatures that diagnose SCI severity. We now hypothesize that these signatures are also functionally involved in SCI and could be targeted to develop therapeutics. To this end, we performed Gene Co-Expression Network Analysis on RNAseq data from blood in humans and rats after SCI. We used RNA from 38 patients with an average time of the blood draw of 25 hours after SCI. In rats, we used RNA from 36 animals undergone cervical SCI of three different severities. Samples were taken before SCI, and at 6, 24, 48, 72 hours, and six weeks post-SCI. The CMap database was used to predict drug candidates targeting the identified transcriptomic signatures. We identified gene modules associated with SCI severity in humans, and similarly, many gene modules were associated with severity and early and long-term recovery in rats. Using homologenes, we identified gene modules conserved between species highly associated with SCI severity in both species. Using the SCI severity-associated conserved gene module's expression pattern in the drug repurposing platform, we identified drug candidates able to reverse the SCI-induced transcriptomic phenotypes. Blood transcriptomic biomarkers may be used as targets for therapeutic interventions after SCI. Using evolutionarily conserved SCI-induced gene signatures significantly increases the probability of an effective preclinical model translatable to human SCI.

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Understanding the heterogeneity in regenerative ability of corticospinal neurons using patch-based single cell RNA-seq

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Abstract

Spinal cord injury (SCI) is a severe condition that results in loss of function in mobility. The corticospinal tract (CST) is a clinically important target for functional recovery after SCI. Multiple molecular pathways including the Pten/mTOR signaling pathway have been revealed to regulate axon regeneration and sprouting from the CST. However, among diverse populations of CST neurons, only a subset regenerates axons following molecular intervention and the number of regenerating neurons declines with age. Here, we have performed single cells RNA-seq using Patch-seq after retrograde tracer injection in animals with Pten/mTOR pathway modification. Through differential gene expression and pathway / network analyses, we identified known and new potential regulators of CST regeneration and found that regenerating transcriptomes differentially map to previously defined neuronal clusters based on single cell seq data. In addition, we found a universal classifier of regenerating neurons that can be applied to any single cell dataset to gauge their regenerative abilities.

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Understanding LZK-mediated reactive astrogliosis and neuronal repair using mouse molecular genetics and transcriptomic profiling

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Abstract

After spinal cord injury (SCI), astrocytes become reactive in a process called astrogliosis. These reactive astrocytes surround the lesion and form an astrocyte scar border. Our lab recently previously discovered that LZK (or MAP3K13) regulates reactive astrogliosis and scar formation after a dorsal spinal cord crush injury. The effect of LZK-mediated astrogliosis on neural repair and regeneration is still not clear. We aim to investigate this question utilizing genetically modified mice and RNA sequencing. We are testing the effect of astrocytic LZK overexpression or deletion on behavioral recovery after a contusion injury. We will also assess the effect of the same LZK manipulations on corticospinal tract (CST) axon regeneration that is induced by Pten suppression or IGF1/OPN overexpression. To identify potential downstream effectors of LZK signaling, we are setting up the RiboTag approach to examine the transcriptomic profiles of LZK-manipulated astrocytes. The relationship between the LZK and STAT3 pathways are also being examined with genetic analyses. Studying LZK-mediated astrogliosis provides an opportunity to dive deep into the mechanisms involved in astrocyte border formation and function. Together, these experiments will provide important insight into the cellular and molecular processes involved in astrogliosis and its functional consequences in SCI, which provides the basis for developing effective therapeutic interventions for spinal cord injury in the future.

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A novel adult neuron screening technology to identify new drugs for treating spinal cord injury

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Abstract

Neurodegenerative diseases and neurotraumatic injuries are age-associated disorders that can reduce neuron survival, neurite outgrowth, and synaptic plasticity leading to loss of cognitive capacity, executive function, and motor control. One such example is spinal cord injury (SCI). Most patients with SCI are over 40 which is a significant issue since the neuroregenerative capabilities are severely reduced with age. Developing therapeutics for promoting recovery has been extremely challenging, with no approved treatment improving functional locomotor recovery after SCI. We believe this lack of clinical success stems from the current high content screens (HCS) in vitro not incorporating age as a biological variable. Indeed, most HCS use cell lines, embryonic neurons, or iPSC-derived neurons, none resembling the aging neurons in patients. The chasm in maturity between the neurons used in drug screens and those in the target population is likely to be a barrier to translational success. It has been historically challenging to culture adult neurons let alone conduct screenings; therefore, age-appropriate drug screenings have previously not been plausible. We developed a new morphology-based screening system using adult cortical neurons from different species, incorporating age and sex as biological variables. Our screen identified novel compounds increasing neuroprotection and neurite outgrowth on adult neurons in vitro. Some of these positive hits were tested in a mouse model of spinal cord injury and were found to promote functional recovery.

