12-2022

Defining the impact of clinically modeled hindlimb stretching, exercise, & inactivity on functional recovery after spinal cord injury.

Greta M. Cesarz
University of Louisville

DEFINING THE IMPACT OF CLINICALLY MODELED HINDLIMB STRETCHING, EXERCISE, & INACTIVITY ON FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

By

Greta M. Cesarz
B.S., University of Louisville, 2015
M.S., University of Louisville, 2017
M.S., University of Louisville, 2020

A Dissertation
Submitted to the Faculty of the
School of Medicine at the University of Louisville in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy in Physiology and Biophysics

Department of Physiology
University of Louisville
Louisville, Kentucky

December 2022

DEFINING THE IMPACT OF CLINICALLY MODELED HINDLIMB STRETCHING, EXERCISE, & INACTIVITY ON FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

By

Greta M. Cesarz
B.S., University of Louisville, 2015
M.S., University of Louisville, 2017
M.S., University of Louisville, 2020

A Dissertation Approved on

November 18, 2022

by the following Dissertation Committee:

_____

Dissertation Director: Dr. David S. K. Magnuson

_____

Dr. Dale Schuschke

_____

Dr. Amanda LeBlanc

_____

Dr. David Rouffet

_____

Dr. Kathryn Harman

_____

Dr. Robert Brainard

DEDICATION

This dissertation is dedicated to every woman in science who has been told she's too much – too loud, too abrasive, too bold, too aggressive.

"Not fragile like a flower, fragile like a bomb."
-Ruth Bader Ginsberg

"They'll tell you you're too loud, that you need to wait your turn and ask the right people for permission. Do it anyway."
-Alexandria Ocasio-Cortez

ACKNOWLEDGEMENTS

I would first like to express my deepest appreciation and gratitude to my parents – Michele and Stefan Cesarz, for their unconditional support and love throughout my graduate school journey. None of this would have been possible without their unwavering dedication to my education from an early age. Thank you to my sister, Gabi, for being an incredible friend, advocate, and fellow educator. Your expertise and dedication to your students will forever be an inspiration to me.

Thank you to Blake, Zola, and Luna for emotionally supporting me and loving me through all these years of school. All the hours spent helping me study, write, and prepare for presentations are finally coming to a close.

Thank you to the Health & Sport Sciences Department at UofL. The amazing educators and mentors in this department encouraged me as a young scientist and have now welcomed me with open arms as a fellow faculty member. A big thank you to Dr. Ann Swank who encouraged me to pursue my PhD and to Dr. Katie Harman for her support in my career as an educator from day one.

To all the members of the Magnuson lab past and present: thank you from the bottom of my heart for all the laughs and good times (and food!). The bonds and friendships I have found in this lab are truly one of a kind. A special thank you to Morgan Sharp, Danni Wood, McKenna Butler, Jenna Wilkerson, and Greg

States. Your support and companionship over the last 5 years has meant the world and I'm lucky to call you all both lab mates and friends. To Alice Shum-Siu – lab mom, IHC magician, and party planner extraordinaire – thank you for always being there for us and cheering us on. I would also be remiss not to acknowledge the hard work of the many bioengineering co-ops and undergraduate volunteers I have had the privilege of mentoring. I could never thank them enough for the hundreds of hours of work they have dedicated to the studies presented in this dissertation.

A heartfelt thank you to the members of the Kentucky Spinal Cord Injury Research Center core – Christine Yarberry, Johnny Morehouse, and Darlene Burke for their assistance with surgeries, animal care, behavioral data collection, and statistical analysis. Likewise, to the staff of the Comparative Medicine Research Unit for always providing excellent care for my animals.

Finally, I would also like to extend my sincere appreciation to all the members of my dissertation committee: Dr. David Magnuson, Dr. Dale Schuschke, Dr. Katie Harman, Dr. Amanda LeBlanc, Dr. David Rouffet, and Dr. Eli Brainard. Your encouragement and scientific expertise have been invaluable over the years. Dr. Magnuson – I am forever grateful for the opportunity to be a part of your lab and its legacy. You challenged and inspired me to be a better scientist, mentor, and educator. Live long and prosper.

ABSTRACT

DEFINING THE IMPACT OF CLINICALLY MODELED HINDLIMB STRETCHING, EXERCISE, & INACTIVITY ON FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

Greta Cesarz

November 18, 2022

Spinal cord injury (SCI) is a devastating, life altering event that affects approximately 282,000 Americans. The most obvious side effect of SCI is paralysis due to damage to the spinal cord that disrupts ascending and descending pathways as well as central pattern generating circuitry. In addition to paralysis, patients suffer from other debilitating side effects including altered cardiovascular function, autonomic dysreflexia, neuropathic pain, spasticity, and contractures.

In contrast to humans, rodents display spontaneous locomotor recovery following incomplete SCI due to in-cage activity/training. Previously, our laboratory has studied the effect of lack of in-cage training by utilizing custom designed rodent wheelchairs. The immobilized SCI animals had poor locomotor function and developed muscle contractures. Additional work by our lab was done to help alleviate the contractures by using clinically-modeled hindlimb stretching. It was found that clinically modeled stretching of rats with a thoracic

SCI does not prevent contractures and surprisingly, causes a dramatic decrease in locomotor function that can persist even after stretching is stopped. Most recently, it has been discovered by our lab that stretching is dependent upon the presence of C-fibers (nociceptive afferents), as injured, stretched animals depleted of TRPV1+ C-fibers do not experience such dramatic detriments to their locomotor recovery. Increased sprouting of these nociceptive afferents occurs spontaneously after injury and has been associated with a myriad of other issues, such as autonomic dysreflexia and neuropathic pain. However, recent work has shown that nociceptive afferent sprouting can be prevented or reduced with increased activity and exercise.

These findings are significant because stretch-based physical therapy is the most common approach for treating spasticity, contractures, and combating muscle atrophy after spinal cord injury in patients. The work presented in this dissertation aims to clarify the potential mechanisms for stretch-induced locomotor dysfunction in rodent models as well as provide rationale for future clinical and translational research that will be able to determine whether stretching has a negative impact in humans post-SCI. The following experiments revealed that the additional of applied exercise to the stretching protocol does not prevent locomotor dysfunction or the sprouting of nociceptive afferents. We also discovered that stretching animals with high thoracic contusion injuries similarly causes a drastic drop in locomotion, but with some key differences in ability to recover locomotor ability. Our studies suggest that stretching is likely

maladaptive for functional locomotor recovery after SCI regardless of injury location or activity status.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

PATHOPHYSIOLOGY AND REHABILITATION STRATEGIES FOLLOWING SPINAL CORD INJURY

Epidemiology & Pathophysiology

Spinal cord injury (SCI) is a life altering event that imposes a substantial financial burden on our healthcare system, both acutely and long term. It has been estimated that the lifetime cost for a person injured at age 25 could be as high as $1.5 million (Cao, Chen, & DeVivo, 2011). In the United States alone, there are approximately 17,800 new cases of SCI per year, according to a 2020 survey conducted by the National SCI Statistical Center (NSCISC, 2020). Statistical modeling completed by the NSCISC indicates that roughly 295,000 people in the US live with SCI, with the average age of injury being 43 years old (Lasfargues, Custis, Morrone, Carswell, & Nguyen, 1995; NSCISC, 2020). Motor vehicle accidents have and continue to be the leading cause of injury, with the next most prevalent cause of injury being traumatic falls (NSCISC, 2020).

The majority of injuries occur in the cervical region of the spinal cord and the most predominantly diagnosed neurological category is incomplete tetraplegia, meaning partial motor and/or sensory loss in both the upper and lower parts of the body (Birmingham, 2020). However, injury can occur to any location within the spinal cord and can vary from complete to incomplete.

Because of the variety of locations and severity of injuries, the acute manifestations of SCI vary widely. Additionally, the disruption of the nervous system leads to chronic impairments that can impact the cardiovascular, urinary, gastrointestinal, musculoskeletal, and other systems, as well as significant metabolic disturbances.

SCI can be divided into two etiologies based on the cause of primary injury: traumatic and non-traumatic. Trauma induced injuries are typically defined as a disruptive, mechanical force to the cord due an external impact like a motor vehicle accident, fall, violence, or a sports-related injury. Non-traumatic SCI occurs when a disease, tumor, or infection causes injury to the spinal cord (Ahuja et al., 2017). The primary injury mainly induces the most damage to the central gray matter (Anjum et al., 2020; Kraus, 1996; Wolman, 1965) but can also result in damage to the vertebrae, blood vessels, and the myelin sheath depending on the cause of injury (Sekhon & Fehlings, 2001). The blood spinal cord barrier is also damaged, along with neurons, oligodendrocytes, and the vasculature of the spinal cord (Ahuja et al., 2017).

The primary injury leads to a cascade of additional events and complications often referred to as the secondary injury. Unfortunately, the results of the secondary injury are often worse than that which was caused by the primary injury. The mechanisms of secondary injury are vast and complex, including the following: vascular changes (ischemia, hemorrhage), ion imbalances (increased intracellular calcium, increased extracellular potassium, and increased sodium permeability), accumulation of neurotransmitters

(extracellular glutamate), free radical production, inflammatory cell infiltration, and edema (Ahuja et al., 2017; Alizadeh, Dyck, & Karimi-Abdolrezaee, 2019). This large inflammatory response makes the local environment very inhospitable, leading to apoptosis of many of the spared neurons. In particular, oligodendrocytes are especially susceptible to apoptotic cell death after SCI (Dumont et al., 2001). Loss of oligodendrocytes results in demyelination which likely contributes to functional deficits by interfering with action potential conduction (Almad, Sahinkaya, & McTigue, 2011; Kwon, Tetzlaff, Grauer, Beiner, & Vaccaro, 2004).

Clinical Manifestations & Secondary Complications

The clinical manifestations of SCI heavily depend on the location and severity of the lesion itself. This may include partial or complete loss of sensory, motor, and autonomic functions below the injury level. Clinically, SCIs can be classified as either complete (no sensory or motor function below the level of injury) or incomplete (partial sensory and/or motor function below the level of injury). However, there are varying grades of completeness of injury, which are most commonly clincally defined by the American Spinal Injury Association Impairment Scale (Roberts, Leonard, & Cepela, 2017). Patients with AIS Grade A scores have no motor or sensory function distal to the injury site. Patients with AIS Grade B scores have sensory function, but lack motor function below the injury. Patients with AIS Grades C & D have some motor function preserved. It is important to note that patients with AIS Grade A, while lacking all sensory and

motor function, do not necessarily have anatomically complete injuries. AIS grades are determined via standardized neurological examinations but it has been suggested that in some patients there is residual function that is not detected by such examinations. One study found that by utilizing neurophysiological testing, like electromyography, between 17-39% of patients previously categorized as “complete” injuries actually demonstrated evidence of residual function (Wahlgren, Levi, Amezcua, Thorell, & Thordstein, 2021) In addition to sensory and motor function issues, patients may also experience other chronic difficulties including cardiovascular complications, respiratory complications, metabolic disturbances, urinary and bowel dysfunction, neuropathic pain, and low bone mineral density leading to increased incidence of fractures (Sezer, Akkuş, & Uğurlu, 2015).

Most relevant to the research presented in this dissertation are the secondary complications associated with the neuromuscular system which include spasticity, muscle contractures, and loss of muscle mass (Maynard, Karunas, & Waring, 1990; Yarkony, Bass, Keenan, & Meyer, 1985). These effects on the neuromuscular system have significant impacts on the patient’s quality of life and activities of daily living like dressing and eating (Cooper, Shwedyk, Quanbury, Miller, & Hildebrand, 1993; Grover, Gellman, & Waters, 1996; L. Harvey & Crosbie, 2001). Most considerably, they can impact transfers into and out of wheelchairs, causing significant issues with independent or assisted mobility and ambulation (L. Harvey & Crosbie, 2001).

Spasticity has been defined as a velocity dependent increase in resistance to muscle stretch (Katz & Rymer, 1989). It is often characterized by hyperreflexia (overresponsive reflexes), clonus (rhythmic, involuntary muscle contractions), and muscle spasms that are often painful (Rabchevsky & Kitzman, 2011) and is estimated to affect up to 70% of individuals with SCI (Gorgey et al., 2010). It is not completely clear at this time the exact physiological mechanism of spasticity, but it appears likely that it is related to alterations in membrane permeability and receptor properties. These changes likely lead to the hyperexcitability of certain neurons, which presents as atypical, involuntary muscular contractions (Roy & Edgerton, 2012). Common treatments for spasticity include antispastic medication like baclofen (a GABA agonist), injectable botulinum toxin, physical therapy, and rarely, surgical interventions (Sezer et al., 2015).

Contractures are another common secondary complication for people with SCI that can be particularly debilitating (Fergusson, Hutton, & Drodge, 2007; L. A. Harvey, Batty, Jones, & Crosbie, 2001). A crucial component of skeletal muscle tissue is its ability to remain elastic, or the capability to return to normal length after being stretched. Certain pathophysiologies decrease this elastic nature of the muscle tissues and surrounding connective tissues like tendons and ligaments. The lack of elasticity often leads to decreases in range of motion, joint rigidness, and potential deformity and disability (L. A. Harvey, Glinsky, Katalinic, & Ben, 2011). After SCI, patients are susceptible to contractures because of the resulting paralysis and consequential immobility (Herbert, 1988). Stretching and other passive ROM therapies are the primary treatment for contractures. Splints

and serial casts are also common treatments for the prevention and reduction of contractures (L. A. Harvey et al., 2011).

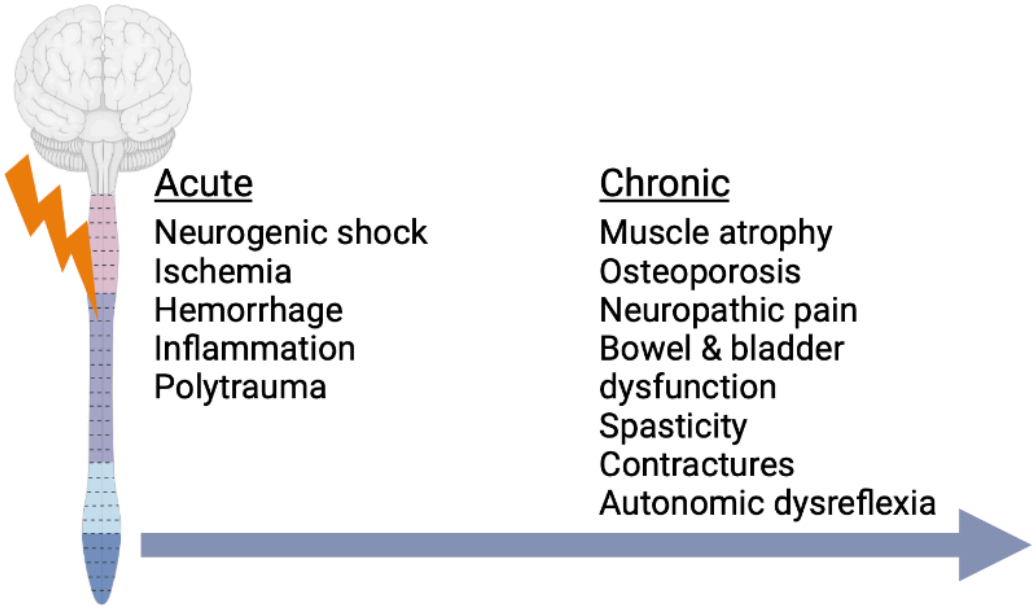


Figure 1. Timeline representing some of the physiological acute and chronic complications resulting from a spinal cord injury. Clinical manifestations are significantly affected by location and severity of injury, but secondary complications are common and often cause significant impacts to activities of daily living.

Human-Animal Model Mismatch

Unlike humans, rats spontaneously recover locomotor ability after incomplete SCI. A predominant theory in our lab is that the disparity in functional outcomes and recovery demonstrated between humans and animal studies is due to the acute increase in activity following injury in animal models. While many human patients are restricted to hospital beds due to polytrauma and secondary injuries, rats must begin to move around their cages and become relatively active within just days of injury. Our lab has developed a way to track this in-cage activity using infrared cameras and specialized kinematic software. Using this method, we can quantify distance traveled per night per animal in meters. We have found that animals with a thoracic, moderately severe injury can travel up to 200 meters per night, which is equivalent to 1600 step cycles, within 1 week of injury (preliminary data). Within 3-4 weeks, the animals have usually plateaued in functional locomotor recovery, which typically presents as weight supported stepping. While uncoordinated and very dissimilar to pre-injury locomotion, the animals still ambulate effectively around the cage. We refer to this increased activity as in-cage retraining, as it is task specific to regaining locomotor function. The Magnuson lab has termed this difference in functional recovery the Human-Animal Model Mismatch (Figure 2).

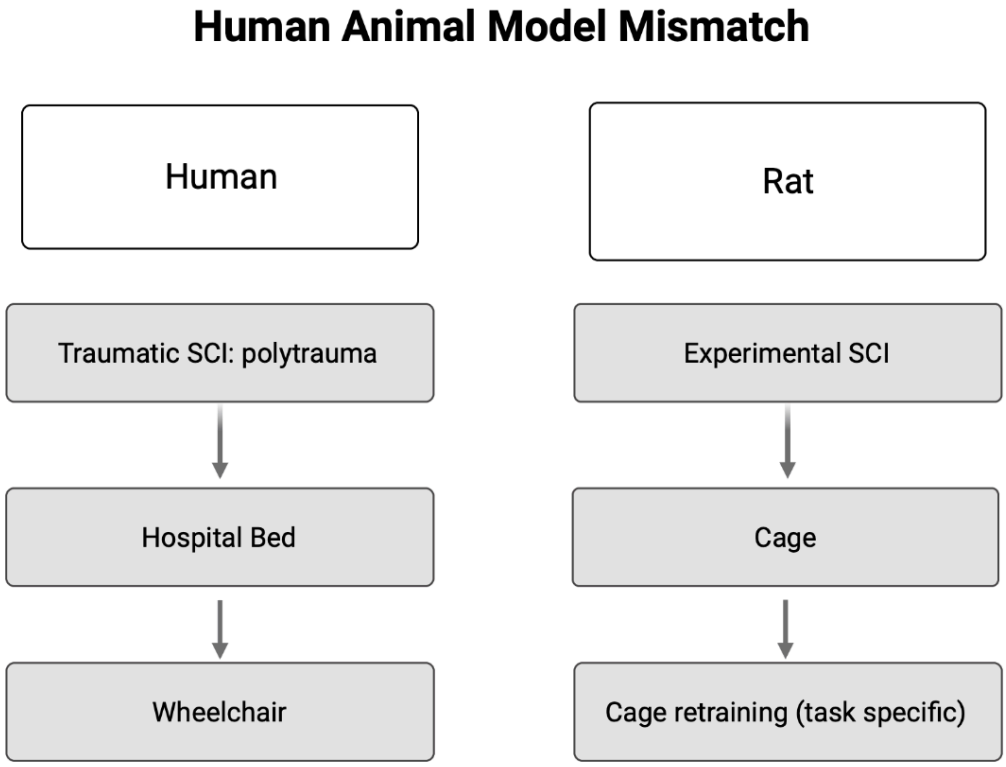


Figure 2. Chart of the timeline describing the differences between human and animal models following spinal cord injury, which our lab has termed the Human Animal Model Mismatch. Humans often experience polytrauma secondary to the SCI, which can lead to long hospital stays and immobility. In contrast, rodent models are given experimental spinal cord injuries with no polytrauma and will begin to move around the cage to seek food and water, leading to task specific cage retraining.

To test the hypothesis that functional recovery in rats is driven by increased in-cage activity, previous graduate student Krista Caudle worked with bioengineering students to develop an animal model of restricted in-cage activity using wheelchairs that would mimic the immobility of a human SCI. She utilized animals with moderate, midthoracic spinal cord injuries and restricted them to the custom-designed wheelchairs for approximately 12 hours overnight (when animals are usually the most active). She discovered that animals bound to wheelchairs had significantly less locomotor recovery than those animals not bound to wheelchairs (Caudle et al., 2011).

An unexpected finding of this study was that animals restricted to wheelchairs developed significant muscle atrophy and contractures, which are not usually seen in rodent models of experimental SCI. After discovering the high incidence of contractures in these animals, our lab developed a clinically-relevant model of hindlimb stretching for rats that is similar to the physical therapy or ROM therapy that SCI patients with contractures would receive. This protocol was developed with the assistance of Physical Therapists at Frazier Rehab Institute, an acute rehab center that provides care for a large population of spinal cord injured patients. Our now standard stretching protocol consists of 6 bilateral, hindlimb stretches held at end ROM for 1 minute and repeated twice so that each animal accumulates approximately 24 minutes of stretching each day. The protocol targets flexion and extension of the hips, knees, and ankles, as well as hip abduction and adduction. Typically, this protocol is conducted 5 days per week, which is also similar to what patients would receive in the clinic. It is

important to note that our stretching protocol was developed in conjunction with a trained physical therapist in order to closely mimic that which would be received in a clinical setting by a patient. Each stretch in the protocol was carefully designed to target the specific muscle group, not the joint or joint capsule. Animals are carefully restrained during the stretching session, so as to limit their movement and therefore, potential to cause harm to the muscles during stretching. However, it was essential that we verify that we were not causing overt muscle damage or applying unnecessary force to the animal. Hindlimb muscles were assessed for fibrosis and centralized nuclei, which are standard markers associated with muscle damage and muscle repair. No significant changes were found in stretched animals vs unstretched controls (A. Keller et al., 2017). A custom-built force sensing glove was also utilized to measure the amount of force being applied to each animal. This was used in conjunction with high resolution video recording and an implanted EMG telemetry device. Analysis indicated that the forces used were well within ranges used on human patients (normalized to rats) (A. Keller et al., 2017).

A 2015 study from our lab implemented the use of this clinically relevant stretching protocol in addition to the wheelchair restriction model to hopefully reduce the incidence of contractures in wheelchair-restricted animals. Surprisingly, it was discovered that not only did stretching not prevent or reduce the incidence of contractures, but control, injured, non-wheelchair animals that received stretching also had significantly reduced locomotor function (Caudle et al., 2015). Following these unexpected findings, another graduate student in our

lab, Anastasia Keller, began the work of investigating the mechanisms behind the phenomenon now known in our lab as the “stretch effect,” or stretch-induced locomotor deficits that we have now shown are a repeated and consistent occurrence.

It was determined by Keller et al. that the stretch effect is present regardless of whether hindlimb stretching is implemented at an acute or chronic timepoint post-injury (A. V. Keller et al., 2017). Animals that received the standard stretching protocol beginning 4 days post-injury (acute) or 10 weeks post-injury (chronic) both saw dramatic drops in locomotor recovery as measured by the Basso, Beattie, and Bresnahan (BBB) Open Field Locomotor Scale (Basso, Beattie, & Bresnahan, 1995). Additionally, Anastasia completed studies that show that dynamic ROM focused therapies, as opposed to our previous model of static stretching only, also induce significant disruption in locomotor function. This type of stretching also appears to induce a novel, clonus-like response in rats that had not been seen before (A. Keller et al., 2017). These studies demonstrated that stretching was detrimental to locomotor recovery regardless of how or when stretching was implemented, but with the absence of muscle damage, the exact mechanism for the stretch effect was still unknown. A 2019 study from our lab revealed insight into the mechanism of stretch-induced locomotor deficits by targeting a phenomenon known as nociceptor sprouting (Keller et al., 2019)

Nociceptor Sprouting following SCI

Nociceptors are afferent neurons that respond to painful or potentially damaging stimuli. Discovered by Charles Sherrington (neurophysiologist and Nobel laureate) in 1906, nociceptors have become a focal point of SCI research in recent years. Nociceptors are found throughout the periphery in any area that may need to sense noxious stimuli including the skin, muscles, joints, bladder, and visceral organs (Julius & Basbaum, 2001). Their cell bodies are found in the dorsal roots ganglia (DRG) and they form synapses in the dorsal horn of the spinal cord. There are two major classes of nociceptors: Aδ fibers and C fibers. Aδ are myelinated and typically conduct acute pain signals (Dubin & Patapoutian, 2010; Julius & Basbaum, 2001). C fibers are small diameter and unmyelinated with a slow conduction velocity. They typically conduct slow, long lasting pain signals. They are also considered polymodal as they react to a variety of stimuli including thermal, mechanical, and chemical (Julius & Basbaum, 2001; Murinson & Griffin, 2004).

Intraspinal sprouting of nociceptors occurs spontaneously after SCI (Nees et al., 2016) and has been associated with a multitude of conditions that people with SCI can experience including autonomic dysreflexia (Hou, Duale, & Rabchevsky, 2009) and neuropathic pain (Detloff, Wade, & Houlé, 2013). In relationship to the stretching effect discussed previously, a recent study from our lab revealed that the stretch effect is dependent on the presence of TRPV1+ C-fibers (Keller et al., 2019). Neonatal rats were given intraperitoneal injections of capsaicin, which is a known technique that effectively depletes C-fibers

expressing TRPV1, the capsaicin receptor. Injured animals that were depleted of TRPV1+ C-fibers had significantly higher locomotor function scores during and after stretching as assessed by the BBB Open Field Scale. Post-sacrifice, the lumbar spinal cord of each animal was assessed for C-fiber sprouting using calcitonin gene-related peptide (CGRP), a peptide primarily localized to unmyelinated, small diameter afferents. The group treated with capsaicin demonstrated successful depletion of C-fibers, as the dorsal horn was almost devoid of CGRP+ fibers. C-fiber intact, stretched, injured animals that received a vehicle injection had significantly greater CGRP+ area, when compared to both C-fiber depleted animals and unstretched, control animals. Injured animals that were not depleted of C-fibers and were stretched had the highest number of C-fibers and they also extended farther into the deeper laminae of the dorsal horn than in control animals. This suggests that stretching can increase spontaneous, aberrant sprouting of nociceptors post-SCI and that the stretching effect is likely dependent upon this aberrant sprouting.