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Opposing roles of the integrated stress response kinases PERK and HRI in oligodendrocyte survival and locomotor recovery after spinal cord injury

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Abstract

After spinal cord injury (SCI), re-establishing cellular homeostasis is critical in facilitating functional recovery. Integral to that response is the mammalian integrated stress response (ISR) pathway mediated by 4 independent kinases: PERK, GCN2, PKR and HRI. Activation of all ISR kinases phosphorylates eIF2α that results in protein synthesis inhibition and in translation of selective stress response mediator proteins such as ATF4 and CHOP. Our prior work, using genetic and pharmacological inhibition of the ISR, demonstrated improvements in hindlimb locomotion after thoracic SCI and implicated oligodendrocyte (OL) survival as a potential mechanism. Here, contusive SCI resulted in acute induction of all four ISR kinases. Global deletion of *Pkr* or *Gcn2* failed to improve locomotor recovery after SCI. Targeted loss of Perk in OL-lineage cells enhanced pro-apoptotic *Atf4* and *Chop* mRNA levels in mice acutely after SCI. Recovery of hindlimb locomotion and spared white matter (SWM) were impaired in OL-*Perk*$^{-/-}$ mice six weeks post-SCI and correlated with greater loss of epicenter OL-lineage cells. In contrast, constitutive *Hri-/-* mice demonstrated reduced Atf4 and Chop mRNA levels acutely and improvement in hindlimb locomotor recovery six weeks post-SCI that correlated with enhanced SWM and reduced loss of OL-lineage cells. Collectively, data show that the ISR kinases differentially contribute to OL loss and implicate PERK and HRI as critical pro- and anti-survival mediators after SCI, respectively. Differential effects of *Perk, Pkr, Gcn2* and *Hri* deletion on functional recovery after SCI potentially reflects fundamental differences in outcome of ISR activation in specific signaling and/or cellular contexts.

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Acute chemogenetic silencing of nociceptive signaling to promote functional recovery following spinal cord injury

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Abstract

Spinal cord injury (SCI) typically results in an immediate loss of neurological function due to massive disruption of endogenous neural circuitry. Previous work in rodent SCI pain models has established that maladaptive hyperactivity within primary nociceptors of the dorsal root ganglion (DRG) occurs as early as 24 hours after SCI, contributing to the onset of neuropathic pain. This not only results in sensory dysfunction but also undermines locomotor recovery. We hypothesized that silencing the activity of nociceptors early after injury will improve long-term functional outcomes. To test this hypothesis, we utilized inhibitory Gi-DREADDs to selectively silence nociceptors during the acute phase of SCI. We delivered AAV6-Gi-DREADD to lumbar DRG nociceptors through bilateral intrasciatic injections, then performed thoracic contusion SCI 4 weeks later. Immediately following SCI and continuing for 14 days post-injury, Gi-DREADDs were activated through oral delivery of agonist clozapine-N-oxide (CNO). We performed sensory and motor behavioral assessments weekly up to 10 weeks post SCI. Gi-DREADD expression was restricted to small-diameter nociceptors including CGRP+, substance P+, and IB4-binding neurons. Through analysis of behavioral outcomes, we observed significantly higher thermal withdrawal thresholds, and greater hindlimb locomotor recovery in subjects that received acute nociceptor silencing, compared to controls. Histological assessments of spinal cord tissue suggest a trend showing reduced lesion volume, increased neuronal sparing and increased CGRP+ axon sprouting in Gi-DREADD treated subjects. Together, these findings suggest that nociceptor silencing early after SCI may promote beneficial plasticity in the acute phase of injury that can impact long-term functional outcomes.

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Sprouting vs. Regeneration: Distinct Modes of Adult Axon Growth are Controlled by Distinct Transcriptional Regulators

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Abstract

Various modes of axon growth/plasticity in adult nervous systems have been qualitatively described, but there are few molecular mechanistic distinctions. Axonal Regeneration is the process severed/injured axons undergoing growth. Collateral Sprouting (CS) is the process of non-injured axons extending new and/or existing axon branches. Although numerous extrinsic factors can influence these processes, we seek to determine intrinsic mechanisms controlling CS and Regeneration.

Adult neurons of both the central and peripheral nervous systems exhibit robust Collateral Sprouting but Regeneration is strong only in the PNS. We therefore compare and contrast these processes using sensory neurons of the Dorsal Root Ganglia. Transcriptomic analysis of sensory neurons undergoing axonal CS or Regeneration reveals largely distinct sets of differentially-expressed genes and distinct patterns of regulation. The on-off switching of gene expression in Regeneration is essentially absent in CS, favoring altered 3’UTR structure of already-expressed genes. In vitro screening of axon outgrowth reveals CS-associated genes capable of promoting axon growth resistant to known inhibitors. In vivo sensory testing and live-imaging of skin innervation using transgenic mice reveals mode-specific effects in knockouts of ATF3 (reduces Regeneration, not CS) or Camk4 (reduces CS, not Regeneration). A novel axonally-localized isoform of Camk4 is constitutively expressed in CS-capable neurons. Camk4 expression is suppressed after axonal injury, concomitant with de-novo expression of ATF3. Accordingly, prior injury reduces CS-capacity.

This demonstrates that different modes of axonal growth likely have distinct molecular signatures and mechanisms, and that the execution of one mode may influence switching to another mode.

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Role of stress granule aggregation in axon regeneration after spinal cord injury.

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Abstract

Our knowledge about neuron-intrinsic control of axon regeneration remains incomplete. One aspect of neuronal response to injury is the cellular stress. As a response to cellular stress, such as axonal injury, cytoplasmic mRNA is sequestered into insoluble ribonucleoprotein granules, known as stress granules. These are membraneless mRNA-protein assemblies that prevent trapped mRNA species to be actively translated. It is hypothesized that chronically aggregated stress granules have a pivotal role in the development of many neurodegenerative diseases. Stress granules are believed to assemble upon non-translating mRNAs which serve as scaffolds for RNA-binding proteins such as TIA1 and G3BP1. These core components of the stress granules are required to nucleate granule formation through protein-protein interactions. Recent findings have demonstrated that such core components of RNA stress granules regulate axon regeneration in C. elegans and the mammalian peripheral nervous system (PNS). The absence or suppression of stress granule core components enhances axon regeneration. Here we show that cortical neurons exhibit stress granule aggregation in vitro upon different cellular stresses. TIA1 and G3BP1 colocalize in granular structures and are predominantly found in the neuronal soma. Spinal cord injury in mice, particularly dorsal hemisection, triggers the aggregation of G3BP1+ granules in the corticospinal projection neurons cell bodies. We are currently setting up to assess genetic loss of function for TIA1 and G3BP1 on axonal sprouting and regeneration after spinal cord injury. We will also identify differentially sequestered mRNA in granules after injury to identify underlying molecular mechanisms.

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Galantamine nanoparticles lead to functional recovery in a rat model of spinal cord injury

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Abstract

Spinal cord injury (SCI) is a condition that provokes great impact on the patient's life, and there are no effective treatments for this disorder. A previous study from the group demonstrated galantamine efficacy in promoting functional improvements in a rat model of SCI. However, systemic drug administration can cause side effects and lead to decreased drug bioavailability at the target tissue. Hence, the aim of this study was to produce and evaluate the therapeutic potential of galantamine nanoparticles for SCI. Galantamine was mixed in a 4% PLGA solution and electrosprayed to produce the nanoparticles. The material was characterized according to its morphology, size, zeta potential and polydispersity index. A contusion model of SCI in rats was used, and the pharmaceutical formulations were applied at the lesion site. The animals were divided into 5 groups: sham, injury, galantamine, PLGA particles and PLGA particles containing galantamine (PG). Animal locomotion was evaluated weekly for 6 weeks using the BBB scale. Three days and six weeks after the injury, the spinal cords were collected. Three days after the injury, the administration of galantamine resulted in a decrease in lipid peroxidation levels, whereas the use of PG improved levels of reactive oxygen species (ROS) and IL-1b, in addition to lipid peroxidation levels. Furthermore, when analyzing the treatment effects 42 days after the injury, galantamine treatment was able to reduce ROS, while PG reduced both ROS and IL-1b. Therefore, PG treatment showed not only inflammatory and oxidant improvements, but also significant functional recovery after SCI.

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Single-cell transcriptomics reveal a pro-regenerative neuron population in adult zebrafish.