We have hypothesized that one potential mechanism of the stretching effect is the interference of additional nociceptor sprouting with the locomotor circuitry (discussed below) present in the lumbar spinal cord. A majority of skeletal muscle innervation is composed of small diameter, presumably nociceptive, afferents, (Schmalbruch, 1986; Stacey, 1969) and nociceptive afferents are indirectly connected to motor neurons and locomotor circuitry via interneurons (Lundeberg, 1995). In addition to CGRP, Keller et al. showed that the number of c-Fos+ nuclei, a marker for novel neuronal activity, was

significantly greater in the lumbar spinal cord in injured, C-fiber intact, stretched animals compared to C-fiber depleted animals. While c-Fos was higher in the whole lumbar spinal cord, it was particularly elevated in the intermediate gray matter, the location of much of the locomotor circuitry. Based on these findings, it is possible that aberrant nociceptive afferent sprouting due to stretching is suppressing the locomotor circuitry and interfering with locomotor recovery (see Figure 3), especially when considering the work from Grau et al. demonstrating the effect of nociceptive stimulation on spinal learning and locomotor recovery (Ferguson et al., 2012; Grau et al., 2017; Grau et al., 2014). Grau and colleagues found that unpredictable, noxious stimuli applied caudal to an animal with a thoracic injury induced maladaptive plasticity in the lumbar spinal cord and which sensitizes nociceptors and interferes with learning (Ferguson, Crown, & Grau, 2006; Ferguson et al., 2012). It is highly likely that clinical stretching is activating nociceptors below the level of injury, even if the animal shows no signs of discomfort or the patient reports no sensation of pain. A 2003 study found that some physical therapists apply forces to patients with SCI that could be perceived as painful in sensate, uninjured people (L. A. Harvey, McQuade, Hawthorne, & Byak, 2003). Additionally, it is known that nociceptive neuron endings in the periphery are sensitized n individuals with SCI (Wu, Yang, Crook, O'Neil, & Walters, 2013). Therefore, even if forces applied during stretching

would not activate nociceptive afferents in a non-injured individual, it is more likely that they will be activated in a person with SCI.

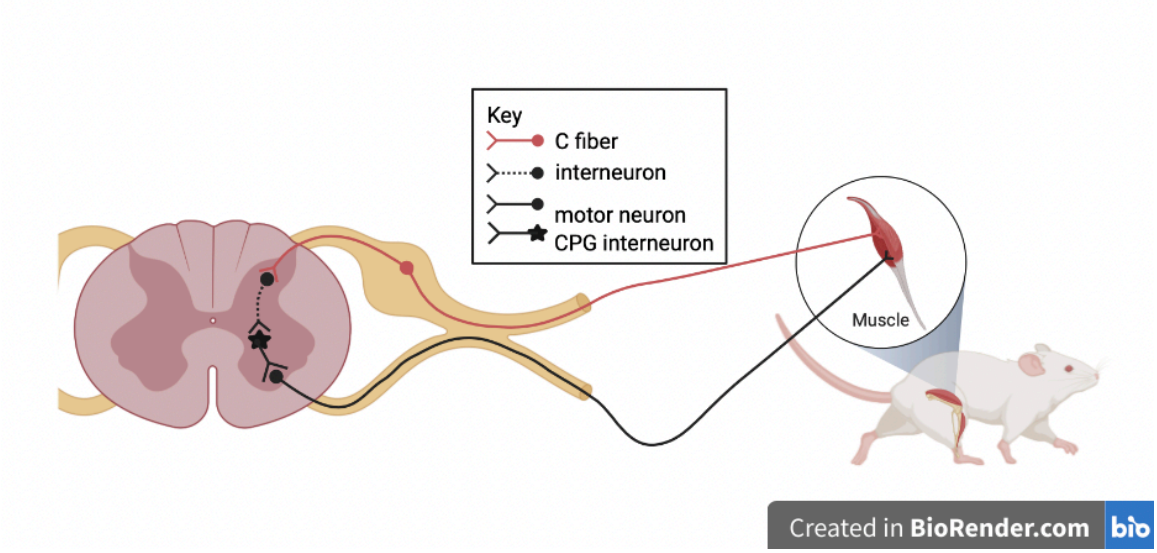


Figure 3. A proposed mechanism for the “stretch effect,” or decreased locomotor function caused by clinically-modeled stretching. We suspect that sensitized nociceptors are suppressing locomotor circuitry found in the lumbar cord known as the central pattern generator (discussed below).

Physical Therapy following SCI in Humans

A review of the top seven SCI rehabilitation centers revealed that stretching and/or range of motion therapy is one of the top three therapies for all SCI patients (Taylor-Schroeder et al., 2011). This review also reported that over 98% of patients with C5-C8 injuries received stretching therapy. It is likely that stretching therapy is so prominent amongst patients and therapists because of its usage in non-injured human populations as a way to manage joint pain, reduce risk of injury, and improve range of motion and movement efficiency (Bixler & Jones, 1992; R. Pope, Herbert, & Kirwan, 1998; R. P. Pope, Herbert, Kirwan, & Graham, 2000). Although stretching has been widely used to manage secondary complications of SCI, research has not been able to determine specific guidelines for the dosage and usage of stretch-based therapy. This is likely due to the fact that most clinical stretching studies to date have implemented a variety of interventions, have subjects with different levels of impairments, and the type and reliability of outcome measures vary greatly (Bovend'Eerdt et al., 2008).

A common technique used to manage contractures is serial casting in which the physician will apply a fiberglass cast to the limb(s) holding the joint and muscles in a slightly stretched position. Additional casts will be removed and re-applied in order to gradually stretch the muscle and improve range of motion. This type of stretching appears to be the most effective in the treatment of contractures, however much of the research has been done in patients with cerebral palsy or traumatic brain injuries. Booth et al. examined the effect of serial casting of the ankle joint in patients with head injuries and found that there

was a statistically significant increase in joint range of motion (Booth, Doyle, & Montgomery, 1983). In patients with cerebral palsy, it appears that knee-flexion contractures were significantly reduced when patients used overnight soft splints for a period of 10 months (Anderson, Snow, Dorey, & Kabo, 1988). A study including stroke, brain injury, and SCI patients found that nightly splinting of thumbs affected by contracture for 3 months showed no meaningful differences in thumb angle in any of the groups (L. Harvey, de Jong, Goehl, & Mardwedel, 2006).

Conversely, the research that has been done on stretching in SCI patients has shown little meaningful improvement. A 2000 study looked at SCI patients with both paraplegia and quadriplegia. The stretching intervention consisted of 30 minutes of passive ankle stretching 5 days per week over 4 weeks. Joint angles were measured at 2, 4, and 5 weeks and found no significant changes in ankle mobility (L. A. Harvey, Batty, Crosbie, Poulter, & Herbert, 2000). Additionally, another study conducted by the same researcher showed that stretching the hamstrings for 30 minutes 5 days per week for 4 weeks produced only a 1 degree difference in extensibility in patients with SCI (L. A. Harvey, Byak, et al., 2003). One of the only stretching studies in SCI rodents not from our laboratory investigated both duration and torque of stretching: 60 minute, 30 minute, or 30 minute intermittent (60 seconds of stretch with 30 second breaks) with either high or low torque. It was determined that high torque + long duration static stretching produced the greatest effect on range of motion, but was not enough to combat

the reduction in ROM that results from SCI (Moriyama, Tobimatsu, Ozawa, Kito, & Tanaka, 2013).

Even with the less than compelling research available, stretching remains a cornerstone treatment for physical therapists and their patients with SCI that experience both spasticity and muscle contractures (Barbosa, Glinsky, Fachin-Martins, & Harvey, 2021; L. A. Harvey et al., 2011; Patrick, Farmer, & Bromwich, 2002). It is often recommended that even those that do not actively have spasticity or contractures receive this type of therapy as a preventative measure (L. A. Harvey & Herbert, 2002). Interestingly, multiple reviews have even established that this type of therapy provides no clinically meaningful changes in joint mobility (Barbosa et al., 2021; Katalinic, Harvey, & Herbert, 2011; Khan, Amatya, Bensmail, & Yelnik, 2019), but with little human research available, the clinical protocols for stretching post-SCI remain the same.

Locomotor Control & Locomotor Training following SCI

It is now widely accepted that the lumbar spinal cord contains a complex network of interneurons that produces coordinated, rhythmic motor output, designated the lumbar central pattern generator (CPG) (Frigon, 2012; Kiehn, 2006, 2016). CPGs have been described in a variety of other bodily functions including respiration (Bellingham, 1998) and swallowing (Jean, 2001). In 1911, Thomas Graham Brown described the ability of an isolated spinal cord to produce step-like patterns without the need for descending control (Brown, 1911). Some of the most convincing evidence for the existence of a mammalian

locomotor CPG came in 1991 from Rossignol and Pearson who described fictive motor patterns in chronic, spinalized cats (Pearson & Rossignol, 1991). More recently, there has been a movement to construct a computational model of the locomotor CPG to help fill the gaps in knowledge (McCrea & Rybak, 2008). Rybak and colleagues have proposed a CPG consisting of 2 half-centers with rhythm generator and pattern formation networks. In this model, the rhythm generator regulates the pattern formation network which describes the pattern for the motor neuron activation (McCrea & Rybak, 2007; Rybak, Dougherty, & Shevtsova, 2015).

Now a focal point of SCI and locomotor recovery research, the field is slowly expanding in our understanding of the function and organization of the CPG and its contributions to locomotion after SCI. Because the majority of SCIs do not result in damage to the lumbar cord, there has been an increase in research studying different therapies and training to target the CPG in hopes of restoring locomotor function. A study by Rossignol et al. demonstrated the ability of the locomotor CPG to adapt to a lack of supraspinal input in the presence of adequate afferent input. Cats with full spinal cord transections at T13 were able to be trained to step on a treadmill and after 3-4 weeks of training, the fully transected cats were able to achieve weight supported stepping (Rossignol & Frigon, 2011) (Bélanger, Drew, Provencher, & Rossignol, 1996). These studies provide a strong rationale for the ability to train the locomotor CPG with task specific exercises in rehabilitation settings for SCI patients.

The benefits of physical activity and exercise have been well established in the SCI population, both clinically and in animal models (Devillard, Rimaud, Roche, & Calmels, 2007; Jacobs, 2009). Benefits include improved motor function, improved muscular strength, enhanced quality of life, and reduction of neuropathic pain (Chhaya, Quiros-Molina, Tamashiro-Orrego, Houle, & Detloff, 2019; Detloff, Smith, Quiros Molina, Ganzer, & Houle, 2014; Hicks et al., 2011; Hicks et al., 2003). In humans, locomotor training is typically accomplished through partial body weight supported stepping on a treadmill. Patients are placed in a harness that is suspended over the treadmill and technicians assist the patient in placing their feet correctly or possibly provide support for the patient to move through the whole ROM. In a study by Behrman and Harkema, 4 subjects with SCI (3 thoracic, 1 cervical) utilized body weight supported treadmill training and overground walking training. All 4 subjects showed improved in treadmill stepping and one subject was able to achieve overground walking when they were unable to previously (Behrman & Harkema, 2000). In another study of only a single bout of body weight supported treadmill training, 4 subjects classified as ASIA D (incomplete injury, some motor function is preserved below the level of injury), were able to increase their self-selected treadmill speed by 26% and maximum overground walking speeds by 25% (Trimble, Behrman, Flynn, Thigpen, & Thompson, 2001). Finally, in a larger study with 64 participants, body weight supported treadmill training also produced improvements in walking speed in patients with chronic, incomplete SCI (Field-Fote & Roach, 2011).

Interestingly, exercise has been shown to be a potent modulator of nociceptor sprouting in rat SCI models. This has been shown to affect the severity of both AD and neuropathic pain that is associated with increased nociceptor sprouting in rats with experimental SCI (Detloff et al., 2014; Hou et al., 2009; West, Crawford, Laher, Ramer, & Krassioukov, 2016). Some notable studies regarding timeline of application of exercise in relationship to injury revealed that exercise must begin acutely post-injury in order to show decreased intraspinal nociceptor sprouting in rats. Detloff et al. began exercise at both 14 and 28 days post-injury and found that after 5 weeks of training, allodynia (perceived pain caused by a usually non-noxious stimulus) was not ameliorated and was associated with an increased number of nociceptive afferents in the lumbar cord (Detloff et al., 2016). However, in a separate study, Detloff et al. examined the effects of beginning exercise at a very acute time point and found much different outcomes. SCI animals that began exercising at just 5 days post-injury were less likely to develop allodynia and had less sprouting of nociceptive afferents in the lumbar cord (Detloff et al., 2014). Based on this literature it appears likely that there is a critical, acute window in which the application of exercise can be a beneficial therapy, but there is likely a chronic timepoint during which exercise therapy has the potential to have adverse effects.

It is possible that input from nociceptor sprouting is interfering with locomotor circuitry in the spinal cord, causing the stretch-induced drop in locomotion seen in our previous studies. It seems likely that this locomotor circuitry is somehow suppressed or cannot respond to non-nociceptor input, but

activity and/or exercise applied acutely could provide appropriate activation of non-nociceptive afferents and reduce maladaptive sprouting. Based on previous literature (Detloff et al., 2014), it seems possible that the addition of activity and/or exercise to our current stretching paradigm could decrease the amount of aberrant nociceptive afferent sprouting, and therefore improve locomotor outcomes.

Cardiovascular Complications following SCI

Post-SCI, cardiovascular complications are common, particularly in patients with high thoracic or above injuries. Common cardiovascular complications include, but are not limited to orthostatic hypotension, autonomic dysreflexia (AD), cardiac atrophy (loss of left ventricular mass), atrial fibrillation, and atherosclerosis. Cardiovascular complications post-injury can be partially attributed to disruption of supraspinal control over preganglionic sympathetic neurons that innervate the heart (Furlan, Fehlings, Shannon, Norenberg, & Krassioukov, 2003; Teasell, Arnold, Krassioukov, & Delaney, 2000). This is demonstrated by the fact that individuals with high level thoracic lesions or above often experience the most severe cardiac dysfunction when compared to individuals with lower thoracic or lumbar injuries.

One of the most severe chronic cardiac complications seen post-injury is autonomic dysreflexia. Autonomic dysreflexia is a potentially life-threatening event that is characterized by severe hypertension and bradycardia (Cragg & Krassioukov, 2012). This typically occurs due to a stimulus that is perceived as

noxious below the level of injury, such as bladder or bowel overdistension, constrictive clothing, or temperature changes. It has been estimated that AD may regularly affect up to 90% of patients with high thoracic or cervical injuries (Elliott & Krassioukov, 2006; Lindan, Joiner, Freehafer, & Hazel, 1980). Relevant to the work in this dissertation is the evidence that AD has been associated with increased sprouting of nociceptors, specifically unmyelinated afferents, within the lumbar spinal cord (Hou et al., 2009).

It is important to note that to date, all experiments involving stretching post-SCI have been completed in animals with a T10 contusion model of injury. This is because this particular model of injury has been the standard for understanding hindlimb function and locomotor recovery since its inception (Basso, Beattie, & Bresnahan, 1996; Basso, Beattie, Bresnahan, et al., 1996). However, mid thoracic contusions do not represent a majority of clinical injuries. They also do not represent the loss of cardiovascular control and resulting issues that many patients with higher level injuries face. Recently, the Magnuson lab and other groups have begun to utilize the high thoracic contusion model which is ideal for studying the autonomic circuitry and sympathetic preganglionic neurons of this area (Harman et al., 2018; Squair et al., 2018; Squair et al., 2017). It is possible that part of the reason we see such detrimental drops in locomotion in our previous stretching studies is due to the usage of the T10 contusion model because the injury epicenter is closer to the locomotor circuitry and CPG located in the lumbar spinal cord. Utilizing a clinically relevant T2 contusion injury and creating greater distance between the injury epicenter and

locomotor circuitry may alter the post-injury sprouting that appears to be a prerequisite to the stretching phenomenon. Nearly all patients with varying levels of SCI receive some form of stretching or range of motion therapy (Taylor-Schroeder et al., 2011), so it is important that a variety of animal models be used to establish clinical relevance.

Additionally, utilizing a T2 contusion model of injury will allow us to explore the cardiovascular outcomes that could be associated with increased nociceptor sprouting. Control of the cardiovascular system via the autonomic nervous system in an uninjured individual depends upon a multifaceted, complex set of interactions between descending supraspinal fibers and sympathetic neurons. Following high thoracic SCI, there is a loss of supraspinal input and afferent stimulation can trigger reflexes leading to preganglionic sympathetic activation from neurons below the lesion. This leads to widespread vasoconstriction and hypertension, causing reflex bradycardia and autonomic dysreflexia, which as mentioned earlier has been associated with increased nociceptor sprouting (Cragg & Krassioukov, 2012; West, Squair, et al., 2016) (Hou et al., 2009).

Dissertation Overview

Specific Aims

Aim 1: Determine if increasing or decreasing spontaneous in-cage activity has a modulatory effect on nociceptive afferent sprouting and locomotor recovery in stretched rats with a T10 contusion. We utilized our clinically-modeled stretching protocol in conjunction with gain or loss of in cage activity in

order to determine the effect that spontaneous activity has on locomotor function recovery. Animals in this study received a T10 moderately severe contusive SCI. All animals were then placed in specially designed cages – either large or tiny cage with a dropped ceiling. After allowing time for function recovery to plateau, we utilized our standard stretching protocol for 3 weeks. Locomotor assessments like the Basso, Beattie, and Bresnahan (BBB) locomotor scale and overground walking kinematics were assessed biweekly. We also assessed sensory function biweekly, as well as determined CGRP+ fiber sprouting in the lumbar spinal cord post-sacrifice. We hypothesized that animals in the large cage paradigm will be less hypersensitive, have better locomotor function, and have less afferent fiber sprouting area in the lumbar cord.

Aim 2: Determine if increasing activity via applied exercise has a positive modulatory effect on nociceptive afferent sprouting and locomotor recovery in stretched rats with a T10 contusion. We utilized our clinically-modeled stretching protocol in conjunction with shallow water stepping in order to determine the effect that exercise has on locomotor function recovery. Animals in this study also received a T10 moderately severe contusive SCI. Animals were housed in large cages in order to maximize activity throughout the experiment. Beginning at 5 days post injury (dpi), we began a shallow water walking exercise paradigm. Shallow water walking was utilized because it is task specific to locomotor recovery, as well as clinically relevant to current rehabilitation strategies. After animals reached a functional plateau, we began the same standard stretching protocol for 3 weeks while continuing with daily shallow water

walking exercise. Locomotor assessments like the BBB locomotor scale and overground walking kinematics were assessed biweekly. We also assessed sensory function biweekly, as well as determined CGRP+ fiber sprouting in the lumbar spinal cord post-sacrifice. We hypothesized that exercised, stretched animals will be less hypersensitive, have better locomotor function, and have less afferent fiber sprouting area in the lumbar cord when compared to non-exercised animals.

Aim 3: Investigate whether locomotor recovery and cardiovascular function are affected in stretched rats with a more clinically relevant, high thoracic contusion injury model. We utilized a moderately-severe T2 contusion to explore the relationship between CGRP+ fiber sprouting, cardiovascular dysfunction, distance from epicenter, and stretch-induced locomotor deficits. After receiving a T2 contusion, animals were placed in standard cages and recovered until reaching a functional plateau. We then began our standard stretching protocol on half of the animals. Locomotor assessments like the BBB locomotor scale and overground walking kinematics were assessed biweekly. We also utilized echocardiography throughout the experiment to track structural changes to the heart and major blood vessels. We hypothesized that stretched animals will have lower locomotor scores, higher CGRP+ fiber sprouting area, and possibly reduced cardiovascular outputs.

Summary

The proposed studies will provide additional insight into the complex relationship between stretching and the recovery of cardiovascular and locomotor function post-SCI. Understanding the impact of injury location as well as the effect of spontaneous activity and applied exercise will provide additional contributions to our current understanding of the relationship between CGRP+ fiber sprouting and stretch-induced locomotor deficits. The goal of this work is to provide rationale and novel framework for future translational and clinical research to determine whether stretching has a negative locomotor impact after SCI in humans.

CHAPTER II

SPONTANEOUS ACTIVITY & APPLIED EXERCISE AS MODULATORS OF STRETCH-INDUCED FUNCTIONAL DEFICITS AFTER SCI IN RATS AFTER INCOMPLETE T10 SCI IN RATS

Disclaimer: Due to COVID-19 related limitations enacted by the University of Louisville, including room density restrictions and personnel reductions, Aims 1 & 2 were combined into one experimental design.

Introduction

Spasticity, contractures, and muscle atrophy are common secondary complications of severe, incomplete spinal cord injury (SCI) (Maynard et al., 1990; Yarkony et al., 1985). Stretching has become the most widely accepted therapy modality for combatting spasticity and contractures in SCI patients (L. A. Harvey et al., 2011). Healthcare providers also use stretching to attempt to increase or maintain range of motion, however, the justification for stretching post-SCI is mainly based on studies involving spinally intact rats as well as clinical studies that are largely inconclusive (L. A. Harvey et al., 2000; L. A. Harvey, Byak, et al., 2003; Williams, 1990). After noticing increasing incidence of contractures following implementation of a hindlimb immobilization protocol in post-SCI rats, our lab developed a stretching protocol designed to mimic clinical therapy. Multiple studies

from our lab have determined that daily clinically-modeled stretching of rats with moderately severe T10 contusion SCIs does not decrease the incidence of contractures. Surprisingly, daily stretching resulted in deficits in locomotor recovery when compared to unstretched SCI control animals (Caudle et al., 2015). Further examination of this phenomenon determined that stretch-induced locomotor deficits occur at both acute and chronic timepoints, as well as during static or dynamic stretching protocols (A. Keller et al., 2017; A. V. Keller et al., 2017).

More recently, it was determined by our laboratory that the stretching effect we see is dependent upon the presence of TRPV1+ unmyelinated afferents (Keller et al., 2019). SCI animals that were depleted of these afferents using neonatal capsaicin injections had significantly higher locomotor function scores during and after stretching as well as decreased afferent sprouting in the dorsal horn of the lumbar cord when compared to SCI stretched animals that did not received capsaicin injections. Intraspinal sprouting of nociceptors occurs spontaneously after SCI and has been associated with other SCI related conditions including autonomic dysreflexia (AD) (Hou et al., 2009) and neuropathic pain (Detloff et al., 2013). However, increased sprouting of nociceptors was associated with higher levels of neuropathic pain and nociception-elicited AD. Therefore, we believe it is possible that input from nociceptor sprouting is interfering with locomotor circuitry in the spinal cord, causing the stretch-induced drop in locomotion seen in our previous studies.

Modulation of nociceptor sprouting via exercise has been shown to affect the severity of both autonomic dysreflexia and neuropathic pain in rats with experimental SCI (Detloff et al., 2014; Hou et al., 2009; West, Crawford, et al., 2016). Therefore, the goal of the current study was to assess whether limiting activity or increasing activity acutely after injury would modulate nociceptor sprouting in the lumbar cord, and subsequently increase locomotor function in animals receiving daily clincally-modeled stretching.

Materials and Methods

Ethical Approval

All animal care and experimental procedures were approved by the University of Louisville Institutional Animal Care and Use Committee and performed in accordance with their surgical and animal care guidelines.

Spinal Cord Injuries and Study Design

Twenty four, adult, female Sprague-Dawley rats (200-250g; Harlan Laboratories, Indianapolis, IN, USA) were included in this study. Upon arrival, all rats underwent a two week period of acclimation, daily handling/gentling, and baseline assessments. After acclimation, animals were given spinal cord injuries by first anesthetizing with a Ketamine (50 mg/kg)/Xylazine (0.024 mg/kg)/Acepromazine (0.005 mg/kg) cocktail given intraperitoneally. Animals were shaved and cleaned with surgical scrub before receiving a midline incision through the skin and musculature from the T7-T12 spinal segments. A single level laminectomy was performed at the T9 vertebral level before positioning, stabilizing, and immobilizing the animal under the NYU Impactor (MASCIS

Impactor Rutgers University, NJ). The NYU Impactor then delivered a 25 g/cm (considered moderately-severe) weight drop contusion injury. Following delivery of the injury, the underlying muscles and skin were sutured, and antibiotic ointment was applied to the incision site. Each rat received meloxicam (5 mg/kg), gentamicin sulfate (20 mg/kg), Lactated Ringer's solution (5mL), and antisedan (1 mg/kg) post-operatively. Animals recovered and were monitored for 1-2 hours on heating pads until awake and alert. Post-operative care consisted of daily subcutaneous injections of gentamicin sulfate for seven days (20 mg/kg), twice daily subcutaneous injections of meloxicam for three days (5 mg/kg) and as needed for pain management, and twice daily 3-5ml subcutaneous injections of Lactated Ringer's solution for three days and as needed for hydration. Manual bladder expression was performed four times per day until spontaneous voiding ability returned.

For one night following injury, animals were single housed to allow for sufficient monitoring and ensure sutures remained intact. Within 48 hours of injury all animals were switched to double-housing environments and placed in their home cages. Animals were randomly assigned to one of three groups prior to injury: tiny cage with dropped ceiling (TCDC, n=8), large cage (LC, n=8), or large cage plus exercise (LC + Ex, n=8). Most assessments were completed on a biweekly basis, with an emphasis on pre-stretching, during stretching, and recovery timepoints. Figure 4 illustrates a timeline of the primary variables and assessments.

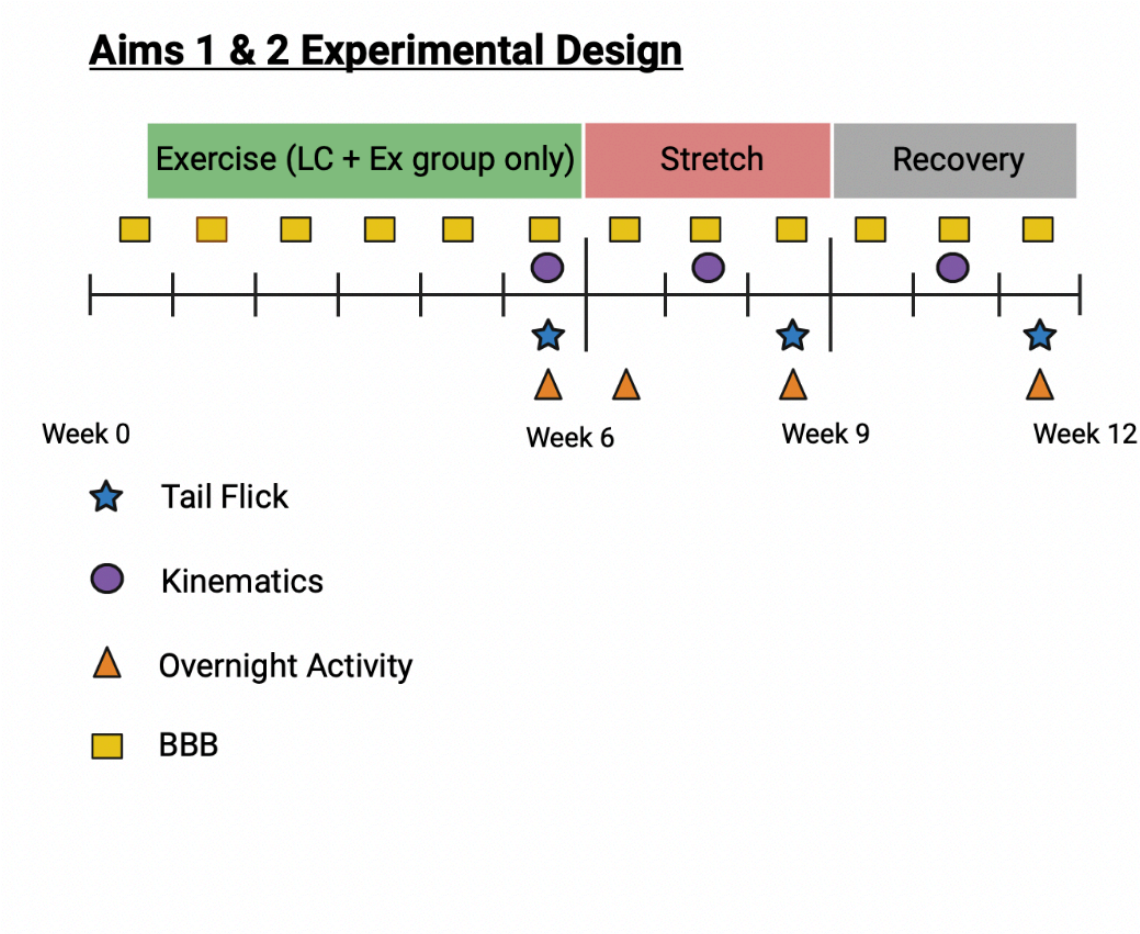


Figure 4. Representative timeline of primary variables and assessments.

Housing Conditions

Animals were placed into their respective housing conditions within 48 hours post injury. Animals were all double housed in custom designed cages, either tiny cages with dropped ceiling (7.5” x 8.5” x 4.5”) or large cages (14” x 18” x 8”). A representative image of the cage sizes is shown below. Previous work from our lab has shown that animals placed in tiny cages travel significantly less distance per night than animals in standard or large cages, effectively limiting their spontaneous activity. Conversely, animals in large cages have significantly higher in cage activity when compared to standard rat cages (preliminary data, Dr. Kathryn Deveau). After noticing that animals in smaller cages spend more time vertically exploring in their cages, specifically leading to more hindlimb support time, we added an adaptor to the tiny cages that created a dropped ceiling to discourage vertical exploration. Figure 5 shows a representative image of the cage sizes.

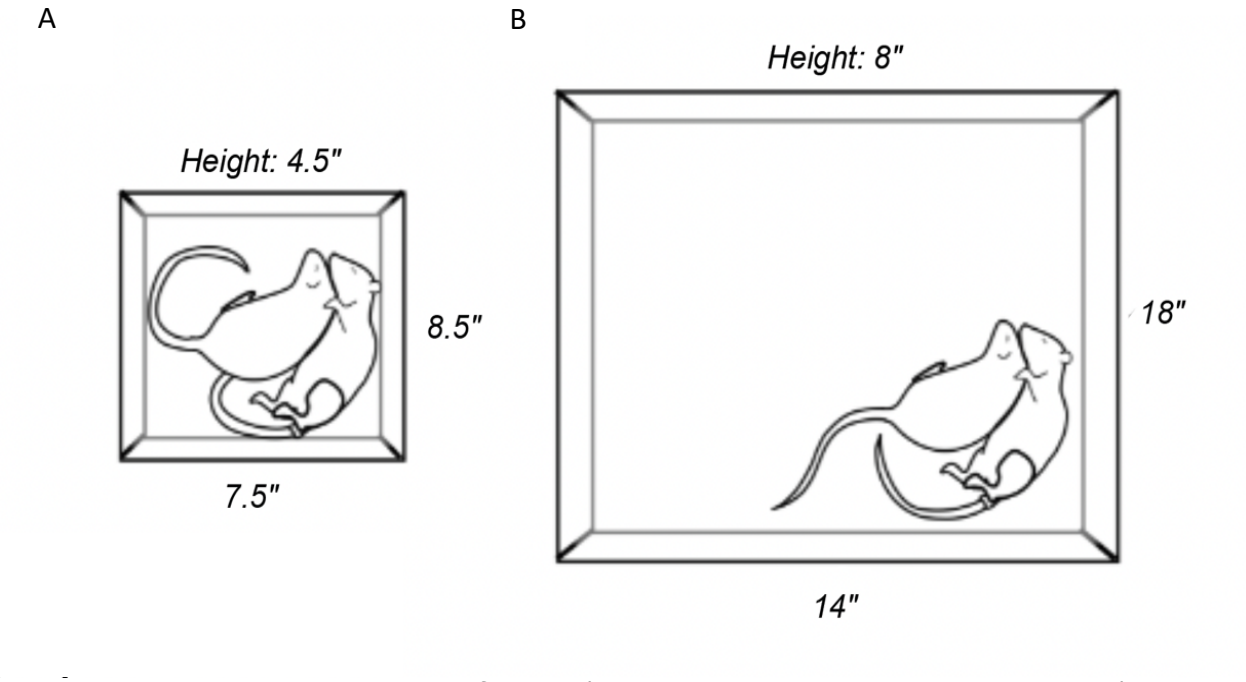


Figure 5. Representative image of both A) tiny cage with dropped ceiling and B) large cage with animal depictions shown for scale. Adapted from Kathryn Deveau (2016).

Locomotor Assessments

Animals were assessed for overground locomotor function using the Basso, Beatie, and Bresnahan (BBB) Open Field Locomotor Scale (Basso et al., 1995). This assessment consists of a minimum of two trained examiners observing each animal for 4 minutes in an open field (plastic wading pool or similar). Rats are encouraged to continuously move about the testing site by the examiners. The scoring scale ranges from 0-21, with a score of 0 representing no observable hindlimb movements and a score of 21 representing consistent coordination, plantar placement, parallel paw placement, and trunk stability that would be consistent with an uninjured, healthy animal. Occasional weight supported stepping begins at a score of 10. Testing was conducted once pre-injury, and then weekly for the first 5 weeks post-injury until animals reached a functional plateau. BBB scores were assessed 3 times per week during weeks of stretching - Monday morning prior to stretching, Monday afternoon after one stretching session, and Friday afternoon after 5 cumulative days of stretching, as described previously (Caudle et al., 2015).

Additionally, digital video recordings were made of each rat walking in the training tank from the ventral viewpoint using a camera (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) mounted below the plexiglass tank. Animals were encouraged to move from one end of the tank to the other and only high quality passes with no speed changes or exploratory behavior were included. These high speed (60 Hz) videos consist of six passes for each animal and were analyzed using the kinematic software MaxTraq and MaxMate (Innovation

Systems Inc., Columbiaville, MI) to identify plantar and dorsal hindlimb steps, forelimb steps, and foot placement and timing for coordination. The primary outcome measures determined from this analysis were Central Pattern Index (CPI), which is calculated as the number of correctly patterned steps (plantar and dorsal) divided by the total number of steps, Regularity Index (RI), which is calculated as the number of correctly patterned plantar steps divided by the total number of steps (plantar and dorsal) and Plantar Stepping Index (PSI), which is calculated as the number of plantar steps over the total number of steps. These indices were determined after analysis with MaxTraq was completed using custom designed Excel macros.

Stretching Protocol

Our standard stretching protocol (Caudle et al., 2015) was implemented beginning 5.5 weeks post-injury. This timeline allowed for the subjects to platueau in their locomotor recovery which can happen as early as 3 weeks, but typically occurs around weeks 4-5 post-injury. This also allowed the LC + Ex group to receive sufficient amounts of exercise treatment prior to beginning stretching. Animals were gently wrapped in small towels, leaving both hindlimbs exposed, and all stretches were performed while the animal was supine. Six different handlers were trained in hindlimb stretching and animals were rotated so that no animal was handled by an individual more than twice in a week. The stretching protocol consists of 6 different stretches that target the major hindlimb muscle groups – ankle, knee, and hip flexors/extensors, and hip adbuctors/adductors. Each of the 6 stretches are performed bilaterally and held at the end range of motion for 1

minute. This protocol is then repeated so that two 12 min sessions are completed, totalling approximately 24 minutes of stretching daily. All animal handlers were trained to recognize signs of distress, pain , and injury in the animals and were instructed to stop stretching if any of these behaviors were observed. Animals were rewarded with cereal before, during, and after stretching sessions. See Figure 6 for a detailed, kinematic analysis of the 6 stretches that were performed. Animals were stretched 5 days a week for 3 weeks and then allowed to recover with no stretching for 3 weeks prior to sacrifice.

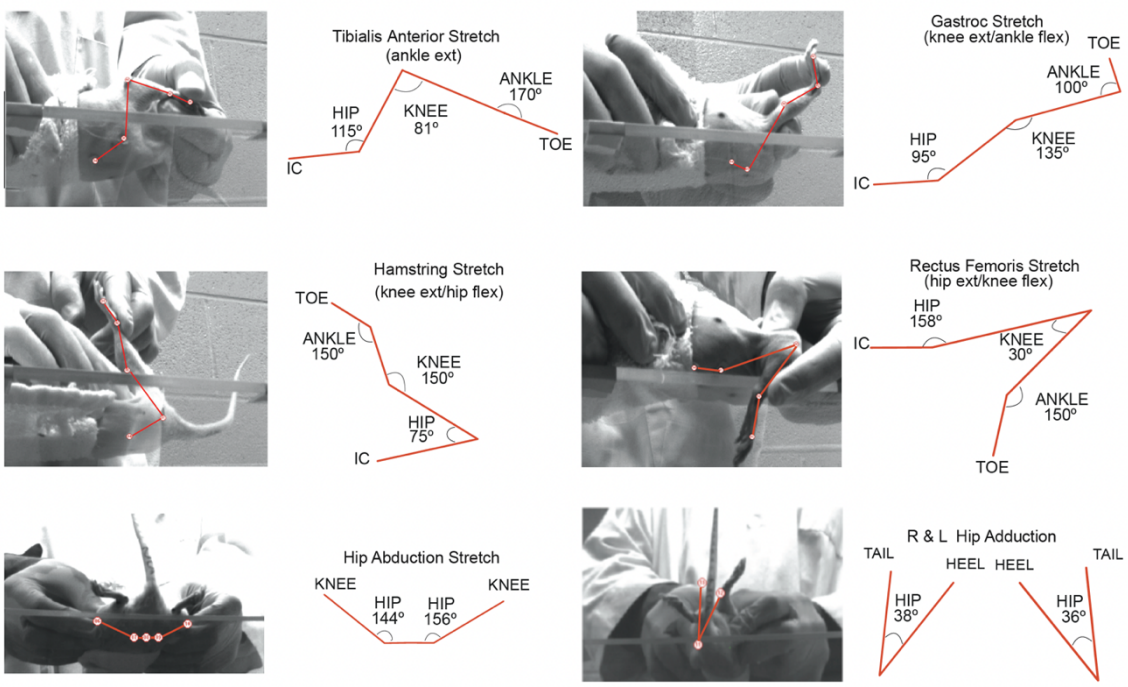


Figure 6. Kinematic analysis of the 6 hindlimb stretches included in the standard stretching protocol. Adapted from Krista Caudle (2012).

Exercise paradigm

The exercise paradigm chosen for this experiment was shallow-water walking, an exercise modality developed to mimic typical rehabilitation tools like body weight supported treadmill training. Two animals at a time were placed in a 60” L x 6.5” W x 18” H clear Plexiglass pool sealed with specialized Plexiglass adhesive and a rubber stopper in the bottom to allow for draining dirty water. Small amounts of water were added to the tank prior to training and the animals were encouraged by the handlers to continuously walk from end to end. See Figure 7 for an image of the exercise tank and an image showing typical animal walking in the tank. The water in the exercise tank was maintained near 35-37°C as normal rat body temperature falls within this range. This temperature avoided any issues with drastic drops or increases in temperature and provided less of a distraction for the animals. Previous studies from our lab have determined that 225g, adult, female Sprague Dawley rats placed in 5 cm deep water displace approximately 130ml of water, which is equivalent to 50-60% body weight support due to buoyancy. Each pair of animals exercised for 6 five minute bouts per day, totaling 30 minutes of exercise. Rest periods between bouts were typically around 6-7 minutes. Animals were consistently encouraged by the handlers to complete as many laps as possible during each bout.

All animals were acclimated to the exercise tank prior to injury. Exercise sessions began 5 days post-injury and were completed 5 days per week for 8 weeks. On average, animals walked approximately 300 ft per day during exercise sessions. The water depth was adjusted on a week by week basis to optimize the

amount and quality of plantar stepping while maintaing a challenging training stimulus.

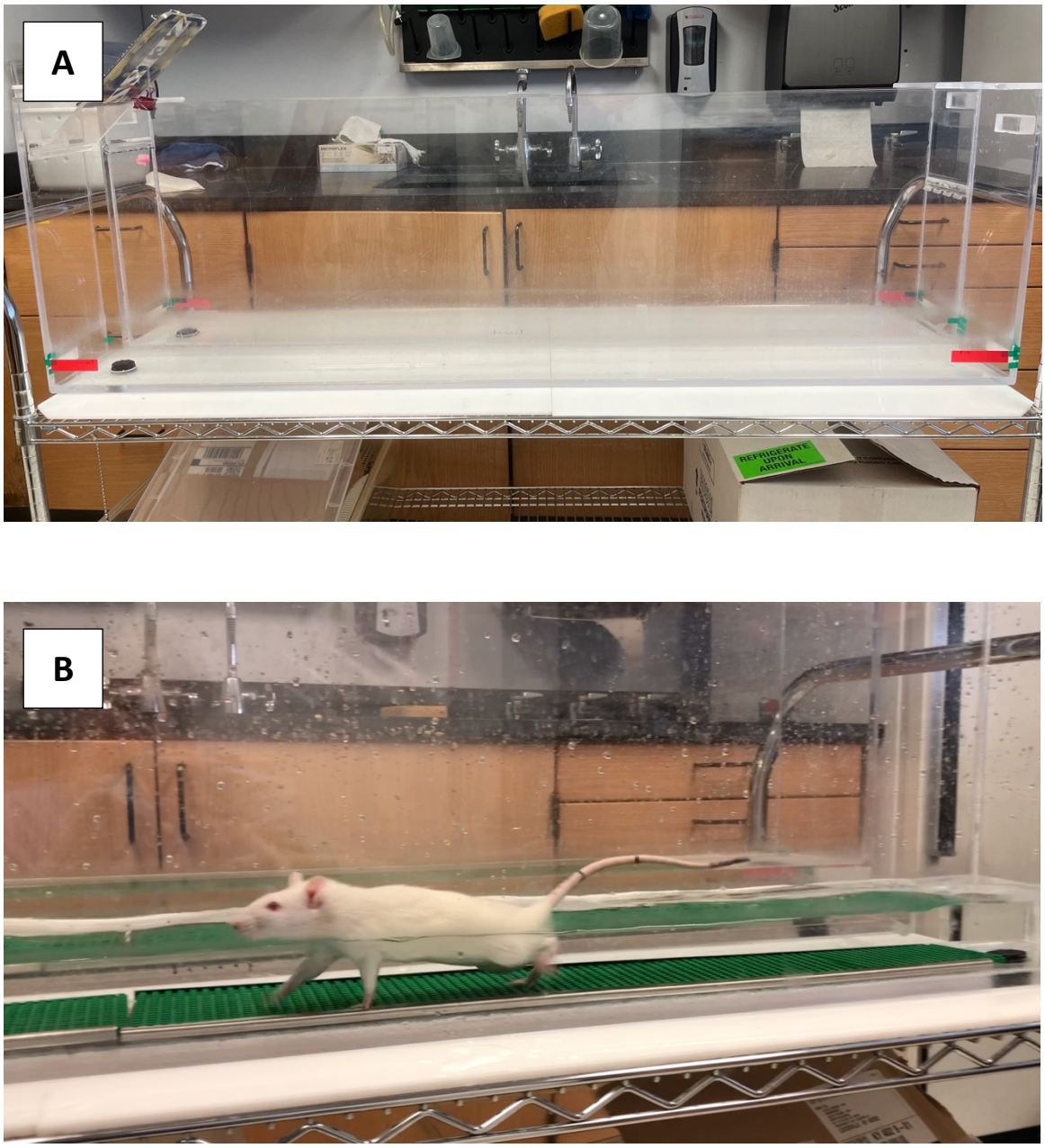


Figure 7. Images of A) shallow water walking tank set-up and B) an animal with a BBB score 8 (unable to bodyweight support step) walking in the tank.

Nocturnal In-Cage Activity

Overnight in-cage activity was recorded the week prior to stretching, during the first week of stretching, during the third week of stretching, and prior to sacrifice using overhead cameras (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) and infrared lights. Animals' lower backs were shaved and a 1 inch diameter tracking dot was placed between the iliac crests using a black marker. Cameras were positioned so that the entire bottom of the cage was recorded to include all animal movements. Animals were recorded for 12 hours per night (6pm-6am) using a custom software designed to acquire high resolution video at 4Hz for 1 minute every 10 minutes. All videos were analyzed using MaxTraq software and a custom designed Excel program determined distance travelled per animal each night. Small movements equivalent to 2 cm per or less were filtered out, thus leaving out most behavior consistent with sleeping or grooming. Figure 8 shows an example of the overnight tracking set-up after being analyzed with MaxTraq.

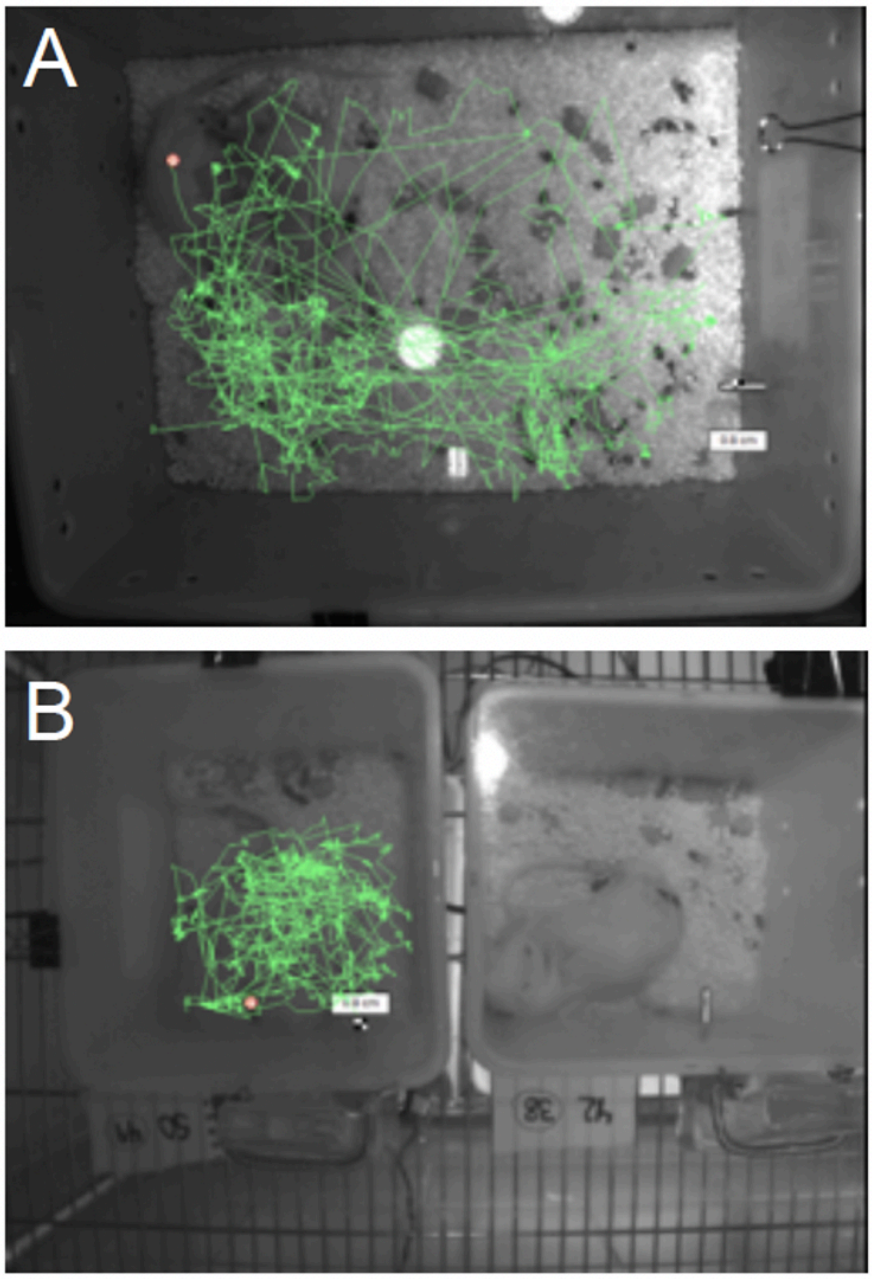


Figure 8. Images of both A) large and B) tiny cage overnight recording after analysis. The green tracking lines show how each animal is followed throughout the night to determine total distance traveled. Adapted from Kathryn Deveau (2016).

Tail Flick

In order to determine thermal sensitivity a tail flick assay was utilized. Animals were gently restrained in a towel and placed on a radiant heat tail flick device (Columbus Instruments, Columbus, Ohio, USA). The base of the tail was placed directly above the heat source sensing groove on top of the instrument. Once placed on the device, the timer automatically starts and the timer stops when the animal flicks its tail away. In uninjured animals, this application of heat to the tail will result in discomfort at some point and the recorded latency reponse is a reliable measure of pain threshold. In an animal with a spinal cord injury, the perception of pain is disrupted, but because the tail withdrawal reflex is a spinal reflex, the latency response to the stimulus is still a valid measure of thermal sensitivity.

Three trials per animal were recorded during each assessment. A latency response was recorded for each trial with a maximum duration of 10 seconds to avoid tissue damage. Tail flick assessments were performed once the week before stretching began, once during the third week of stretching, and once before sacrifice during the third week of recovery.

Magnetically Evoked Potentials from Tail Stimulation

Gastrocnemius motorneuron pool excitability was assessed via EMG responses to tail stimulation pre-stretching, during the third week of stretching, and after the 3 week recovery period. As described previously, animals were restrained to a pine board with a clock stockinette. A MagStim 200 (MagStim Ltd., Whitland, UK) machine was used to stimulate the base of the tail using a 25mm figure-8

magnetic coil at 80% of maximum intensity. This stimulus is not enough to induce direct muscle or motor neuron activation, but provides sufficient stimulus to induce a plateau response. Responses from the gastrocnemius muscle were recorded from both left and right sides using a 26 gauge needle electrode. All EMG responses were analyzed for onset latency and peak amplitudes.

Euthanasia and Tissue Histology

All animals were sacrificed utilizing an overdose of ketamine (50mg/kg)/xylazine (0.024 mg/kg)/acepromazine (0.005 mg/kg) cocktail and transcardially perfused with phosphate buffer followed by 4% paraformaldehyde (PFA). The right side hindlimb muscles (medial and lateral gastrocnemius, tibilias anterior, and soleus) of each animal were carefully dissected out and weighed.

Spinal cords were dissected and post-fixed in 4% PFA for 6 hours before being transferred to 30% sucrose solution for storage. The lesion site was confirmed by carefully examining the spinal column and verified by counting spinal cord dorsal roots. An 8 mm long section containing the injury epicenter was cut and placed in freezing medium. 50 μm transverse sections were cryosectioned and stained for spared white matter using eriochrome cyanine. After imaging, cross-sectional area of white matter was traced and quantified using specialized ImageJ software.

CGRP Immunohistochemistry and Analysis

The lumbar spinal cord was dissected out, post-fixed in 4% PFA, and cryopreserved in 30% sucrose. Levels L1-L5 were transverse cryosectioned at 20

μm for immunohistochemical analysis of CGRP. After sectioning, slides were stored at 4°C . To begin staining, slides were first warmed at 37°C for 30 minutes before removing the excess blocking media and applying a hyrdrophobic PAP pen border. Next, slides were rinsed multiple times and blocked for 1 hour. Slides were then incubated with CGRP primary antibody (rabbit polyclonal anti-CGRP, 1:1000, batch #3611083, Calbiochem) overnight in 4°C, before being rinsed and incubated with secondary antibody (Alexa Fluor 594-conjugated Donkey anti rabbit, 1:200, Lot #158327, Jackson ImmunoResearch Laboratories) for 1 hour. The sections were then rinsed and coverslipped with fluoromount.

Sections of interest were imaged using a Nikon Eclipse Ti2 microscope at 10x objective. After imaging, CGRP+ area was quantified within the dorsal horn region using a specially designed ImageJ program as described previously (States, Keller, Shum-Siu, Petruska, & Magnuson, 2022). Thresholds were chosen based on control images and then utilized to quantify total CGRP+ area of each section.