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Abstract

Unlike mammals, zebrafish have the innate ability to regenerate spinal cord tissues within 6-8 weeks of injury. To achieve a comprehensive understanding of the pro-regenerative cell identities that direct SC repair, we performed single nuclear RNA sequencing at 0, 1, 3 and 6 weeks post-injury. Our analysis revealed dynamic changes in cell populations and signaling pathways across time points. By analyzing neurotransmitter gene expression, both *in silico* and *in vivo*, we characterized the excitatory and inhibitory landscape during successful SC regeneration. Analysis of neuronal subclusters identified one injury-induced population of neurons that is exclusively present at 1 week post-injury and that we refer to as iNeurons. HCR *in situ* hybridization confirmed the expression of iNeurons genes *in vivo* and high-efficiency CRISPR/Cas9 mutagenesis elucidated their importance during SC repair. Interestingly, cross-species transcriptomic comparisons identified an analogous cell population after mouse SCI, indicating conservation of iNeurons in mammals. Our study provides a comprehensive resource of cell populations that direct innate SC regeneration, cross-species comparisons of SCI responses in zebrafish and mammals, and novel therapeutic targets for neural repair.

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Acute lumbosacral spinal cord epidural stimulation improves cerebral hemodynamics during orthostatic stress in individuals with chronic spinal cord injury

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Abstract

Introduction: Orthostatic hypotension (OH), defined as a substantial drop in arterial blood pressure (BP) when assuming an upright position, commonly occurs after spinal cord injury (SCI). OH causes cerebral hypo-perfusion and is difficult to manage. We previously showed that lumbosacral spinal cord epidural stimulation (scES), optimized for cardiovascular function (CV-scES) mitigates OH in individuals with chronic SCI. This study evaluated the immediate effects of CV-scES on cerebral blood flow velocity (CBFv). Methods: Ten individuals with chronic cervical SCI and severe cardiovascular dysfunction had a 16-electrode array implanted over the lumbosacral spinal segments. Personalized stimulation parameters were identified to maintain systolic BP between 110-120 mmHg. Orthostatic tolerance was tested with a 70° head-up tilt maneuver lasting up to 30 minutes, with and without CV-scES; Participants were tilted down to supine position if OH symptoms developed before the 30th minute of 70° tilt. Beat-to-beat CBFv at the middle cerebral artery and BP at the finger were monitored simultaneously. Results: Compared to tilt without stimulation, CV-scES increased tilt time (10±3 vs. 30±0 minutes), mitigated the OH symptoms, reduced the fall in systolic BP (-45±9 vs. -4±17 mmHg), in mean BP (-28±4 vs. -2±4 mmHg) and in mean CBFv (-16±2 vs. -8±1 cm/s). Conclusions: Maintenance of BP immediately following CV-scES use during orthostatic stress is associated with improved cerebral hemodynamics and prolonged orthostatic tolerance in individuals with chronic cervical SCI.

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Single-cell RNA sequencing indicates LPS-mediated inflammation in chronic spinal cord injury induces a microglial state distinct from acute injury

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Abstract

Spinal cord injury (SCI) results in motor, autonomic and sensory deficits below the injury site. Motor training is an effective method to promote recovery following SCI, with lower training efficacy in the chronic stage. The increased training efficacy during the subacute period is associated with a transient adaptive state induced by the SCI. A potential link is SCI induced inflammation, as injection of lipopolysaccharide (LPS; a proinflammatory compound) alongside training improves recovery in chronic SCI, suggesting LPS could reopen a window of plasticity late after injury. Microglia may play a role in LPS-mediated plasticity as they are activated by LPS and implicated in facilitating recovery following nervous system injuries. However, it is unknown how microglia change in response to LPS following SCI, knowledge that could provide new treatment avenues to promote neuroplasticity. Here we used single-cell RNA sequencing to examine if microglia respond to an LPS injection by recapitulating the inflammatory response following acute SCI in rats. We show that LPS in chronic SCI induces a distinct microglial state from that following acute injury. With LPS injection, there appears to be an upregulation of genes associated with translation. As well, distinct metabolic pathways are active following acute SCI in comparison to LPS injections in the chronic state, suggesting distinct mechanisms underlying the plasticity-inducing effects. Receptor-ligand analysis identified microglial-neuronal pathways as potential mediators of this plasticity. Our results contribute to our understanding of how microglia and LPS-induced neuroinflammation contribute to plasticity and recovery following SCI.