Statistical Analysis

Data was analyzed using SPSS (IBM SPSS Statistics for Windows, Versions 26/27, IBM, Armonk, NY). All data are presented as group means +/- standard deviation. Differences between groups or across time were considered statistically significant when $p \leq 0.05$.

Results

Locomotor Function

The locomotor open field assessment (BBB) showed a drastic drop in locomotion for all groups at one week post-SCI. However, by weeks 4 and 5 all groups had reached a plateau prior to beginning the stretching protocol. At the onset of stretching in week 6, all groups showed a drop in locomotor function which was expected based on previous work. Surprisingly, animals receiving acute exercise therapy (LC + Ex) did not show statistically significant locomotor improvement in the first 5 weeks post-injury when compared to the tiny cage (TCDC) and large cage only (LC) groups. Beginning at week 9, BBB scores began to improve as the stretching protocol was no longer being implemented. By week 11, average BBB scores were similar to the plateau seen prior to stretching as animals experienced locomotor recovery. Repeated Measures ANOVA did not show any significant differences between groups at any point during the study.

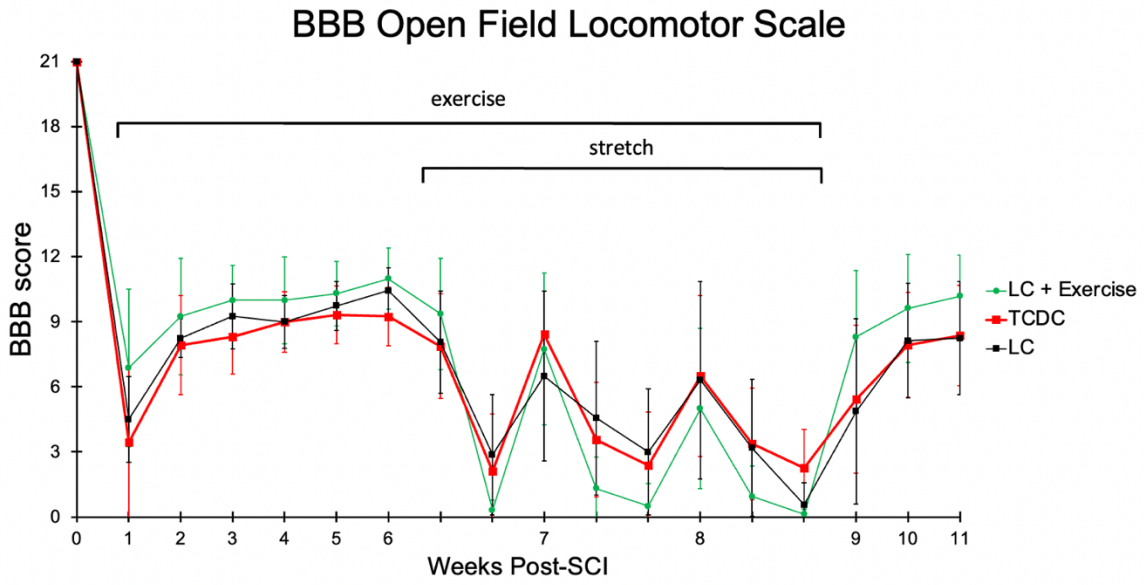
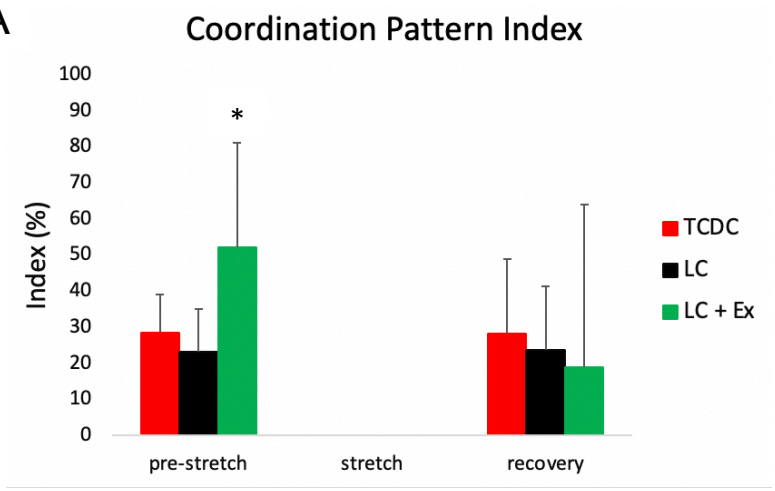


Figure 9. Locomotor function scores of post-injury animals in response to modulation of cage size, exercise, and stretching therapy. No significant differences were observed between groups at any point in the study.

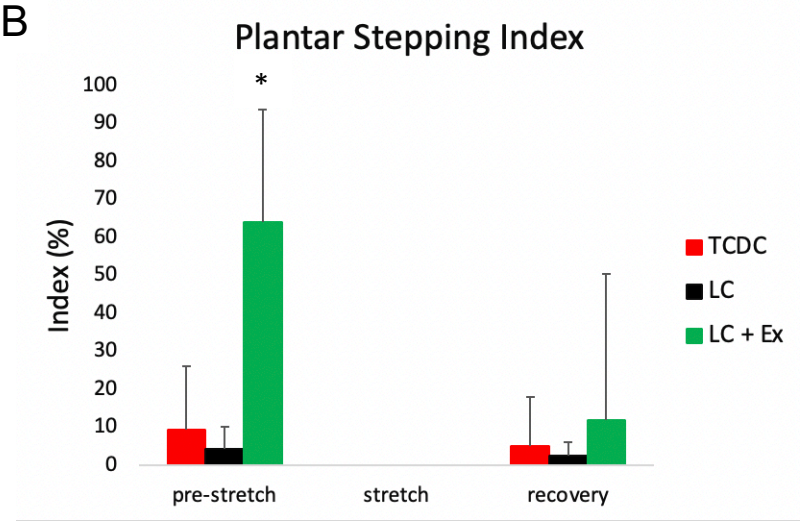
Kinematics and gait analysis of locomotor function

After kinematic videos were recorded and processed, the data points were analyzed for the gait measures previously described (CPI, PSI, RI). Repeated Measures ANOVA revealed significant differences across time for all groups and significant differences between groups at pre-stretching time points ($p < 0.05$). During stretching weeks, all animals in all groups were unable to achieve any type of body weight supported stepping, thus resulting in CPI, PSI, and RI scores of 0. However, at 3 weeks post-stretching (recovery) most animals had regained at least some bodyweight supported stepping capabilities, thus leading to significantly higher CPI, PSI, and RI scores at this timepoint.

A


Coordination Pattern Index
Index (%)
100
90
80
70
60
50
40
30
20
10
0
*
pre-stretch
stretch
recovery
TCDC
LC
LC + Ex

B


Plantar Stepping Index
Index (%)
100
90
80
70
60
50
40
30
20
10
0
*
pre-stretch
stretch
recovery
TCDC
LC
LC + Ex

C

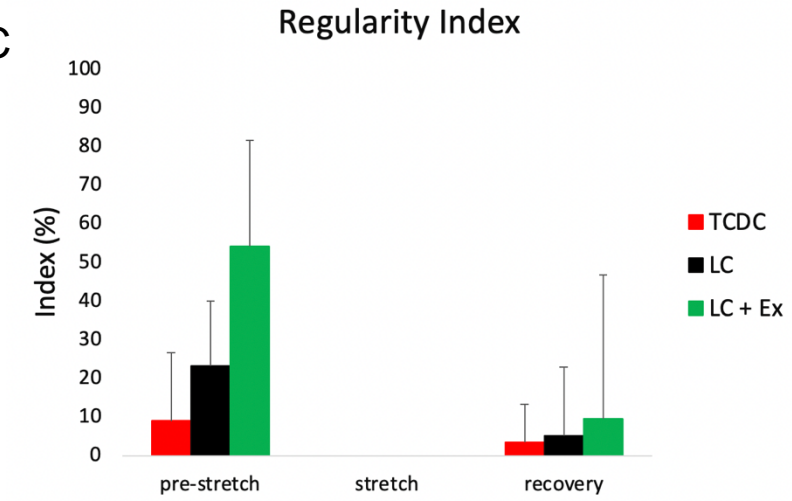

Regularity Index
Index (%)
100
90
80
70
60
50
40
30
20
10
0
pre-stretch
stretch
recovery
TCDC
LC
LC + Ex

Figure 10. Gait analysis of overground kinematics of post-injury animals in response to cage size, exercise, and stretching therapy. A) Coordination Pattern Index comparisons between groups and over time. B) Plantar Stepping Index comparisons between groups and over time. C) Ratio Index comparisons between groups and over time. Significant differences are indicated by (*) for values greater in LC + Ex group when compared to the LC and TCDC groups.

Overnight Activity

Nocturnal in-cage activity was recorded and analyzed for distance traveled per night as previously described. Repeated Measures ANOVA revealed significant differences across time as well as between groups. Stretching did not appear to have an effect on nocturnal activity of animals in the TCDC group or LC + Ex group until the recovery time point. By the last week of stretching (week 8) the LC group showed a significant decrease in overnight activity when compared to week 5. Interestingly, animals in the LC + Ex group moved significantly less per night than the animals in the LC only group. We hypothesize that the addition of exercise decreased nocturnal activity in these animals due to the increased activity received during the daytime. After 3 weeks of recovery (no stretching) both the TCDC and LC + Ex group showed significant improvement in overnight activity.

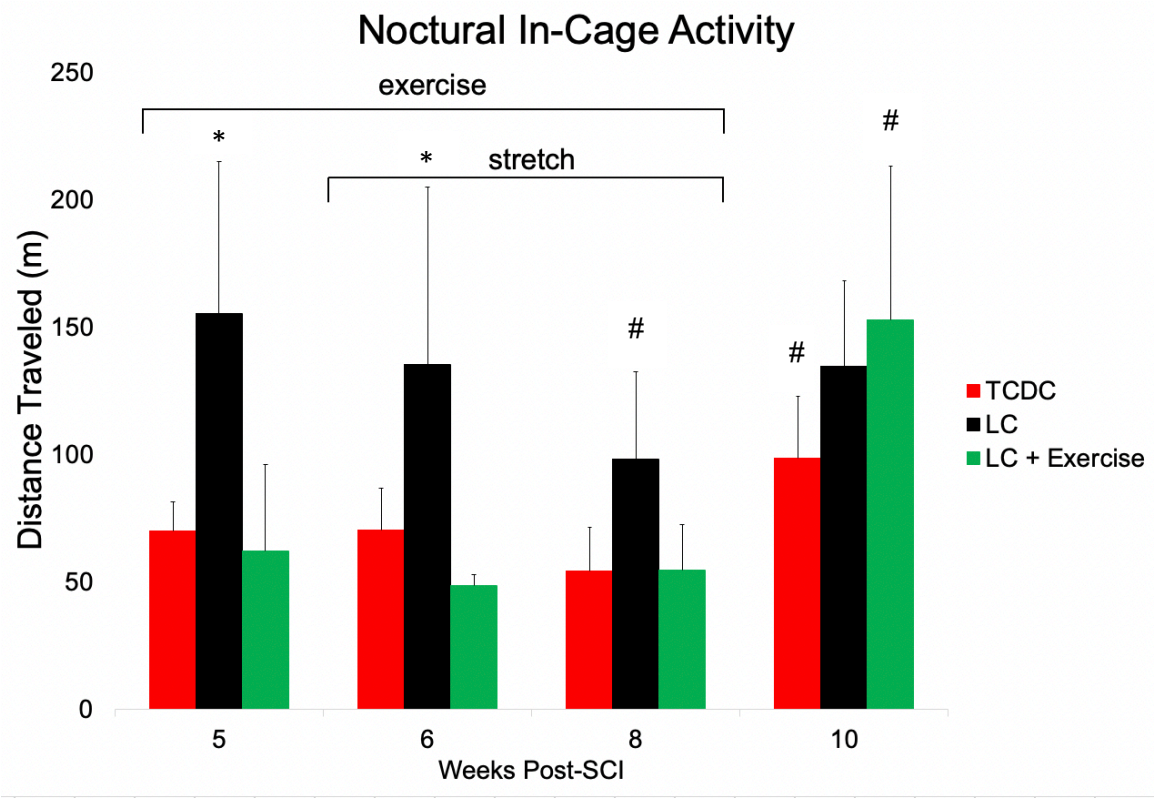


Figure 11. Analysis of nocturnal in-cage activity revealed significant differences between groups as well as over time. Significant differences between groups are indicated by (*) when $p < 0.05$. Significant differences over time are indicated by (#) when $p < 0.05$.

Tail Flick

Tail flick assessments were administered at week 3 (pre-stretching), week 6 (during stretching), and week 11 (post-stretching). Time to respond to the thermal stimulus was recorded as latency, measured in seconds. Repeated Measures ANOVA revealed no significant differences across time within any of the groups. However, week 6 (during stretching) assessments showed a significant difference between the LC + Ex group and the TCDC group. During this time, all groups were being stretched, so it appears that stretching caused increased thermal hypersensitivity in the TCDC group and decreased thermal sensitivity in the LC + Ex group.

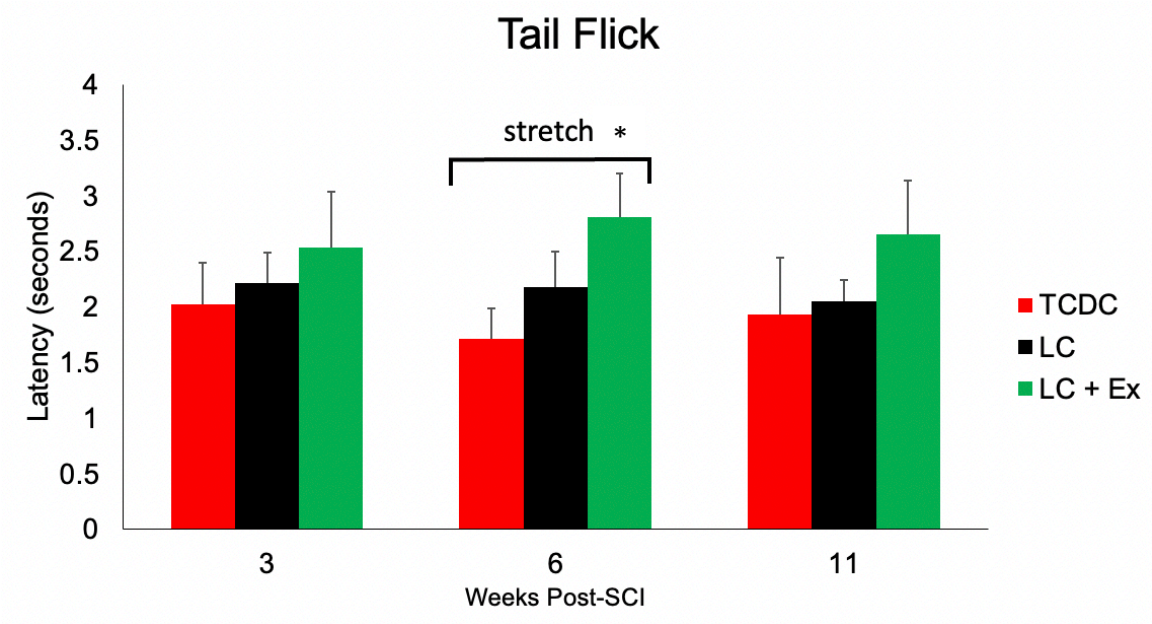
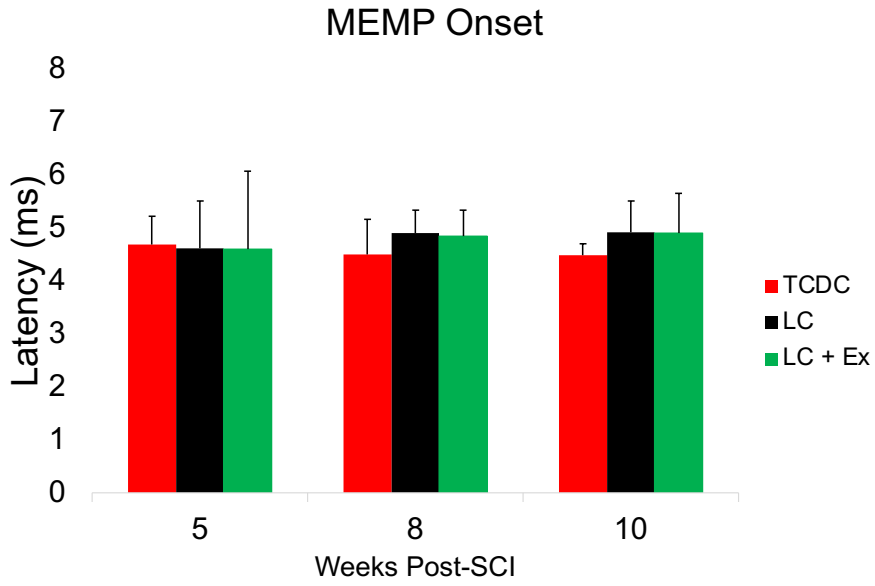


Figure 12. Tail flick latency time of animals in response to modulation of cage size, exercise, and stretching. There was a significant difference between the LC + Ex group and the TCDC group during week 6 as indicated by (*) when $p < 0.05$.

Magnetically Evoked Potentials from Tail Stimulation

Onset and amplitude were recorded as factors of electromyographic responses from the gastrocnemius muscle in response to magnetic stimulation of the base of the tail. Repeated Measures ANOVA revealed no differences between groups or across time during any point in the study for either onset or amplitude. Onset time remained extremely similar between groups and between time points. Amplitude varied greatly with high levels of variability within each group.



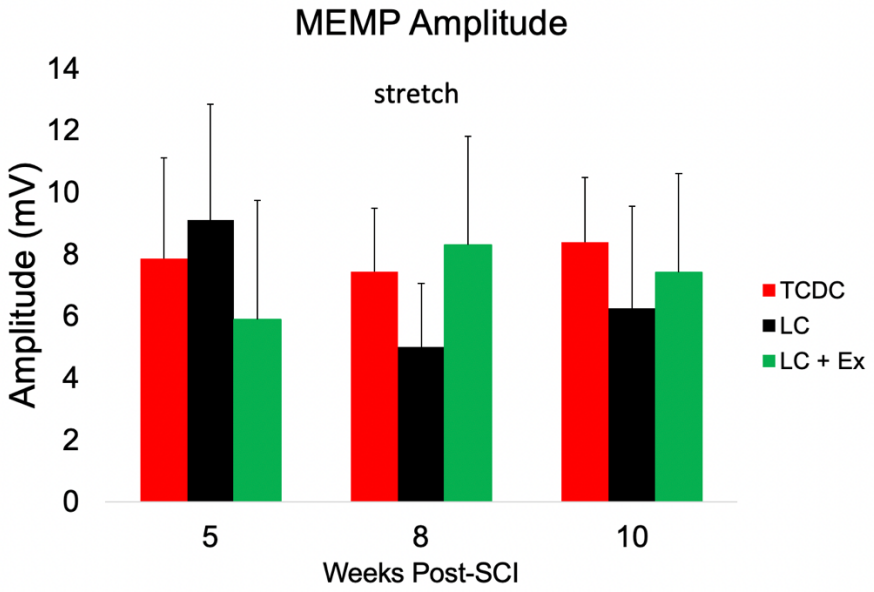


Figure 13. Magnetically evoked muscle potentials of animals in response to modulation of cage size, exercise, and stretching. No significant differences were reported.

Muscle Weights

Following sacrifice, right side hindlimb muscles were dissected and weighed. Muscles dissected included lateral gastrocnemius, medial gastrocnemius, tibialis anterior, and soleus. Each muscle weight was normalized to the rat's body weight (g/g of BW). One way ANOVAs revealed a significant smaller average weight of the medial gastrocnemius and tibialis anterior of the LC + Ex group when compared to the other two groups. We hypothesize that the lack of body weight support time in the LC + Ex group (as revealed by the lack of overnight activity detailed above) led to muscle atrophy of this group.

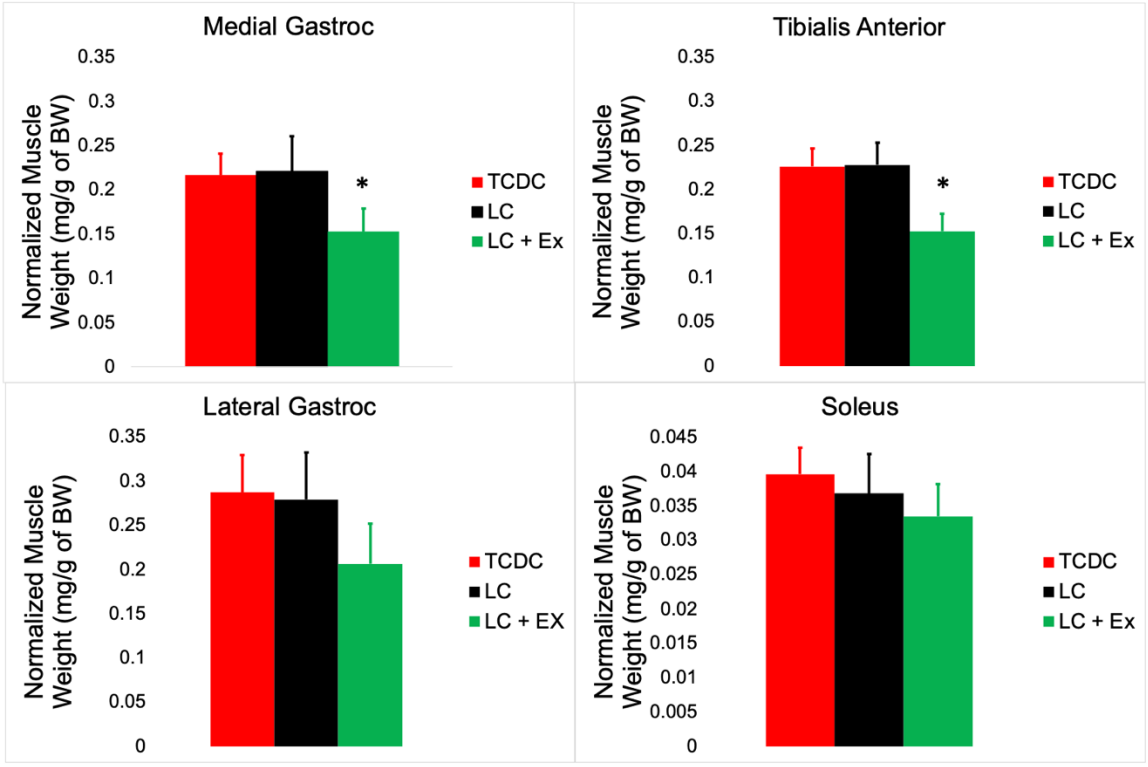


Figure 14. Normalized hindlimb muscle weights of the animals in response to modulation of cage size, exercise, and stretching. The LC + Ex group had significantly smaller medial gastrocnemius and tibialis anterior as indicated by (*) when $p < 0.05$.

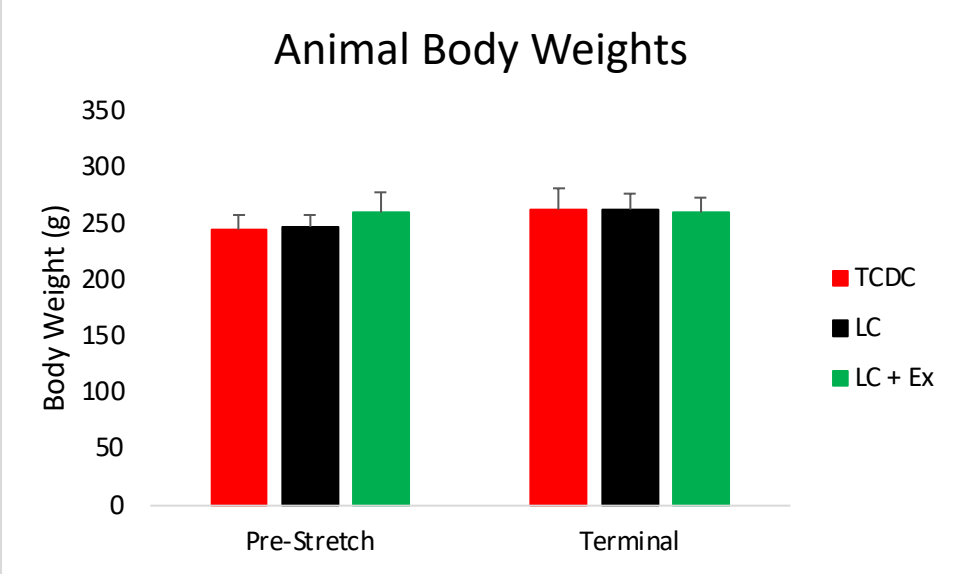


Figure 15. Animal body weights in grams at pre-stretch and terminal time points. No significant differences were found between groups or over time.

Immunohistochemistry

After completion of immunohistochemistry, images were analyzed for both total CGRP positive area in the dorsal horn as well as total sprouting area. Sprouting area was determined as any CGRP positive area that extended past laminas 1 and 2 into the deeper lamina. Analysis was done using a specialized ImageJ software. One way ANOVAs revealed no significant differences between groups for neither total area nor sprouting area.

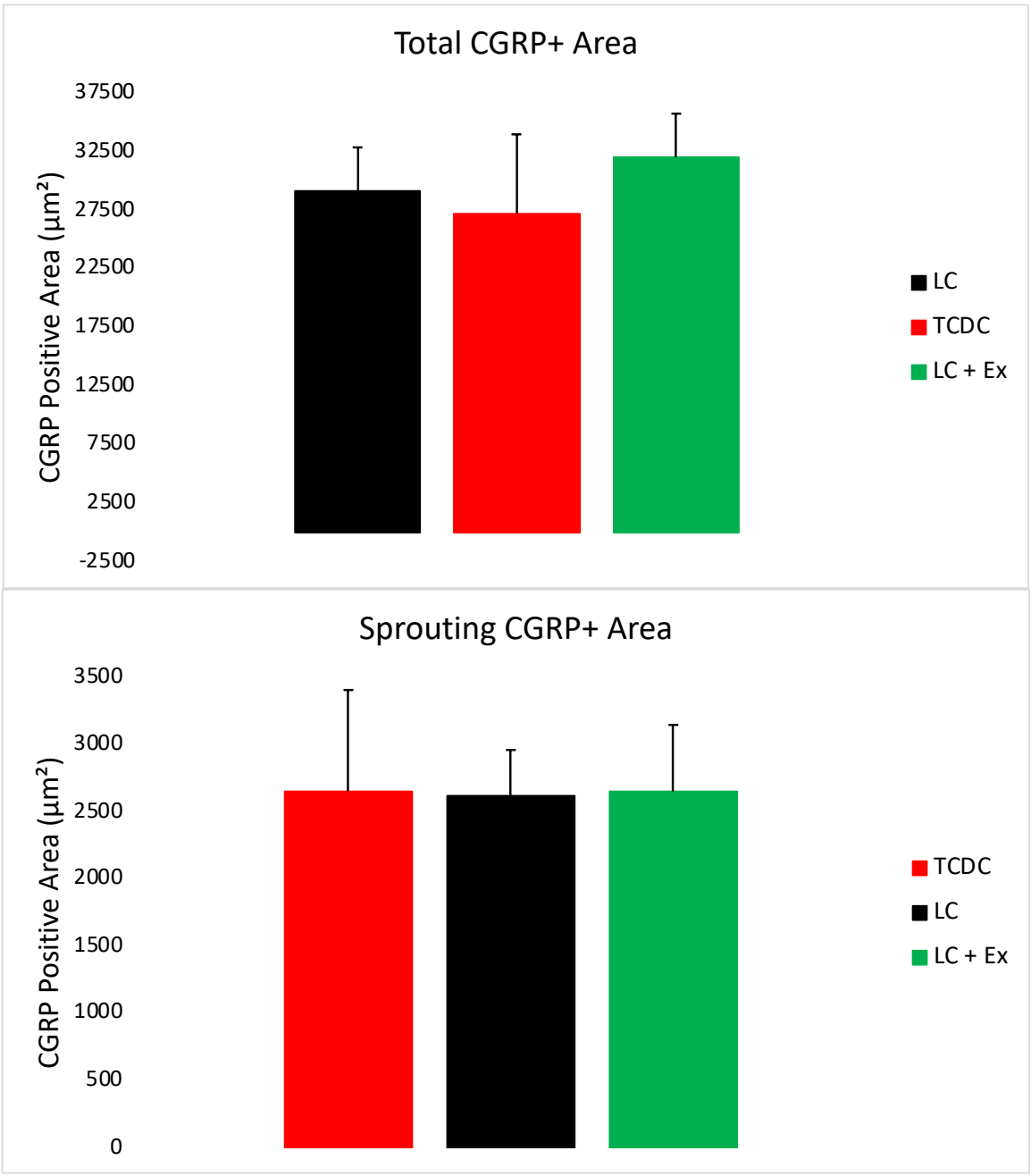


Figure 16. Total CGRP+ Area and Sprouting CGRP+ Area for all groups. No significant differences were found between groups for either measure.

Histology

After sacrifice, spinal cords were sectioned and stained for spared white matter at the epicenter. One way ANOVA showed no significant group differences.

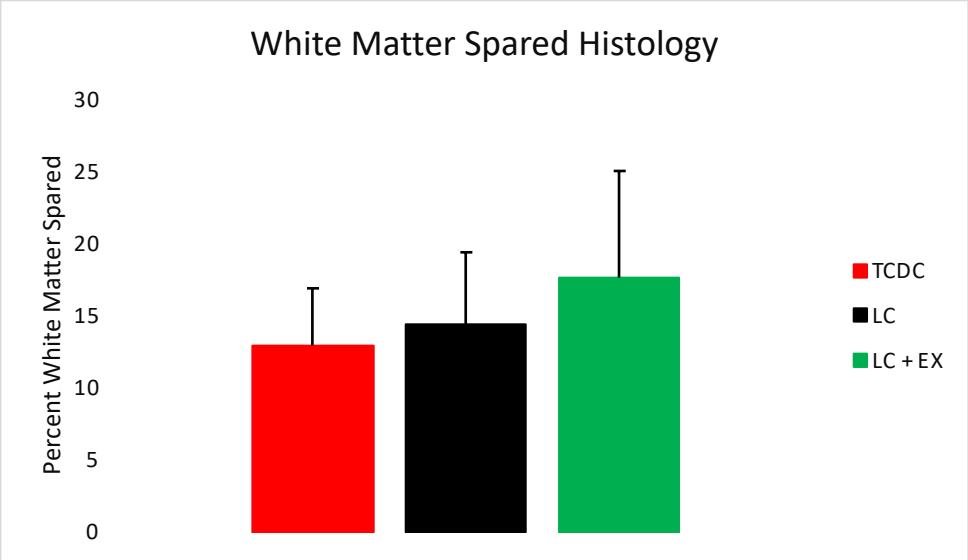


Figure 17. Percent of white matter spared at spinal cord injury epicenter for each group. No significant differences reported.

Preliminary Discussion

Summary of main findings

Our findings are consistent with previous work in that stretching initiated after locomotor ability plateau causes significant disturbances in locomotor recovery following SCI. We also observed similar patterns to this disruption in that locomotor function seemed to recover over the weekend when the animals were not stretched for two consecutive days. Surprisingly, the addition of acute shallow water walking (a task specific exercise for recovering stepping ability), did not induce changes in BBB scores in the first 5 weeks of recovery post-SCI, although there exists a possible trend. Further exploration of these data did reveal differences in regards to gait wherein plantar stepping was lowest and coordination was greatest in the exercised group, suggesting that acute task-specific exercise may result in changes in locomotion that are too nuanced for the BBB scale to detect.

In-cage overnight activity was highly varied between groups and throughout the study, most likely due to the addition of the shallow water walking. Animals that engaged in shallow water walking traveled significantly less distance per night even compared to animals housed in the same, large cage size. Interestingly, it appears that the difference in distance traveled per night between the LC only and LC + Ex group is equal to the distance accumulated during the exercise sessions each day. The average shallow water walking distance covered was around 100m per day and the approximate difference in distance traveled per night between the two groups was also around 100m. Once

the exercise and stretching interventions were completed, the LC + Ex group increased their overnight activity to look more like the LC only group. This suggests the possibility of a sort of “physiological ceiling” to the amount of distance an animal will travel in a 24 hour time period without additional interventions. This lack of time spent body weight supported stepping also likely resulted in the hindlimb muscle atrophy seen in the LC + Ex group.

Activity and exercise effects on afferent sprouting

A primary goal of the present study was to determine whether increased in-cage activity alone (similar to increasing activities of daily living or non-exercise activity thermogenesis in humans) would have similar positive effects on modulation of aberrant afferent sprouting as intentional exercise that has been previously described (Detloff et al., 2014; Nees et al., 2016). It appears that the chosen exercise paradigm for this current study failed to reproduce the effects seen in previous work. We hypothesize that this could be due to a variety of reasons. First, the experimental injuries described in previous literature are much different and milder than the injury model used in the current study. Previous work utilized injury models that still allowed for body weight supported stepping very early on in post-injury recovery. Detloff and Nees were able to use forced wheel paradigms and treadmill training, respectively to accomplish acute exercise that was loaded and task specific. This loaded, weight supported exercise seems to produce a different effect on nociceptor sprouting as well, possibly due to the difference in afferent input from body weight supported stepping compared to shallow water stepping. The more severe, contusion

injuries utilized in this study did not allow the animals to recover occasional stepping capabilities until 4-5 weeks post-injury, so shallow water stepping was one of the only viable exercise paradigms. While this exercise paradigm is clinically relevant and the injury chosen is an excellent model for studying locomotion, the combination of the two factors may have been the cause for the different outcomes.

Afferent sprouting and rehabilitation strategies

As expected based on previous literature, stretching caused an increase in afferent sprouting in the dorsal horn of the spinal cord. Contrary to our original hypothesis, the addition of acute exercise did not decrease or modulate nociceptor afferent sprouting. Increases in in-cage activity via increased cage size also failed to decrease nociceptor afferent sprouting. As discussed above, it is possible that the chosen exercise paradigm of shallow water stepping did not provide enough of a load bearing stimulus to cause decreases in sprouting. It is also possible that shallow water stepping in and of itself provided too much afferent input. The stimulus of the water contacting the animal's skin and fur, changes in temperature from being in the water to outside of the water, and the texture of the training tank bottom may have caused unintended overstimulation of the already hypersensitized post-injury afferents.

Future work in this specific area of interest should focus on the outcomes resulting from different types of exercise as well as time course changes. For example, it is possible that shallow water walking did have some positive effect on nociceptor sprouting early on, but that effect was washed out once the

stretching paradigm was initiated. It would be important to compare which types of exercise resulted in the greatest difference in sprouting pre-stretching and post-stretching, as it is possible that exercise intensity, volume, and task specificity play a role in the reduction (or not) of sprouting.

CHAPTER III

CARDIOVASCULAR AND FUNCTIONAL RECOVERY FOLLOWING STRETCHING OF RATS WITH A CLINICALLY RELEVANT MODEL OF SPINAL CORD INJURY

Introduction

A review of the top seven SCI rehabilitation centers revealed that stretching and/or range of motion (ROM) therapy are one of the top three therapies for all SCI patients.(Taylor-Schroeder et al., 2011) This review also reported that over 98% of patients with C5-C8 injuries received stretching therapy. Although stretching has been widely used to manage secondary complications of SCI, research has not been able to determine specific guidelines for the dosage and usage of stretch-based therapy. This is likely due to the fact that most clinical stretching studies to date have implemented a variety of interventions, have subjects with different levels of impairments, and the type and reliability of outcome measures vary greatly (Bovend'Eerdt et al., 2008). Nevertheless, stretching remains a cornerstone treatment for physical therapists and their patients with SCI that have both spasticity and muscle contractures (L. A. Harvey et al., 2011; Patrick et al., 2002). It is often recommended that even those that do not actively have spasticity or contractures receive this type of therapy as a preventative measure (L. A. Harvey & Herbert, 2002). Interestingly,

recent reviews have established that this type of therapy provides no clinically meaningful changes in joint mobility (Katalinic et al., 2011).

It is important to note that to date, all experiments involving stretching have been completed in animals with a T10 contusion model of injury. This is because this particular model of injury has been the standard for understanding hindlimb function and locomotor recovery since its inception (Basso, Beattie, & Bresnahan, 1996; Basso, Beattie, Bresnahan, et al., 1996). However, mid thoracic contusions do not represent a majority of clinical injuries. They also do not appropriately model the loss of cardiovascular control and resulting issues that many patients with higher level injuries face. Recently, the Magnuson lab and other groups have begun to utilize the high thoracic contusion model which is ideal for studying the autonomic circuitry and sympathetic preganglionic neurons of this area (Harman et al., 2018; Squair et al., 2018; Squair et al., 2017). It is possible that part of the reason we see such detrimental drops in locomotion in our previous stretching studies is due to the usage of the T10 contusion model because the injury epicenter is closer to the locomotor circuitry and CPG located in the lumbar spinal cord. Utilizing a clinically relevant T2 contusion injury and creating greater distance between the injury epicenter and locomotor circuitry may alter the post-injury sprouting that appears to be a prerequisite to the stretching phenomenon. It has yet to be established if the observed effects are limited to low thoracic injuries. Nearly all patients with varying levels of SCI receive some form of stretching or range of motion therapy (Taylor-Schroeder et al., 2011), so it is important that a variety of animal models

be used to establish clinical relevance. Even if we find that the results of this study are not directly applicable to the clinical realm, the outcomes will still provide important information for understanding plasticity and sprouting post-SCI.

Additionally, utilizing a T2 contusion model of injury will allow us to explore the cardiovascular outcomes that could be associated with increased nociceptor sprouting. Control of the cardiovascular system via the autonomic nervous system in an uninjured individual depends upon a multifaceted, complex set of interactions between descending supraspinal fibers and sympathetic neurons. Following high thoracic SCI, there is a loss of supraspinal input and afferent stimulation can trigger reflexes leading to preganglionic sympathetic activation from neurons below the lesion. This leads to widespread vasoconstriction and hypertension, causing reflex bradycardia. This condition is known as autonomic dysreflexia (Cragg & Krassioukov, 2012; West, Squair, et al., 2016). Increased sprouting of nociceptive afferents have been implicated in increased bouts of AD (Hou et al., 2009). We hypothesize that increased sprouting of nociceptive afferents via stretching could lead to cardiovascular dysfunction in animals with a high thoracic SCI.

Materials and Methods

Ethical Approval

All animal care and experimental procedures were approved by the University of Louisville Institutional Animal Care and Use Committee and performed in accordance with their surgical and animal care guidelines.

Spinal Cord Injuries and Study Design

Twenty six, adult, female Sprague-Dawley rats (200-250g) were included in this study. Upon arrival, all rats underwent a two week period of acclimation, daily handling/gentling, and baseline assessments. After acclimation, animals were given spinal cord injuries by first anesthetizing with a Ketamine (50 mg/kg)/Xylazine (0.024 mg/kg)/Acepromazine (0.005 mg/kg) cocktail given intraperitoneally. Animals were shaved and cleaned with surgical scrub before receiving a midline incision through the skin and musculature from the C8-T4 spinal segments. A laminectomy was performed at the T2 vertebral level before positioning and immobilizing the animal under the NYU Impactor (MASCIS Impactor Rutgers University, NJ). The NYU Impactor then delivered a 25 g/cm (considered moderately-severe) weight drop contusion injury. Following delivery of the injury, the underlying muscles were sutured, the incision was closed using surgical staples, and antibiotic ointment was applied to the site. Animals recovered and were monitored for 1-2 hours on heating pads until awake and alert. Post-operative care consisted of daily subcutaneous injections of gentamicin sulfate for seven days (20 mg/kg), twice daily subcutaneous injections of meloxicam for three days (5 mg/kg) and as needed for pain management, and twice daily 3-5ml subcutaneous injections of Lactated Ringer's solution for three days and as needed for hydration. Manual bladder expression was performed four times per day until spontaneous voided ability returned.

For one night following injury, animals were single housed to allow for sufficient monitoring and ensure sutures and staples remained intact. Within 48 hours of injury all animals were switched to double-housing environments and

placed in their home cages. Animals were randomly assigned to one of two groups prior to injury: injured control (CON, n=14) or injured and stretched (STRETCH, n=12). Most assessments were completed on a biweekly basis, with an emphasis on pre-stretching, during stretching, and recovery timepoints. Figure 18 illustrates a timeline of the primary variables and assessments.

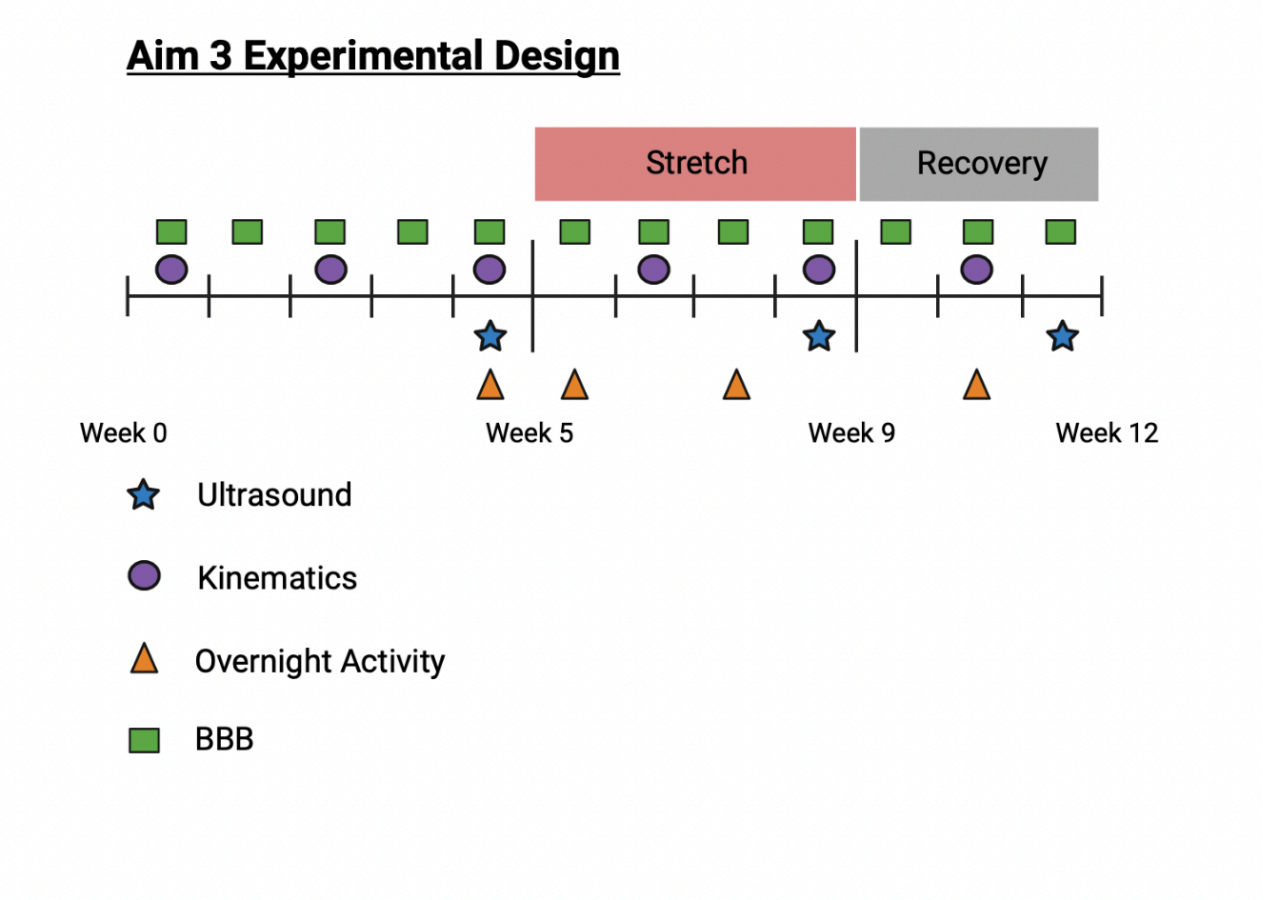


Figure 18. Representative timeline of primary variables and assessments.

Locomotor Assessments

Animals were assessed for overground locomotor function using the Basso, Beatie, and Bresnahan (BBB) Open Field Locomotor Scale (Basso et al., 1995). This assessment consists of a minimum of two trained examiners observing each animal for 4 minutes in an open field (plastic wading pool or similar). Rats are encouraged to continuously move about the testing site by the examiners. The scoring scale ranges from 0-21, with a score of 0 representing no observable hindlimb movements and a score of 21 representing consistent coordination, plantar placement, parallel paw placement, and trunk stability. Occasional weight supported stepping begins at a score of 10. Testing was conducted once pre-injury, and then weekly for the first 5 weeks post-injury until animals reached a functional plateau. BBB scores were assessed 3 times per week during weeks of stretching - Monday morning prior to stretching, Monday afternoon after one stretching session, and Friday afternoon after 5 cumulative days of stretching, as described previously (Caudle et al., 2015).

Additionally, digital video recordings were made of each rat walking in the training tank from the ventral viewpoint using a camera (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) mounted below the plexiglass tank. Animals were encouraged to move from one end of the tank to the other and only high quality passes with no speed changes or exploratory behavior were included. These high speed (60 Hz) videos consist of six passes for each animal and were analyzed using the kinematic software MaxTraq and MaxMate (Innovation Systems Inc., Columbiaville, MI) to identify plantar and dorsal hindlimb steps,

forelimb steps, and foot placement and timing for coordination. The primary outcome measures determined from this analysis were Central Pattern Index (CPI), which is calculated as the number of correctly patterned steps (plantar and dorsal) divided by the total number of steps, Regularity Index (RI), which is calculated as the number of correctly patterned plantar steps divided by the total number of steps (plantar and dorsal) and Plantar Stepping Index (PSI), which is calculated as the number of plantar steps over the total number of steps. These indices were determined after analysis with MaxTraq was completed using custom designed Excel macros.

Stretching Protocol

Our standard stretching protocol (Caudle et al., 2015) was implemented beginning 5.5 weeks post-injury. This timeline allowed for the subjects to platueau in their locomotor recovery which can happen as early as 3 weeks, but typically occurs around weeks 4-5 post-injury. Animals were gently wrapped in small towels, leaving both hindlimbs exposed, and all stretches were performed while the animal was supine. Six different handlers were trained in hindlimb stretching and animals were rotated so that no animal was handled by an individual more than twice in a week. The stretching protocol consists of 6 different stretches that target the major hindlimb muscle groups – ankle, knee, and hip flexors/extensors, and hip adbuctors/adductors. Each of the 6 stretches are performed bilaterally and held at the end range of motion for 1 minute. This protocol is then repeated so that two 12 min sessions are completed, totalling approximately 24 minutes of stretching daily. All animal handlers were trained to recognize signs of distress, pain , and injury in

the animals and were instructed to stop stretching if any of these behaviors were observed. Animals were rewarded with cereal before, during, and after stretching sessions. See Figure 6 for a detailed, kinematic analysis of the 6 stretches that were performed. Animals were stretched 5 days a week for 4 weeks and then allowed to recover with no stretching for 3 weeks prior to sacrifice.

Nocturnal In-Cage Activity

Overnight in-cage activity was recorded the week prior to stretching, during the first week of stretching, during the third week of stretching, and prior to sacrifice using overhead cameras (Basler acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) and infrared lights (CM-IR30 IR Illuminator; C&M Vision Technologies Inc, Houston, TX). Specially designed cages with clear tops were placed on wire racks fitted with cameras (with wide view lenses) and lights mounted over each cage. Animals were recorded for 12 hours per night (6pm-6am) using a custom LabView software designed to acquire high resolution video at 4Hz for 1 minute of every 10 minutes of real time.

Animals' lower backs were shaved and a 1 inch diameter tracking dot was placed between the iliac crests using a black Sharpie marker. Cameras were positioned so that the entire bottom of the cage was recorded to include all animal movements. Following recording, videos were able to be analyzed using MaxTraq software and a custom designed Excel program which determined distance travelled per animal each night. Small movements equivalent to 2 cm per or less were filtered out, thus leaving out most behavior consistent with sleeping or grooming.

In Vivo Echocardiography

Cardiac and vascular echocardiogram assessments were performed using a high resolution ultrasound machine (VisualSonics, Vevo 3100, Toronto, CA). Animals were anesthetized using isofluorane and shaved to expose the thorax, abdomen, neck, and medial hindlimb, before being placed in a supine position on a physiological monitoring unit to maintain temperature. Body temperature as well as heart rate and respiration rate were monitored throughout the assessment.

Medical grade ultrasound gel was applied liberally to the skin before placing transducers in the correct location. Images of left ventricular structure and function were obtained using the parasternal long axis (PSLAX) and short axis (SAX) views at approximately the mid-ventricular level. We also obtained brightness mode (B-mode) images for determining anatomical measures as well as movement mode (M-mode) for measuring systolic function and flow. Blinded analysis was completed using VEVO® LAB software and the results from five cardiac cycles were averaged.

Euthanasia and Tissue Histology

All animals were sacrificed utilizing an overdose of ketamine (50mg/kg)/xylazine (0.024 mg/kg)/acepromazine (0.005 mg/kg) cocktail and transcardially perfused with phosphate buffer followed by 4% paraformaldehyde (PFA). The right side hindlimb muscles (medial and lateral gastrocnemius, tibilias anterior, and soleus) of each animal were carefully dissected out and weighed.

Spinal cords were dissected and post-fixed in 4% PFA for 6 hours before being transferred to 30% sucrose solution for storage. The lesion site was confirmed by carefully examining the spinal column and verified by counting spinal cord dorsal roots. An 8 mm long section containing the injury epicenter was cut and placed in freezing medium. 50 μm transverse sections were cryosectioned and stained for spared white matter using eriochrome cyanine. After imaging, cross-sectional area of white matter was traced and quantified using specialized ImageJ software.

CGRP Immunohistochemistry and Analysis

The lumbar spinal cord was dissected out, post-fixed in 4% PFA, and cryopreserved in 30% sucrose. Levels L1-L5 were transverse cryosectioned at 20 μm for immunohistochemical analysis of CGRP. After sectioning, slides were stored at 4°C . To begin staining, slides were first warmed at 37°C for 30 minutes before removing the excess blocking media and applying a hyrdrophobic PAP pen border. Next, slides were rinsed multiple times and blocked for 1 hour. Slides were then incubated with CGRP primary antibody (rabbit polyclonal anti-CGRP, 1:1000, batch #3611083, Calbiochem) overnight in 4°C, before being rinsed and incubated with secondary antibody (Alexa Fluor 594-conjugated Donkey anti rabbit, 1:200, Lot #158327, Jackson ImmunoResearch Laboratories) for 1 hour. The sections were then rinsed and coverslipped with fluoromount.

Sections of interest were imaged using a Nikon Eclipse Ti2 microscope at 10x objective. After imaging, CGRP+ area was quantified within the dorsal horn

region using a specially designed ImageJ program. Thresholds were chosen based on control images and then utilized to quantify total CGRP+ area of each section.

Statistical Analysis

Data was analyzed using SPSS (IBM SPSS Statistics for Windows, Versions 26/27, IBM, Armonk, NY). All data are presented as group means +/- standard deviation. Differences between groups or across time were considered statistically significant when $p \leq 0.05$.

Results

Locomotor Function

The locomotor open field assessment (BBB) showed a drastic drop in locomotion for all groups at one week post-SCI. Repeated Measures ANOVAs revealed that once stretching began at week 5 post-SCI, the STRETCH group had significantly decreased locomotor function at multiple timepoints. Interestingly, STRETCH animals recovered to baseline locomotor function over the weekend which has previously not been seen in animals with T10 injuries.