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Targeting Matrix Metalloproteinases as Putative Modulators of Stretch-Induced Bladder Wall Remodeling and Loss of Compliance After Spinal Cord Injury

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Abstract

A reduction in bladder compliance after spinal cord injury (SCI) limits ability to store urine, with increased risk of bladder/kidney infections from repeat catheterizations. Abnormal stretch of the denervated bladder wall, leads to increased extracellular matrix production, detrusor hypertrophy and/or hyperplasia, and loss of bladder compliance. We hypothesize that matrix metalloproteinases (MMPs), well known for tissue remodeling, act directly on the bladder wall and that this gives rise to a loss of bladder compliance. Consistent with this hypothesis, we find that spinal cord injured mice, treated systemically with a general MMP inhibitor within the first 3 days(d) post-injury, show long-term improvement in voiding efficiency ($p<0.001$) coincident with a reduction in detrusor area ($p<0.05$), compared to vehicle controls. Gelatin and *in situ* zymography confirm early upregulation of MMP-2 and MMP-9 ($p<0.001$) and rtPCR shows dynamic regulation of multiple MMP family members in the bladder wall within the first week post-SCI ($p<0.001$). Mechanical testing of the bladder wall reveals increased bladder wall stiffness at 3d ($p<0.01$), followed by a decrease at 7d ($p< 0.001$), compared to shams. Similarly, there is temporal variability in viscoelasticity, as evidenced by a decrease in Tau2 ($P<0.0001$) and Tau3 ($p<0.05$) at 3d, followed by an increase in Tau2 at 7d ($p<0.05$). These collective findings suggest a novel role for MMPs after SCI through their action on bladder wall dynamics, functioning as early determinants of long-term urological recovery.

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Mechanosensitive, IB4-positive nociceptive afferent input interferes with forelimb motor function after spinal cord injury

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Abstract

Spinal cord injury (SCI) not only damages vital sensorimotor pathways, but it also creates an environment that causes maladaptive plastic changes within the cord, which further limit recovery. For example, there is evidence of increased density and distribution of nociceptive primary afferent fibers within the spinal cord after SCI. Although it has been well studied that primary afferent input emanating from body movement and/or external stimuli can drive plasticity and recovery of motor function after SCI, the role of primary nociceptive input on motor control is overlooked. This experiment investigated whether increased mechanical nociceptive input is detrimental to motor function after SCI. Female, Sprague-Dawley rats with a C5 unilateral contusion or a sham hemilaminectomy received ipsilateral C7-8 intraganglionic injections of vehicle or rIB4-conjugated saporin, to ablate mechanosensitive nociceptive inputs. Immunohistochemistry and sensory testing confirmed ablation of these fibers in rIB4-conjugated saporin treated rats. Nociceptor ablation did not elicit differences in reaching and grasping objects between uninjured, sham groups. However, ablation following SCI improved the rats' pull force and ability to grasp food pellets. This indicates that injury-altered mechanosensitive primary nociceptors relay information from peripheral receptive fields to spinal motor circuits that control specific behaviors. A follow up study to determine whether primary nociceptors interfere with strength training as a treatment for forelimb motor control is underway. Data from the initial experiment and preliminary data will be presented to demonstrate that these mechanosensitive nociceptors interfere with motor control post-SCI and with rehabilitative strength training.

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Infiltrating Macrophages and Microglial Activation Correlates with SCI-Induced Pain and Depressive-Like Behavior

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Abstract

Spinal cord injury (SCI) affects ~500,000 people worldwide every year, with the majority developing chronic neuropathic pain. Following SCI, approximately 60% of individuals are diagnosed with comorbid pain and mood disorders, while only ~21% of the general population will experience mood dysfunction within their lifetime. We hypothesize that nociceptive and depressive-like dysregulation occurs after SCI and is associated with aberrant macrophage infiltration along the pain pathway. We completed moderate unilateral C5 contusions on LysM-eGFP reporter mice to visualize infiltrating macrophages following SCI. At 6-weeks post-SCI, both male and female mice exhibit nociceptive (mechanical and thermal) and depressive-like dysfunction compared to naïve and sham mice. There were no statistically significant differences between sexes, indicating that sex is not a factor driving nociceptive or depressive-like behaviors after SCI. Utilizing an unbiased clustering method, hierarchical cluster analysis, we were able to subgroup mice based on nociceptive and depressive-like behavior scores at 6-weeks post-SCI. SCI mice displayed significantly increased macrophage infiltration (LysM+/CD68+) into the ipsilateral C7-8 DRGs and lesion epicenter when compared to naïve and sham mice. Additionally, SCI mice displayed microglial activation (IBA1+) in the lesion epicenter, anterior cingulate cortex and primary somatosensory cortex when compared to sham and naïve mice. In conclusion, SCI caused the development of pain and depression in a subset of mice and these behavioral changes can be attributed to immune system activation in the DRG and spinal cord. Future directions include investigation of macrophage activation state, cytokine signaling and altered neuronal properties along the pain pathway.