Spinal cord injury also had a significant impact on gait measures, however post-plateau of locomotor recovery no significant differences between groups or over time were detected in response to stretching. It does however appear that there is a trend towards decreased CPI, increased DPI, and decreased PSI in the stretched animals which is related to the decrease in BBB scores mentioned above.

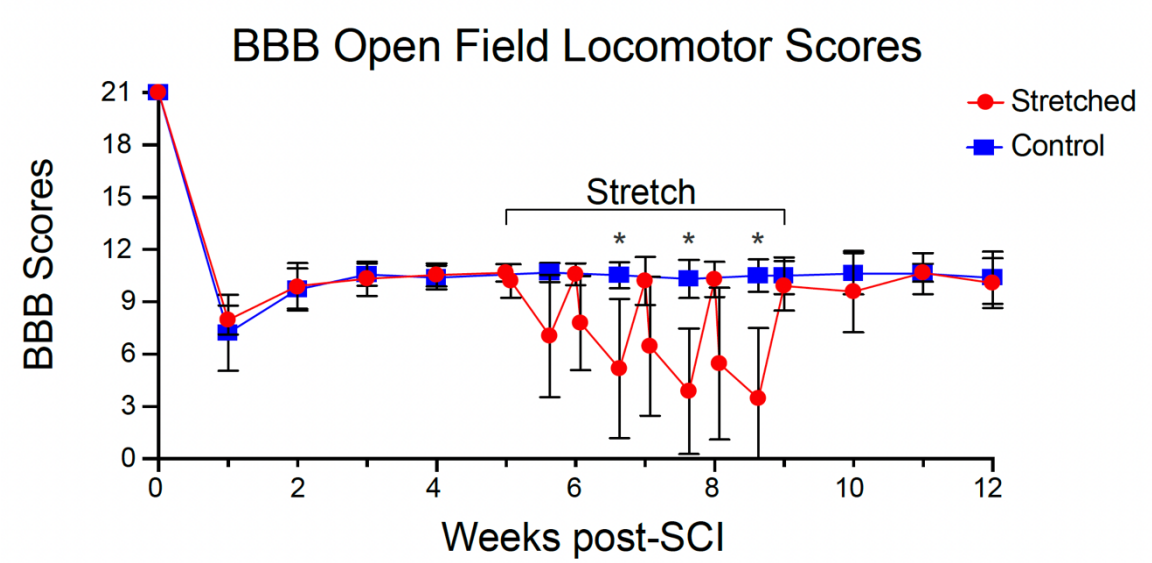


Figure 19. Locomotor scores in response to stretching in animals with T2 injuries. Stretching induced a significant drop in BBB scores on weeks 6, 7, and 8 as indicated by (*) when $P < 0.05$. However, significant recovery occurred over the weekend. No differences were found between groups during the recovery period.

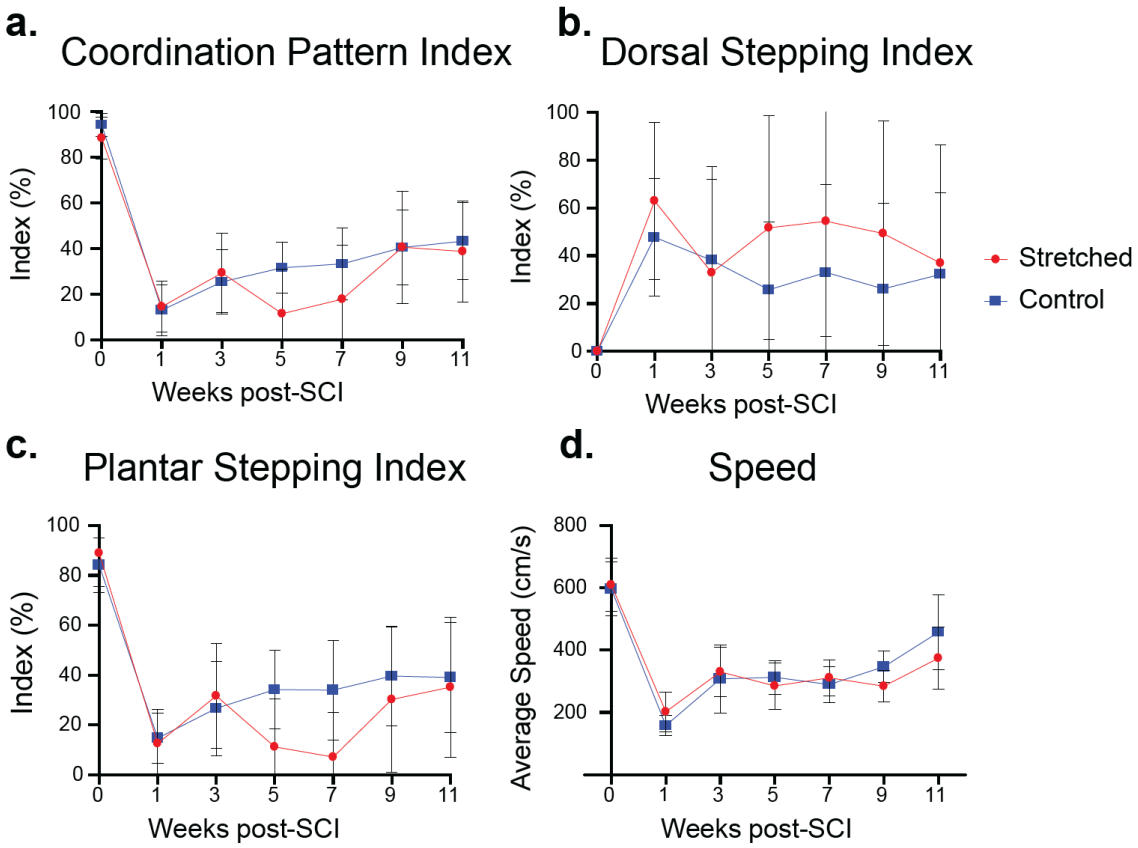


Figure 20. Gait analysis of overground kinematics of post-injury animals in response to stretching or no stretching. No significant differences were found between stretched and control animals in overground kinematics analysis. However, there does appear to be a trend towards decreased CPI, increased DPI, and decreased PSI in the stretched animals.

Overnight Activity

Nocturnal in-cage activity was recorded and analyzed for distance traveled per night as previously described. Repeated Measures ANOVA revealed significant differences across time for the stretched group. Stretching decreased nocturnal activity of animals during stretching weeks when compared to the pre-stretching timepoint. After recovery (no stretching), the stretching group showed significant improvement in overnight activity. The control animals (injured, no stretching) did not have any significant differences in nocturnal in-cage activity.

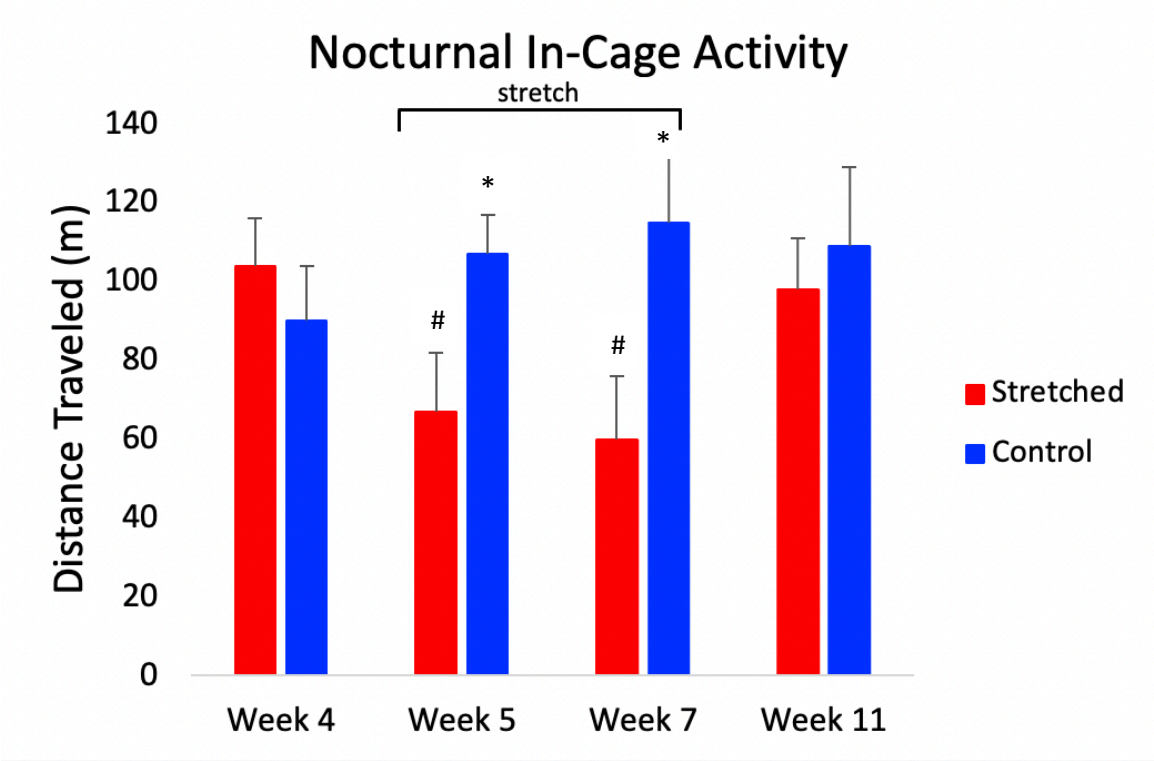


Figure 21. Analysis of nocturnal in-cage activity revealed significant differences between groups as well as over time. Significant differences between groups are indicated by (*) when $p < 0.05$. Significant differences compared to pre-stretching timepoint (week 4) are indicated by (#) when $p < 0.05$.

Cardiovascular Function

Echocardiography assessments were completed prior to stretching, after stretching, and after recovery. Cardiac measurements gathered during ultrasound included ejection fraction, stroke volume, and cardiac output. The primary vascular measurement gathered during ultrasound was end diastolic volume (EDV) of the superior mesenteric artery (SMA), carotid artery (CCA), and femoral artery (FA). All data was normalized to baseline cardiovascular measurements. Repeated Measures ANOVA revealed no significant differences between groups or over time for either of the groups.

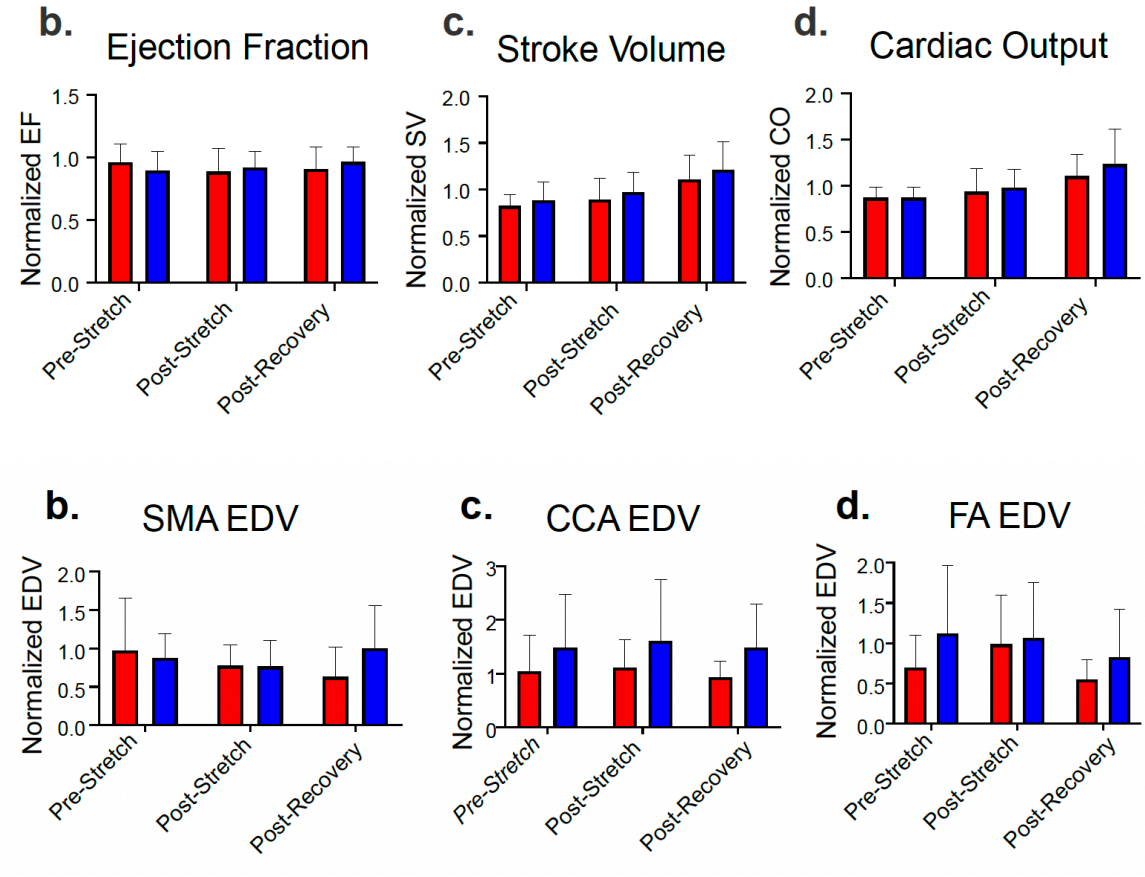


Figure 22. Stretched and control animals showed no significant differences between groups or over time in regards to cardiac or vascular function.

Muscle Weights

Following sacrifice, right side hindlimb muscles were dissected and weighed. Muscles dissected included lateral gastrocnemius, medial gastrocnemius, tibialis anterior, and soleus. Each muscle weight was normalized to the rat's terminal body weight (g/g of BW). One way ANOVAs revealed no significant differences between groups, however it does appear that there is a trend towards smaller muscle weights in the stretched animals when compared to control.

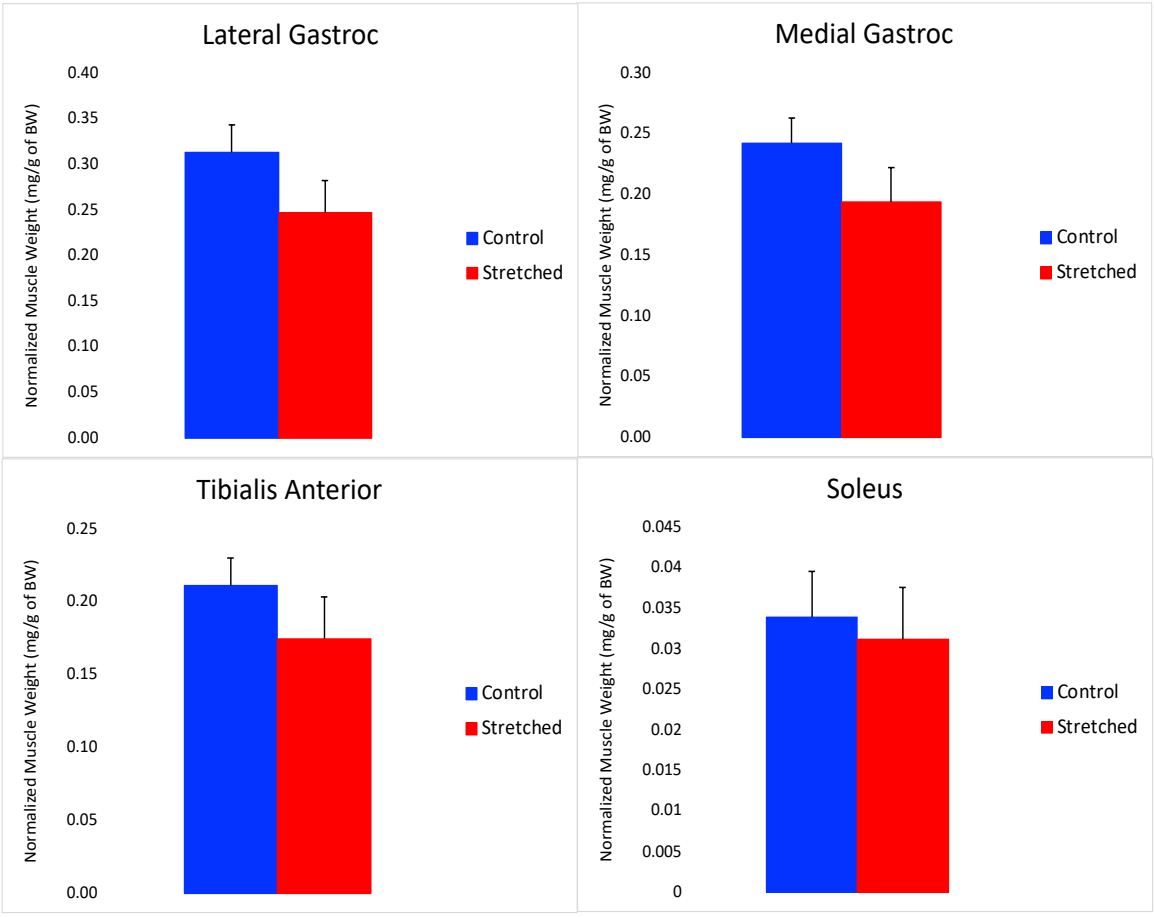


Figure 23. Normalized hindlimb muscle weights of the stretched and control animals. No significant differences were discovered between groups.

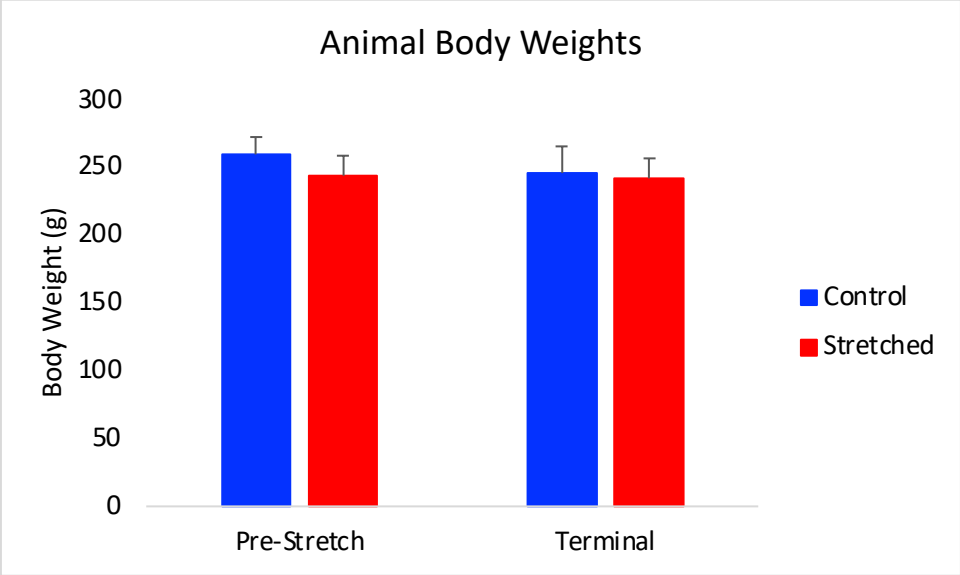
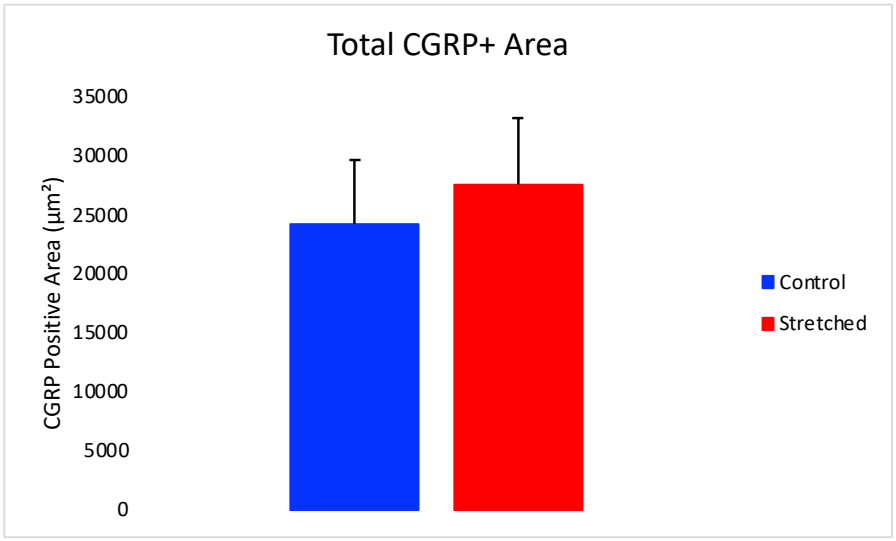


Figure 24. Animal body weights in grams at pre-stretch and terminal time points. No significant differences were discovered between groups or across time.

Immunohistochemistry

After completion of immunohistochemistry, images were analyzed for both total CGRP positive area in the dorsal horn as well as total sprouting area. Sprouting area was determined as any CGRP positive area that extended past laminas 1 and 2 into the deeper lamina. Analysis was done using a specialized ImageJ software. One way ANOVAs revealed no significant differences between groups for neither total area nor sprouting area.



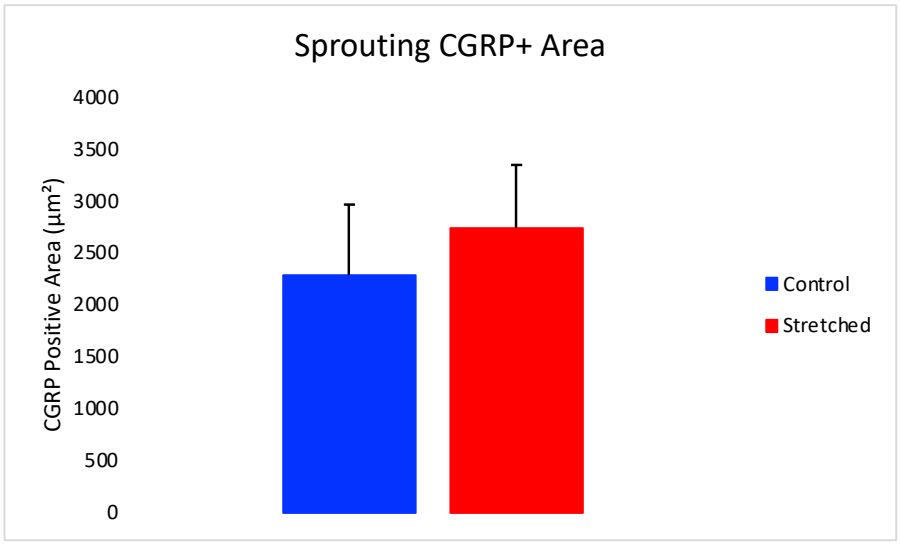


Figure 25. Total CGRP+ Area and Sprouting CGRP+ Area for both groups. No significant differences were found between groups for either measure.

Histology

After sacrifice, spinal cords were sectioned and stained for spared white matter at the epicenter. One way ANOVA showed no significant group differences.

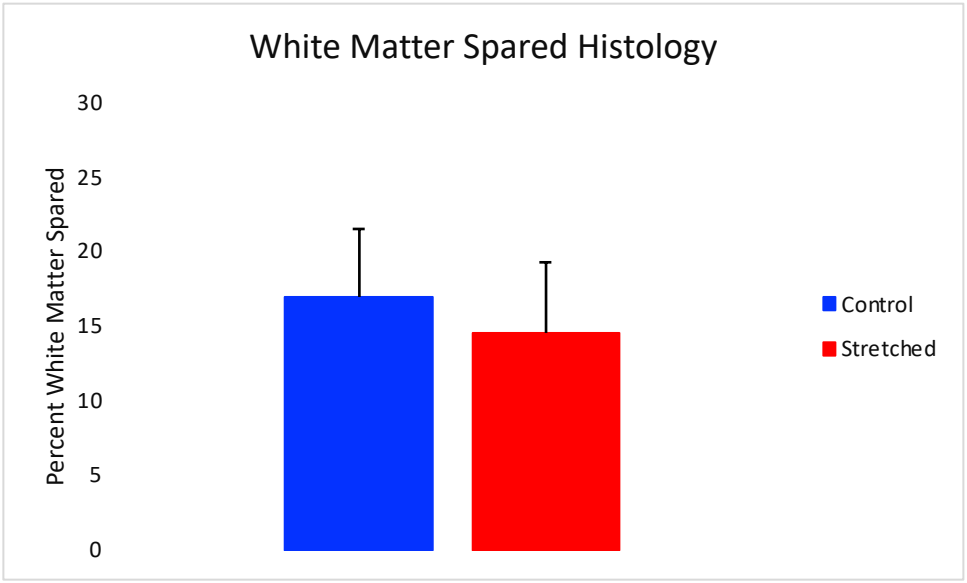


Figure 26. Percent of white matter spared at spinal cord injury epicenter for both groups. No significant differences reported.

Preliminary Discussion

Summary of main findings

Here we report on the impact of clinically-modeled hindlimb stretching in rodents with T2 moderately-severe, contusion spinal cord injuries. All previous work has been completed in animals with T10 injuries so as to most accurately determine locomotor function post-injury. Locomotor outcomes were mostly consistent with previous work done in animals with T10 level injuries, although surprisingly the T2 injured animals demonstrated much greater weekend recovery than has been seen previously. These animals showed an impressive recovery to near baseline, pre-stretching BBB scores after only two days of no stretching. We hypothesize that this impressive weekend recovery could be due to the distance from injury epicenter to CPG and stretched hindlimb muscles.

Functional recovery and plasticity following stretched, T2 injured animals

The existence of the above mentioned phenomenon in which T2 injured animals recover locomotor function over the weekend following stretching, suggests the presence of a transient mechanism for the reduction of locomotor function in stretched animals. Previous work by Keller (Keller et al., 2019) determined that locomotor dysfunction in stretched, T10 injured animals was at least partly due to the increased nociceptor sprouting in the lumbar cord. However, the animals in this present study showed no differences in sprouting area or total CGRP+ area when compared to controls, while also showing drops in locomotor function throughout the week. The locomotor dysfunction seen in

the T2 injured animals appears to be at least partially mechanistically different than the locomotor dysfunction demonstrated in T10 injured animals based on these findings. While the correlation between CGRP+ fiber sprouting and locomotor dysfunction is still valid, further research is necessary in order to determine the cause of the transient drops in locomotor function demonstrated here.

To our knowledge, this is also the first study to examine CGRP+ fiber sprouting in the lumbar cord following a clinically-relevant T2 contusion injury. When using the same specialized analysis program to determine CGRP+ area in the dorsal horn, it appears that the amount of sprouting present in our T2 injured, control and stretched animals is similar to the amount of CGRP+ area seen in T10 injured , stretched animals discussed in Chapter 2 of this dissertation as well as previous work from our lab (Keller et al., 2019). Interestingly, Keller found that the amount of nociceptor sprouting in the lumbar cord in control, non-stretched, T10 injured animals is around half of that of their stretched counterparts. In the current study we found no differences between stretched and non-stretched animals and the physical amount of sprouting looks similar to that of a T10, stretched animal in both groups. This suggests that a T2 contusion injury results in more afferent sprouting than a T10 injury, even if the animals receive no stretching intervention.

Cardiovascular function and stretching

Previous work has described the consistent drop in in-cage, overnight activity in animals that have been stretched. This decrease in distance traveled

per night is likely due to the lack of locomotor ability that is typically demonstrated after stretching and in-cage activity almost always returns back to baseline once stretching is stopped. We hypothesized that this decreased locomotor ability and subsequent drop in overnight activity could have an effect on cardiovascular function. Analysis of echocardiography data showed no differences between groups or over time. However, it does appear that the there was a trend in the vascular data to suggest that stretched animals have lower end diastolic volumes, particularly in the carotid artery. This suggests that the stimulus of decreasing in-cage activity only (similar to decreasing activities of daily living or non-exercise activity thermogenesis in humans) is not a powerful enough stimulus to cause permanent changes in cardiovascular remodeling or function, at least not in the time period discussed in this study. It is possible that extended decreases in in-cage activity would have a more robust effect and cause true cardiovascular dysfunction.

CHAPTER IV

CONCLUDING REMARKS

Summary of Main Findings

Spinal cord injury is a devastating, life altering event that can have major impacts on locomotion, sensory function, and cardiovascular control. Notably, many musculoskeletal complications, like spasticity and contractures, are currently being treated with clinical stretch-based physical therapy. Despite the evidence from our lab that correlates stretch-based physical therapy with increased nociceptive afferent sprouting in the lumbar spinal cord and decreased locomotor function in rodent models, stretching remains the gold standard for treating these patients. The goal of this dissertation was to provide rationale and novel framework for future translational and clinical research to determine whether stretching has a negative locomotor impact after SCI in humans.

In summary, the above studies outline the decline in locomotor function following stretching regardless of the addition of exercise and/or activity or the location of the spinal cord injury. We determined that increasing and decreasing activity via cage size and applying an exercise intervention did not have any effect on the previously described locomotor deficits that occur with stretching. Interestingly, we found that moving the injury to a more clinically relevant, high

thoracic location did alter the pattern of locomotor deficit we typically see with stretching. These animals experienced locomotor deficits only throughout the week but had remarkable recovery over the weekend. We also found that the sprouting of nociceptive afferents that has been described previously and has been correlated with poor functional recovery, also occurs to a similar degree regardless of exercise intervention or a high thoracic injury.

In-Cage Activity vs Applied Exercise

Our current understanding of spontaneous locomotor recovery following incomplete SCI in rodents is based on the idea of in-cage training. We believe the survival instincts to pursue food and water within the animals' cage lead the rats to begin a form of task-specific self-training almost immediately post-injury that helps the animal accomplish some degree of weight-supported stepping within just a few weeks of injury. This concept was supported by previous work in our lab that showed that SCI animals restrained in wheelchairs acutely after injury had significantly disrupted locomotor recovery that persisted even when animals were removed from the wheelchairs (Caudle et al., 2011). Interestingly, the above studies appear to show that when activity is limited via a smaller cage size rather than a wheelchair there is no impact on locomotor recovery even when compared to animals in cages twice the size. Even though in-cage overnight activity was significantly lower in the tiny cage group, the small amount of in-cage activity they did receive was enough to ameliorate any negative locomotor impacts like in previously described wheelchair groups. It is possible

that there is a “ceiling effect,” meaning that the level of in-cage training needed to actually impact locomotion, is so low that extreme restriction of stepping (like the wheelchair model) is the only way to accomplish a disruption of locomotor recovery. In the future, we should consider alternative ways to decrease in-cage activity/self-training, or return to the wheelchair models we previously utilized. There are currently other models of inducing decreased in-cage activity and muscle atrophy, such as tail suspension hindlimb unloading and casting (Herbison, Jaweed, & Ditunno, 1978; Morey-Holton, Globus, Kaplansky, & Durnova, 2005; Nemoto & Goyagi, 2021; Tomiya et al., 2019), however, these methods have not been thoroughly explored in animals with SCI and would require significant modifications in order to be practical interventions for an SCI rodent model.

Our applied exercise model, while clinically relevant and task-specific, was relatively low intensity. Because we do not fully understand the exact mechanism by which applied exercise has decreased afferent sprouting in previous studies (Detloff et al., 2014; Nees et al., 2016) it is possible that the low intensity, partially unloaded exercise that we chose was not enough of a stimulus to induce less sprouting. Detloff and colleagues utilized a moderate, unilateral C5 contusion injury that allowed for significant stepping abilities post-injury. The exercise paradigm utilized in this study was 20 continuous minutes per day on a forced exercise wheel walking system and the authors report that all animals in the study were able to achieve the maximum speed of 14 m/min (Detloff et al., 2014). In contrast, our animals were able to cover a distance of approximately 100

meters in 30 minutes of shallow water walking. Our animals also exercised in bouts of 5 minutes, with 5-6 minutes of rest in between each bout. Nees and colleagues utilized a mild T11 contusion injury in a mouse model. Their exercise paradigm of choice was a motorized treadmill at a moderate intensity. The authors state that speeds were selected that allowed the animals to run without signs of stress (Nees et al., 2016). Again, in contrast, our animals were walking with weight support and were never able to achieve a gait that could be described as running, suggesting that the intensity of our exercise paradigm was much lower. Additionally, because our exercised animals traveled much less during in-cage activity and their exercise was only partially loaded, it seems that we inadvertently induced hindlimb muscle atrophy in this particular group. Future research should be careful to consider the impact of unintentionally choosing combinations of exercise and activity that would encourage animals to gather only minimal time spent weight supporting.

Even though the differences in gait and kinematic outcomes were not statistically significant between groups of this study, it does appear that there is a trend for the exercised animals to have better gait characteristics. We believe this is due to the task specificity of shallow water walking and the translatability to overground walking. Had we not begun the stretching protocol, it is likely that the exercised group would have continued to improve in both gait outcomes and BBB scores. Preliminary data does suggest that animals housed in the TCDC condition that gathered more in-cage nocturnal activity had higher BBB scores. Figure 27 shows the correlation between BBB score and overnight activity in the

TCDC and LC groups. This preliminary data in conjunction with the previously presented data suggests that there is likely a “ceiling effect” of overnight activity, in which adding in-cage activity does not necessarily guarantee higher locomotor scores. Additionally, our modality of applied exercise does appear to have some sort of beneficial effect on locomotor scores after injury, but this improvement was dampened by the stretching effect, likely because our modality was not as great of a physiological stressor as utilized in previous literature.

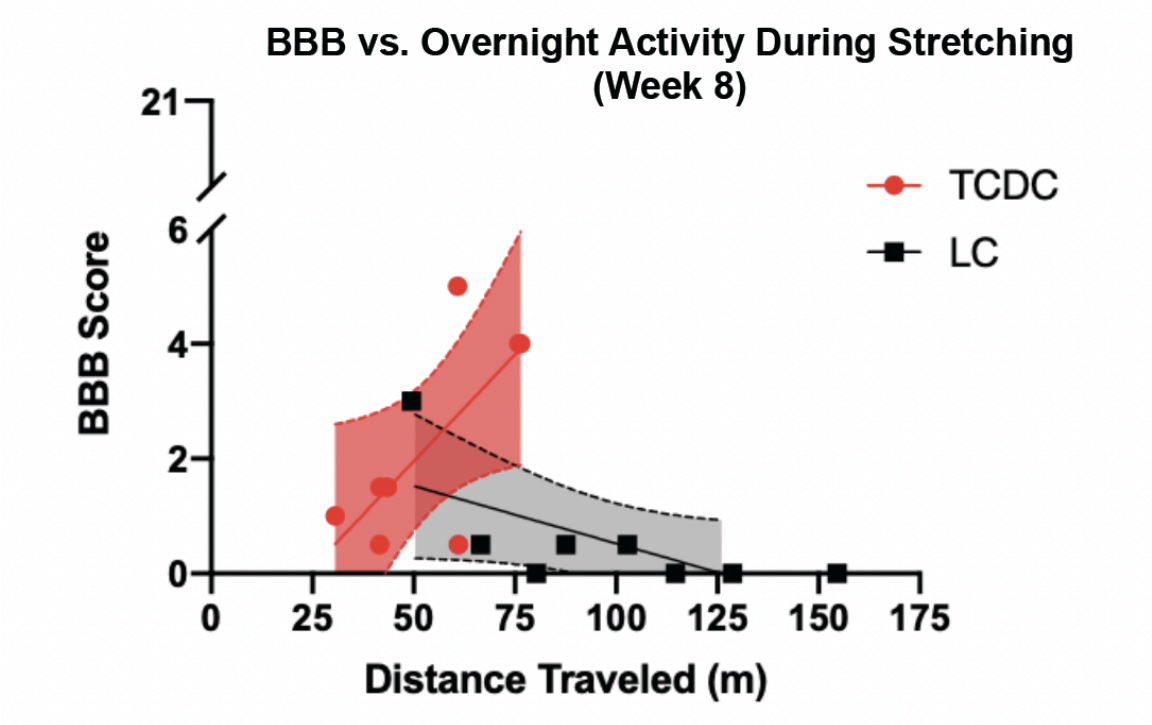


Figure 27. Cage size did not have a significant main effect on BBB scores, but overnight activity did affect BBB scores ($F(1, 59) = 4.9674$, $p < 0.05$). Locomotor scores were higher for animals with greater overall overnight activity in the TCDC group, but lower for animals in the LC group.

Another area of interest, specifically within the SCI research community, is defining the terms activity and exercise as it applies to those impacted by SCI. Currently, in both the animal and clinical research realms, we do not have a direct definition of these terms. Should we define the difference in the two based on elevation of heart rate as a function of intensity? Possibly by the intentionality of the activity? Exercise can be modulated based on frequency, intensity, duration, and type of training, however, we as a field do not clearly define where the shift from activity to exercise occurs. “Training” is also another common term used within the field. Typically, within the exercise science field training refers to intentional exercise with the purpose of achieving a specific performance goal. Training is highly structured and often implements incremental, short-term goals throughout the program. The SCI field often uses terminology like training and exercise interchangeably, and while the two are similar, we should be sure to point out the differences and nuances. A clear definition of these terms and how they translate clinically, will help bring clarity to the research.

Modulation of Overall Activity in Rodent Models

A novel finding of the current studies is the demonstration of the modulation of in-cage, overnight activity in response to applied exercise in rodent SCI models. We demonstrated a significant decrease in overnight activity in the LC + Ex group, suggesting that animals will self-regulate their own daily activity when allowed to increase other activity outputs via exercise. Figure 28 below shows the previously presented nocturnal in-cage activity graph with the addition of exercise distance

traveled for the LC + Ex group. To our knowledge, this is not a phenomenon that has been documented elsewhere. We believe it is an important consideration for those researchers implementing exercise interventions in SCI rodent models to consider. There have been only a few studies that have investigated the relationship between exercise and activities of daily living in the human SCI population. Ginis et al. found that participants that demonstrated a higher level of aerobic fitness (measured via estimated VO2max) tended to spend more time participating in certain activities of daily living (Hetz, Latimer, & Ginis, 2009). Another review was focused on examining the relationship between exericse, activities of daily living, and quality of life in people with spinal cord injuries. It was noted that people with SCI that participate in intentional physical activity/exercise spent more time doing activities of daily living and also experienced higher quality of life as determined by questionnaires (Kawanishi & Greguol, 2013).

It appears that the phenomenon we demonstrated in the current study of decreased in-cage activity in response to applied exercise is in direct opposition to what happens clinically. We believe that more researchers in the pre-clinical SCI and exercise field should consider monitoring in-cage activity of their animals in order to provide a more clear picture of the exercise and activity relationship in this model. Future studies should consider utilizing additional enrichment or possibly housing more animals per cage in order to encourage more in-cage activity to more closely mimic the clinical scenario.

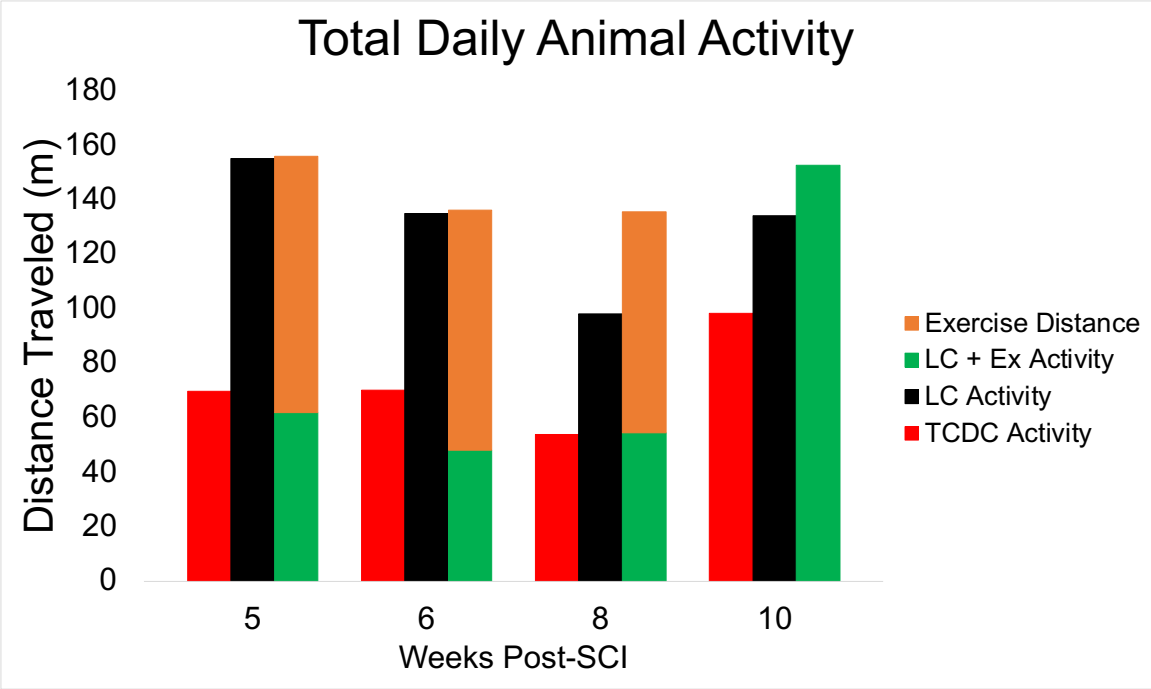


Figure 28. Average distance traveled overnight for each group with the addition of average exercise distance completed for the LC + Ex group.

Nociceptive Afferents and Locomotor Recovery

Our findings regarding nociceptive afferent sprouting following injury and stretching were consistent with previous findings from our lab. In addition to the muscle spindle-based proprioception, skeletal muscles are heavily innervated by small diameter, predominantly nociceptive afferents (Schmalbruch, 1986; Stacey, 1969). These afferents can be classified as group III (thin, myelinated, Aδ fibers) or group IV (thin, unmyelinated, C fibers). Both group III and IV afferents carry nociceptive information and are activated by stimuli that could be potentially tissue damaging. They have cell bodies in the dorsal root ganglia and send axons to the periphery to innervate effector organs. They project into the superficial lamina of the spinal cord dorsal horn before synapsing with secondary neurons. These secondary neurons can excite or inhibit interneurons that synpase onto motor neurons (typically to mediate spinal reflexes like the withdrawal reflex or crossed extensor reflex). Previous work done in cat models has shown that activating these afferents results in activation and modulation of fictive locomotor patterns (Kniffki, Schomburg, & Steffens, 1981; Schomburg, Steffens, & Wada, 2001).

Our laboratory has previously reported that the stretch-induced locomotor deficits in SCI rat models is dependent on nociceptive afferent activation. Animals that were depleted of TRPV1+ C fibers do not experience the drastic drops in locomotion associated with stretching when compared to control animals. The studies presented in this dissertation also support this hypothesis as all groups that received stretching had higher than baseline levels of CGRP+ fiber area and also experienced signficant locomotor dysfunction. Keller et al. also reported on

the increased activation of nociceptive afferents leading to an increase in c-Fos+ (marker of novel neuronal activity) neurons in the intermediate gray matter, specifically areas that are related to generation of motor control (Keller et al., 2019). This observation implies that the activation of locomotor centers via nociceptive afferents is somehow inherently different from the activation of the locomotor center that occurs with normal stepping.

Interestingly, throughout most of our stretching related studies we have consistently noted that the stretching of hindlimb muscles illicites motor responses from both the limb being stretched (ipsilateral) and the unstretched (contralateral) limb. They are typically either spastic or rhythmic in nature. We have denoted these movements as kicking, spasms, and “air stepping.” Air stepping refers to a rhythmic and repetitive flexion and extension of the hips and knees of the contralateral, unstretched limb that is similar to a stepping pattern. Previous work done by Keller and colleagues explored the electromyographic responses of each leg during stretching sessions. The authors reported that the “kicking” pattern we typically see (both ipsilateral and contralateral) has a strong EMG pattern that is similar to clonus in human patients. The frequency and strength of these EMG patterns increased throughout the stretching weeks. Air stepping did not result in detectable EMG bursts in most instances, only when the air stepping was classified as more intense by the therapist, The authors also reported spasm-like patterns that typically occurred near the end of the 1 minute stretch hold (Keller et al., 2018). Other researchers have also reported on rhythmic motor patterns in response to nociceptive stimuli. A 2014 paper demonstrated that rats with thoracic contusions

engaged in spastic, kicking-like behavior (measured via EMG) when mechnically stimulated (van Gorp et al., 2014). Because we now believe that stretching activates nociceptive afferents, it is possible that the responses we see during stretching are the consequence of nociceptive signaling. Based on the current data, we hypothesize that the nociceptive afferents in the dorsal horn have an excitatory effect on the locomotor circuitry in the lumbar cord, likely via secondary interneurons.

Possible Mechanism for Acute Decreases in Locomotor Function Due to Stretching

Research done on human subjects demonstrates decreased force and power output after both intense exercise and flexibility training (Ogura, Miyahara, Naito, Katamoto, & Aoki, 2007; Powers & Jackson, 2008; Robbins & Scheuermann, 2008). This phenomenon has been attributed to a few different mechanisms, including central fatigue and peripheral fatigue. Central fatigue is a type of fatigue that is driven by changes within the central nervous system (McKenna & Hargreaves, 2008). Typically, central fatigue is associated with a decrease in voluntary activation of the skeletal muscles due to reduced drive from the motor cortex leading to decreases in frequency of firing and synchronization of motor neurons (Zając, Chalimoniuk, Maszczyk, Gołaś, & Lngfort, 2015) (Davis & Bailey, 1997; Rattray, Argus, Martin, Northey, & Driller, 2015; Tornero-Aguilera, Jimenez-Morcillo, Rubio-Zarapuz, & Clemente-Suárez, 2022). The most widely recognized theories underlying central fatigue involve the monoamine neurotransmitters: serotonin, dopamine, and noradrenaline (Meeusen, Watson,

Hasegawa, Roelands, & Piacentini, 2006). It has been suggested that a high ratio of serotonin to dopamine is correlated with perceptions of fatigue and tiredness in human subjects. Serotonin receptor agonists have also been found to reduce motor neuron excitability (D'Amico et al., 2017). On the other hand, peripheral fatigue (sometimes also referred to as muscle fatigue) occurs when the skeletal muscle no longer reacts to a stimulus with the same level of contractile activity output. This is often due to changes at or beyond the neuromuscular junction. Decreases in bioenergetic substrates, accumulation of metabolic byproducts, and a decrease in calcium reuptake by the sarcoplasmic reticulum have all been associated with peripheral fatigue (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016; Zając et al., 2015). Interestingly, group III (Aδ fibers) and IV (C fibers) afferents have also been implicated in fatigue mechanisms. Feedback from these afferents during exercise increases cardiorespiratory output as well as skeletal muscle blood flow in order to ensure adequate oxygen delivery to working tissues (Amann, Sidhu, Weavil, Mangum, & Venturelli, 2015). Furthermore, group III and IV afferents have been shown to have inhibitory effects on motor neurons as well as an involvement with central fatigue via decreased corticospinal drive and reduced motor output (Sidhu et al., 2017). If stretching is activating group III and IV afferents it is possible that the acute drop in BBB scores (typically within minutes to hours after stretching) could be mediated by these central fatigue mechanisms, while chronic locomotor deficits are being mediated by sprouting in the dorsal horn.

Anecdotally, our team members often report a feeling of resistance against the stretch they are placing on the animal. Naturally, this occurs most often in

animals with higher functional abilities. It also seems to occur more frequently at the beginning of the 1 minute stretch hold as well as during the first week of stretching. It is possible that this “fighting” of the stretch is causing eccentric and/or isometric contractions of the muscle attempting to be stretched. Eccentric contractions have been highly correlated with delayed onset muscle soreness (DOMS) which typically occurs 24-72 hours after a bout of exercise (Kanda et al., 2013). Acute reductions in muscle strength and power outputs have also been associated with DOMS (Smith, 1992). Therefore, it is plausible to assume that animals engaging in eccentric and/or isometric contractions during stretching sessions could be experiencing DOMS that results in acute decreases in BBB scores. While the exact mechanisms underlying DOMS are not fully understood, the discomfort is often attributed to microtrauma to the muscle tissue (Connolly, Sayers, & McHugh, 2003). The initial injury is a disruption of the sarcomeres that usually initiates an inflammatory response (Gleeson et al., 1995; Warren, Hayes, Lowe, Prior, & Armstrong, 1993). Due to the association between DOMS and muscle damage, it is important to reiterate that our stretching protocol has never been shown to cause frank muscle damage. Keller et al. determined that stretching did not induce an increase in centralized nuclei or fibrosis of the skeletal muscles after stretching, which are both indicators of muscle damage (A. V. Keller et al., 2017).

A popular form of stretching known as proprioceptive neuromuscular facilitation (PNF) stretching could also provide insight into the acute disruptions in locomotion we see post-stretching. PNF stretching involves a series of contraction

and relaxation of the muscles. The two most popular techniques are the contract-relax method and the contract-relax antagonist-contract method (Hindle, Whitcomb, Briggs, & Hong, 2012). The contract-relax method involves the participant engaging in an isometric contraction of the target muscle while pushing against a therapist before relaxing into the desired stretch. The contract-relax antagonist-contract method is similar except there is a second isometric contraction after the relaxed stretch period. This PNF technique could be similar to the reports mentioned above of possible antagonistic muscle force production and contracting against the intended stretches. PNF has been shown to decrease athletic performance when performed prior to exercise (Franco, Signorelli, Trajano, Costa, & de Oliveira, 2012; Kirmizigil, Ozcaldiran, & Colakoglu, 2014). The exact mechanism by which PNF stretching brings about changes in range of motion is currently unknown, but there are multiple theories being explored including autogenic inhibition and reciprocal inhibition. The autogenic inhibition theory suggests that activation of the Golgi tendon organs via the production of tension and force during the contract phase of PNF results in activation of the Golgi tendon reflex and subsequent relaxation of the agonist muscle. The reciprocal inhibition theory suggests that when the antagonist motor neuron pool is activated, the agonist motor neurons will be inhibited. This helps to prevent agonist and antagonist muscles from working in opposition to each other. In the contract-relax-antagonist contract method this would assist with relaxation of the target muscle by allowing the muscle fibers to elongate even further (Hindle et al., 2012). If either or both of these theories are applicable to our stretching protocol, it would be

feasible to assume that activation of either autogenic inhibition and/or reciprocal inhibition would result in decreased force output and thereforce decreased locomotor scores in our animals.

Future Direction: Unilateral & Single Joint Stretching

We have now established that our full stretching protocol results in increased CGRP+ fiber sprouting as well as decreased locomotor function after SCI in a variety of conditions. In order to aid in establishing an in-depth understanding of this mechanism, future studies should consider utilizing either single-sided stretching or single-joint stretching. Specifically, we should focus on any functional changes in contralateral limbs. For example, would stretching only the right side hindlimb result in deficits on only the right side (suggesting a local mediator) or would it induce deficits on both sides (suggesting CGRP+ fiber sprouting in both left and right dorsal horns or some other global mediator)? If deficits occurred on both sides within the first few days of stretching, we would need to explore central mechanisms that could explain the anatomical and functional specificity of the stretch effect. Additionally, it would be important to track these changes over time. It is possible that acute changes in locomotion could be attributed to local mediators, while long term, chronic changes could be the result of sprouting and/or global mediators. We hypothesize that at the onset of stretching, deficits would be localized to the side being stretched, but over time as fiber sprouting begins to happen more bilateral deficits would occur.

Additionally, future studies should consider stretching only one joint of the hindlimb. Currently, our stretching protocol focuses on stretching the hips, knees, and ankles. Anecdotally, we have seen that poor control of the hips seems to be the first functional side effect of stretching to appear and the last ability animals regain after stopping stretching. After the first week of stretching, we have consistently noted that animals will have decent control and mobility of the ankles and knees (as indicated by BBB scores, kinematics, and laboratory notes), but will struggle to flex and adduct the hips in order to achieve plantar placement of the feet. Knees and hips will move in a sweeping fashion, but the hips will stay in a flat, abducted position with little movement. After stopping stretching, we have noticed a similar pattern as animals regain locomotor function, meaning that hip control is the last to return but knee and ankle control return much quicker. It is possible that the hips seems to have a greater effect on locomotion because their movement helps initiate the hip flexion and extension needed to begin the swing phase of the gait cycle. It is also possible that because the hips are innervated by a nerve supply that is higher in the lumbar spinal cord and therefore, closer to the CPG that it is causing a greater effect on locomotion than the knees and ankles. Again, it would be important to track these changes over time to determine how long deficits persist for each joint. We hypothesize that animals that receive hips-only stretching would have poor locomotor scores when compared to animals that received ankles-only stretching.

Future Direction: Timing & Type of Exercise

Previous studies have established the time course for afferent sprouting reduction via exercise intervention (Detloff et al., 2014) as well as the time course for the stretching effect on locomotion (A. V. Keller et al., 2017). In the current studies, we attempted to utilize this established knowledge by implementing acute exercise as a "primer" prior to beginning stretching, meaning that we hoped the applied exercise that began early and continued throughout the stretching protocol would ameloriate the locomotor deficits because we had induced a reduction in the CGRP+ fiber sprouting that occurs spontaneously after spinal cord injury. Unfortunately, either the exercise modality and/or injury model we chose was unresponsive to the acute exercise or the stretching effect on fiber sprouting was so strong that we did not see differences in CGRP+ fiber sprouting or locomotion at the end of the study.

In order to address these potential pitfalls, future studies should consider altering the time course of interventions. For example, we could consider a group that begins both exercise and stretching at acute time points after injury as well as a group that begins exercise acutely, but stops exercise intervention at the time of stretching beginning. The acute exercise + acute stretching group would help determine if stretching and exercise have a more competitive effect when implemented at the same time period when we know that afferent plasticity is occuring spontaneously. The acute exercise followed by stretching group would help us further understand why we saw such drastic drops in locomotion in our LC + Ex group in the current study. If an acute exercise followed by stretching group

showed less drastic decreases in locomotor function, we could speculate that stretching and exercise combined provided too much afferent feedback which led to the poor outcomes. A study like this could also help us identify potential mechanisms for the stretch effect. We have yet to isolate whether the mechanism that drives spontaneous afferent sprouting post-injury is similar to the mechanism that drives stretching-induced afferent sprouting.

As discussed previously, the type of exercise paradigm we chose likely accounts for the different outcomes of the current studies vs previous literature. The shallow water walking protocol is a partially loaded exercise because of the body weight support provided by the water in the tank. It would be interesting to see the differences when utilizing a fully loaded exercise protocol, like wheel walking or treadmill training, and a fully hindlimb unloaded exercise, like swimming. Both of these modalities are likely to be a higher intensity than the paradigm we chose as well. In order to utilize the fully hindlimb loaded exercises we would likely need to consider a less severe injury model in order to avoid complications and injuries in the animals. Another exercise modality that could be explored while utilizing the same moderate-severe contusion model described in these studies is passive hindlimb cycling. While this paradigm would be low intensity because the cycle is motorized, it is extremely clinically relevant and has been shown to be beneficial in regards to cardiovascular outcomes after spinal cord injury (DeVeau et al., 2017).

Relevance of Injury Location & Severity in Regards to Stretching Therapy

Prior to the current studies, we hypothesized that a greater distance between injury epicenter and the lumbar circuitry that is being activated during stretching could possibly alter the abherrant plasticity that seems to be a prerequisite for stretch-induced locomotor deficits. We had hypothesized that we may see less drastic drops in locomotor function in our animals with high thoracic (T2) injuries that received stretching therapy. Interestingly, we did see drastic drops in locomotor function that were similar to previous studies done in T10 injured animals, however the animals showed much more significant recovery over the weekend break than we had seen before. These animals also had similar amounts of CGRP+ fiber sprouting when compared to our previous T10 animals. These results suggest the idea that some other mechanism is modulating the weekend recovery, not the afferent sprouting (or lack thereof) itself.

Based on the data presented in this dissertation and anecdotal evidence from our lab, we believe that while stretching does negatively impact locomotor recovery via nociceptive afferents, there are likely additional mechanisms modulating the decreases in locomotor activity seen acutely. Acute drops in BBB scores occur as soon as 1 hour post-stretching and scores continue to decline throughout the first week of stretching. It is highly unlikely that afferent sprouting is occuring this soon after stretching. One potential mechanism that could be mediating the weekend recovery phenomenon is inflammation caused by stretching-induced DOMS. A 2013 study reported that upregulation of cyclooxygenase (COX2) and glial cell line-derived neurotrophic factor (GDNF)

after lengthening contractions is associated with in increase in DOMS. COX2 is an enzyme utilized in the production of prostaglandins and is associated with inflammatory and pain pathways. It is constituitively expressed in the CNS (Burian & Geisslinger, 2005). However, the addition of a COX2 inhibitor prior to exercise suppressed the development of DOMS (Murase et al., 2013). Interestingly, our laboratory recently completed a pilot study demonstrating the beneficial effects of the admistration of ibuprofen (a nonselective COX inhibitor) prior to stretching. Rats that were administered ibuprofen prior to stretching had improved locomotor scores when compared to non-medicated rats (preliminary data, Morgan Sharp). We hypothesize that any lengthening contractions occuring during stretching could be resulting in DOMS that causes inflammation and reduced force production. Because DOMS usually resolves within a few days with no additional intervention, this could be why we see improved BBB scores on Monday mornings after 2 days of recovery and no stretching.

Additionally, we also saw that the unstretched, T2 injured group had a similar amount of CGRP+ fiber sprouting. To our knowledge, this is the first study to quantify CGRP+ fiber sprouting in the lumbar cord of T2 injured animals, so it appears that stretching these animals did not induce any additional sprouting. However, both groups had similar sprouting area to our previous T10 injured and stretched animals, suggesting that T2 animals naturally have a higher level of spontaneous afferent sprouting post-injury. We believe it is possible that the reduced in-cage activity and lack of body weight support demonstrated in our T2 injured animals could be the cause of the increased spontaneous sprouting.

Because increases in activity have been implicated in the reduction of decreased afferent sprouting in other studies, it is likely that inactivity has a role in increased sprouting. While the data presented in this dissertation does not indicate that housing animals in tiny cages produces increased sprouting, it is important to note that our overnight activity monitoring system does not fully differentiate between stepping/body weight support and non-stepping movement. Our animals with more severe high thoracic injuries may have traveled similar distances overnight when compared to T10 injured animals housed in tiny cages, but the actual activity looks much different with the T2 injured animals unable to achieve consistent, coordinated plantar stepping.

If peripheral inflammation is being induced in our stretched animals as previously discussed, it is important to also discuss the relationship between nociceptors and the immune system. Nociceptor terminals in peripheral tissues (like skeletal muscles) express receptor sites for inflammatory mediators like cytokines and chemokines that are released from nearby immune cells in response to injury (McMahon, La Russa, & Bennett, 2015). The exact mechanisms are not fully understood, but binding of these inflammatory mediators to nociceptors causes increased excitability and hypersensitivity of the nociceptor, likely due to enhanced voltage- and ligand-gated ion channel activation (Marchand, Perretti, & McMahon, 2005). In addition, neurogenic inflammation can arise via release of local inflammatory mediators from the afferent terminals such as Substance P and CGRP (Geppetti, Nassini, Materazzi, & Benemei, 2008). This release of inflammatory mediators triggers vasodilation and increased capillary permeability,

leading to additional migration of macrophages and white blood cells to the area. They also directly activate the innate and adaptive immune systems, which in this case could amplify maladaptive immune responses by causing release of additional inflammatory mediators that sensitize the nociceptors (Corrigan, Mander, Leonard, & Vink, 2016; Marchand et al., 2005).

Interestingly, COX 1/2 secretes prostaglandins that act on receptors sites on nociceptors leading to increased sodium flux and sensitized nerve terminals (Marchand et al., 2005; McMahon et al., 2015). As previously discussed, COX is upregulated in response to DOMS, so if our stretching-induced DOMS theory is correct it is feasible to assume that this is a contributing factor to sensitized nociceptors that are interfering with locomotor function. This also provides additional evidence for the improved locomotion we saw in response to pre-stretching administration of ibuprofen. In the future, we should consider measuring prostagladin levels post-stretching to determine if DOMS could be a contributing factor to the stretch effect.

Finally, if peripheral inflammation is driving the hypersensitization of nociceptors, this could help explain the differences in weekend recovery we have demonstrated. Both DOMS and exercise have been implicated in increased peripheral inflammation (Febbraio, 2007). Because our T10 injured animals were capable of more stepping than our T2 injured animals, the increased activity could have been contributing to increased peripheral inflammation leading to worse locomotor outcomes. This also helps to explain why we saw such poor locomotor function in our animals receiving exercise intervention. On the other hand, our T2

injured animals experienced much less activity of the hindlimbs which could have lead to decreased inflammation and improved post-weekend recovery BBB scores.

Stretching Rodents vs Humans and the Future of Stretching after SCI

There have been multiple studies to date that have determined that stretching previously immobilized muscles in rodent models leads to prevention of muscle shortening (Williams, 1988, 1990). However, these types of studies are done in non-SCI rodent models and thus do not have the clinical, locomotor and functional outcomes of our current studies. Additionally, the sprouting of nociceptors is not likely to be occuring in these non-injured animal models. However, studies similar to these have been used to inform clinical practice and provide rationale for stretching humans even with their lack of relevance.

Another common rationale for including stretching in rehabilitation protocols in humans with SCI is the data found in non-injured human subjects literature. As was previously discussed in the introduction, it is a well-established fact that in healthy, able-bodied individuals, stretching brings about rapid increases in range of motion. There are a few commonly recognized mechanisms that are implicated in stretch-induced increases in range of motion, however the actual field has not reached a consesus. First it was suggested that deformation of the skeletal muscles themselves and tissues at the muscle-tendon junction can bring about increased range of motion (de Weijer, Gorniak, & Shamus, 2003; Weppler & Magnusson, 2010), typically refered to as the viscoelastic and/or plastic

deformation theories. Neuromuscular relaxation, as implicated above in propioceptive neuromuscular facilitation stretching, is also a commonly discussed mechanism. Interestingly, a new proposed mechanism has emerged in recent years known as the sensory theory of muscle flexibility. This theory proposes that increases in range of motion of skeletal muscle are due to changes of sensation, and not due to changes in muscle length (Weppler & Magnusson, 2010). Typically, the amount by which a subject can force elongation of the muscle is regulated by neurological safeties, meaning that muscle spindles will cause contraction of the agonist muscle if too much stretch in the muscle is sensed. The sensory theory implies that the nervous system can adapt and respond to stretching in order to allow the muscle to elongate farther simply by tolerating the stretch more. When applying the above theories to a person with spinal cord injury, however, it becomes clear that the mechanisms by which stretching brings about increases in range of motion are likely disrupted when compared to non-injured individuals. Lack of strength, control, and paralysis of the skeletal muscles brought about by SCI would likely disrupt the mechanisms described in the viscoelastic and plastic theories. Furthermore, both the neuromuscular relaxation and sensory theories rely on the presence of an intact nervous system. It is clear that any of the proposed mechanisms by how stretching works in able-bodied individuals cannot be directly applied to SCI patients and likely explains the differences in outcomes between the two populations.

While stretching my be useful for temporarily reducing spasticity and contractures, the literature is clear that there are no long-term benefits to stretching

SCI patients. Furthermore, all of the data from our lab suggests that stretching may have quite the opposite effect, especially in regards to locomotor recovery and motor function post-injury. It is important that clinical researchers now establish the translatability and relevance of this pre-clinical model research. The data presented in this dissertation in combination with the previous data from our lab strongly argues that stretching has a negative impact on locomotor function and these findings should be further explored in humans in order to provide the best care possible for SCI patients.

REFERENCES

Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., & Fehlings, M. G. (2017). Traumatic spinal cord injury. *Nat Rev Dis Primers, 3*, 17018. doi:10.1038/nrdp.2017.18

Alizadeh, A., Dyck, S. M., & Karimi-Abdolrezaee, S. (2019). Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. *Front Neurol, 10*, 282. doi:10.3389/fneur.2019.00282

Almad, A., Sahinkaya, F. R., & McTigue, D. M. (2011). Oligodendrocyte fate after spinal cord injury. *Neurotherapeutics, 8*(2), 262-273. doi:10.1007/s13311-011-0033-5

Amann, M., Sidhu, S. K., Weavil, J. C., Mangum, T. S., & Venturelli, M. (2015). Autonomic responses to exercise: group III/IV muscle afferents and fatigue. *Auton Neurosci, 188*, 19-23. doi:10.1016/j.autneu.2014.10.018

Anderson, J. P., Snow, B., Dorey, F. J., & Kabo, J. M. (1988). Efficacy of soft splints in reducing severe knee-flexion contractures. *Dev Med Child Neurol, 30*(4), 502-508. doi:10.1111/j.1469-8749.1988.tb04777.x

Anjum, A., Yazid, M. D., Fauzi Daud, M., Idris, J., Ng, A. M. H., Selvi Naicker, A., . . . Lokanathan, Y. (2020). Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. *Int J Mol Sci, 21*(20). doi:10.3390/ijms21207533

Barbosa, P. H. F. A., Glinsky, J. V., Fachin-Martins, E., & Harvey, L. A. (2021). Physiotherapy interventions for the treatment of spasticity in people with spinal cord injury: a systematic review. *Spinal Cord, 59*(3), 236-247. doi:10.1038/s41393-020-00610-4

Basso, D. M., Beattie, M. S., & Bresnahan, J. C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma, 12*(1), 1-21. doi:10.1089/neu.1995.12.1

Basso, D. M., Beattie, M. S., & Bresnahan, J. C. (1996). Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol, 139*(2), 244-256. doi:10.1006/exnr.1996.0098

Basso, D. M., Beattie, M. S., Bresnahan, J. C., Anderson, D. K., Faden, A. I., Gruner, J. A., . . . Young, W. (1996). MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multicenter Animal Spinal Cord Injury Study. *J Neurotrauma, 13*(7), 343-359. doi:10.1089/neu.1996.13.343

Behrman, A. L., & Harkema, S. J. (2000). Locomotor training after human spinal cord injury: a series of case studies. *Phys Ther, 80*(7), 688-700.

Bellingham, M. C. (1998). Driving respiration: the respiratory central pattern generator. *Clin Exp Pharmacol Physiol, 25*(10), 847-856. doi:10.1111/j.1440-1681.1998.tb02166.x

Birmingham, U. o. A. a. (2020). National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance.

Bixler, B., & Jones, R. L. (1992). High-school football injuries: effects of a post-halftime warm-up and stretching routine. *Fam Pract Res J, 12*(2), 131-139.

Booth, B. J., Doyle, M., & Montgomery, J. (1983). Serial casting for the management of spasticity in the head-injured adult. *Phys Ther, 63*(12), 1960-1966. doi:10.1093/ptj/63.12.1960

Bovend'Eerdt, T. J., Newman, M., Barker, K., Dawes, H., Minelli, C., & Wade, D. T. (2008). The effects of stretching in spasticity: a systematic review. *Arch Phys Med Rehabil, 89*(7), 1395-1406. doi:10.1016/j.apmr.2008.02.015

Brown, T. G. (1911). The Intrinsic Factors in the Act of Progression in the Mammal In (Vol. 84): Proceedings of the Royal Society of London.

Burian, M., & Geisslinger, G. (2005). COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites. *Pharmacol Ther, 107*(2), 139-154. doi:10.1016/j.pharmthera.2005.02.004

Bélanger, M., Drew, T., Provencher, J., & Rossignol, S. (1996). A comparison of treadmill locomotion in adult cats before and after spinal transection. *J Neurophysiol, 76*(1), 471-491. doi:10.1152/jn.1996.76.1.471

Cao, Y., Chen, Y., & DeVivo, M. (2011). Liftetime Direct Costs After Spinal Cord Injury. In (Vol. 16): Topics in Spinal Cord Injury Rehabilitation.

Caudle, K. L., Atkinson, D. A., Brown, E. H., Donaldson, K., Seibt, E., Chea, T., . . . Magnuson, D. S. (2015). Hindlimb stretching alters locomotor function after spinal cord injury in the adult rat. *Neurorehabil Neural Repair, 29*(3), 268-277. doi:10.1177/1545968314543500

Caudle, K. L., Brown, E. H., Shum-Siu, A., Burke, D. A., Magnuson, T. S., Voor, M. J., & Magnuson, D. S. (2011). Hindlimb immobilization in a wheelchair alters functional recovery following contusive spinal cord injury in the adult rat. *Neurorehabil Neural Repair, 25*(8), 729-739. doi:10.1177/1545968311407519

Chhaya, S. J., Quiros-Molina, D., Tamashiro-Orrego, A. D., Houle, J. D., & Detloff, M. R. (2019). Exercise-Induced Changes to the Macrophage Response in the Dorsal Root Ganglia Prevent Neuropathic Pain after Spinal Cord Injury. *J Neurotrauma, 36*(6), 877-890. doi:10.1089/neu.2018.5819

Connolly, D. A., Sayers, S. P., & McHugh, M. P. (2003). Treatment and prevention of delayed onset muscle soreness. *J Strength Cond Res, 17*(1), 197-208. doi:10.1519/1533-4287(2003)0172.0.co;2

Cooper, J. E., Shwedyk, E., Quanbury, A. O., Miller, J., & Hildebrand, D. (1993). Elbow joint restriction: effect on functional upper limb motion during performance of three feeding activities. *Arch Phys Med Rehabil, 74*(8), 805-809. doi:10.1016/0003-9993(93)90005-u

Corrigan, F., Mander, K. A., Leonard, A. V., & Vink, R. (2016). Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. *J Neuroinflammation, 13*(1), 264. doi:10.1186/s12974-016-0738-9

Cragg, J., & Krassioukov, A. (2012). Autonomic dysreflexia. *CMAJ, 184*(1), 66. doi:10.1503/cmaj.110859

D'Amico, J. M., Butler, A. A., Héroux, M. E., Cotel, F., Perrier, J. M., Butler, J. E., . . . Taylor, J. L. (2017). Human motoneurone excitability is depressed by activation of serotonin 1A receptors with buspirone. *J Physiol, 595*(5), 1763-1773. doi:10.1113/JP273200

Davis, J. M., & Bailey, S. P. (1997). Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc, 29*(1), 45-57. doi:10.1097/00005768-199701000-00008

de Weijer, V. C., Gorniak, G. C., & Shamus, E. (2003). The effect of static stretch and warm-up exercise on hamstring length over the course of 24 hours. *J Orthop Sports Phys Ther, 33*(12), 727-733. doi:10.2519/jospt.2003.33.12.727

Detloff, M. R., Quiros-Molina, D., Javia, A. S., Daggubati, L., Nehlsen, A. D., Naqvi, A., . . . Houle, J. D. (2016). Delayed Exercise Is Ineffective at Reversing Aberrant Nociceptive Afferent Plasticity or Neuropathic Pain After Spinal Cord Injury in Rats. *Neurorehabil Neural Repair, 30*(7), 685-700. doi:10.1177/1545968315619698

Detloff, M. R., Smith, E. J., Quiros Molina, D., Ganzer, P. D., & Houle, J. D. (2014). Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF- and artemin-responsive) c-fibers after spinal cord injury. *Exp Neurol, 255*, 38-48. doi:10.1016/j.expneurol.2014.02.013

Detloff, M. R., Wade, R. E., & Houlé, J. D. (2013). Chronic at- and below-level pain after moderate unilateral cervical spinal cord contusion in rats. *J Neurotrauma, 30*(10), 884-890. doi:10.1089/neu.2012.2632

DeVeau, K. M., Harman, K. A., Squair, J. W., Krassioukov, A. V., Magnuson, D. S. K., & West, C. R. (2017). A comparison of passive hindlimb cycling and active upper-limb exercise provides new insights into systolic dysfunction after spinal cord injury. *Am J Physiol Heart Circ Physiol, 313*(5), H861-H870. doi:10.1152/ajpheart.00046.2017

Devillard, X., Rimaud, D., Roche, F., & Calmels, P. (2007). Effects of training programs for spinal cord injury. *Ann Readapt Med Phys, 50*(6), 490-498, 480-499. doi:10.1016/j.annrmp.2007.04.013

Dubin, A. E., & Patapoutian, A. (2010). Nociceptors: the sensors of the pain pathway. *J Clin Invest, 120*(11), 3760-3772. doi:10.1172/JCI42843

Dumont, R. J., Okonkwo, D. O., Verma, S., Hurlbert, R. J., Boulos, P. T., Ellegala, D. B., & Dumont, A. S. (2001). Acute spinal cord injury, part I: pathophysiologic mechanisms. *Clin Neuropharmacol, 24*(5), 254-264. doi:10.1097/00002826-200109000-00002

Elliott, S., & Krassioukov, A. (2006). Malignant autonomic dysreflexia in spinal cord injured men. *Spinal Cord, 44*(6), 386-392. doi:10.1038/sj.sc.3101847

Febbraio, M. A. (2007). Exercise and inflammation. *J Appl Physiol (1985), 103*(1), 376-377. doi:10.1152/japplphysiol.00414.2007

Ferguson, A. R., Crown, E. D., & Grau, J. W. (2006). Nociceptive plasticity inhibits adaptive learning in the spinal cord. *Neuroscience, 141*(1), 421-431. doi:10.1016/j.neuroscience.2006.03.029

Ferguson, A. R., Huie, J. R., Crown, E. D., Baumbauer, K. M., Hook, M. A., Garraway, S. M., . . . Grau, J. W. (2012). Maladaptive spinal plasticity opposes spinal learning and recovery in spinal cord injury. *Front Physiol, 3*, 399. doi:10.3389/fphys.2012.00399

Fergusson, D., Hutton, B., & Drodge, A. (2007). The epidemiology of major joint contractures: a systematic review of the literature. *Clin Orthop Relat Res, 456*, 22-29. doi:10.1097/BLO.0b013e3180308456

Field-Fote, E. C., & Roach, K. E. (2011). Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: a randomized clinical trial. *Phys Ther, 91*(1), 48-60. doi:10.2522/ptj.20090359

Franco, B. L., Signorelli, G. R., Trajano, G. S., Costa, P. B., & de Oliveira, C. G. (2012). Acute effects of three different stretching protocols on the wingate test performance. *J Sports Sci Med, 11*(1), 1-7.

Frigon, A. (2012). Central pattern generators of the mammalian spinal cord. *Neuroscientist, 18*(1), 56-69. doi:10.1177/1073858410396101

Furlan, J. C., Fehlings, M. G., Shannon, P., Norenberg, M. D., & Krassioukov, A. V. (2003). Descending vasomotor pathways in humans: correlation between axonal preservation and cardiovascular dysfunction after spinal

cord injury. *J Neurotrauma, 20*(12), 1351-1363. doi:10.1089/089771503322686148

Geppetti, P., Nassini, R., Materazzi, S., & Benemei, S. (2008). The concept of neurogenic inflammation. *BJU Int, 101 Suppl 3*, 2-6. doi:10.1111/j.1464-410X.2008.07493.x

Gleeson, M., Almey, J., Brooks, S., Cave, R., Lewis, A., & Griffiths, H. (1995). Haematological and acute-phase responses associated with delayed-onset muscle soreness in humans. *Eur J Appl Physiol Occup Physiol, 71*(2-3), 137-142. doi:10.1007/BF00854970

Gorgey, A. S., Chiodo, A. E., Zemper, E. D., Hornyak, J. E., Rodriguez, G. M., & Gater, D. R. (2010). Relationship of spasticity to soft tissue body composition and the metabolic profile in persons with chronic motor complete spinal cord injury. *J Spinal Cord Med, 33*(1), 6-15. doi:10.1080/10790268.2010.11689669

Grau, J. W., Huang, Y. J., Turtle, J. D., Strain, M. M., Miranda, R. C., Garraway, S. M., & Hook, M. A. (2017). When Pain Hurts: Nociceptive Stimulation Induces a State of Maladaptive Plasticity and Impairs Recovery after Spinal Cord Injury. *J Neurotrauma, 34*(10), 1873-1890. doi:10.1089/neu.2016.4626

Grau, J. W., Huie, J. R., Lee, K. H., Hoy, K. C., Huang, Y. J., Turtle, J. D., . . . Garraway, S. M. (2014). Metaplasticity and behavior: how training and inflammation affect plastic potential within the spinal cord and recovery after injury. *Front Neural Circuits, 8*, 100. doi:10.3389/fncir.2014.00100

Grover, J., Gellman, H., & Waters, R. L. (1996). The effect of a flexion contracture of the elbow on the ability to transfer in patients who have quadriplegia at the sixth cervical level. *J Bone Joint Surg Am, 78*(9), 1397-1400. doi:10.2106/00004623-199609000-00016

Harman, K. A., States, G., Wade, A., Stepp, C., Wainwright, G., DeVeau, K., . . . Magnuson, D. S. K. (2018). Temporal analysis of cardiovascular control and function following incomplete T3 and T10 spinal cord injury in rodents. *Physiol Rep, 6*(6), e13634. doi:10.14814/phy2.13634

Harvey, L., & Crosbie, J. (2001). Effect of elbow flexion contractures on the ability of people with C5 and C6 tetraplegia to lift. *Physiother Res Int, 6*(2), 76-82. doi:10.1002/pri.216

Harvey, L., de Jong, I., Goehl, G., & Mardwedel, S. (2006). Twelve weeks of nightly stretch does not reduce thumb web-space contractures in people with a neurological condition: a randomised controlled trial. *Aust J Physiother, 52*(4), 251-258. doi:10.1016/s0004-9514(06)70004-6

Harvey, L. A., Batty, J., Crosbie, J., Poulter, S., & Herbert, R. D. (2000). A randomized trial assessing the effects of 4 weeks of daily stretching on ankle mobility in patients with spinal cord injuries. *Arch Phys Med Rehabil, 81*(10), 1340-1347. doi:10.1053/apmr.2000.9168

Harvey, L. A., Batty, J., Jones, R., & Crosbie, J. (2001). Hand function of C6 and C7 tetraplegics 1 - 16 years following injury. *Spinal Cord, 39*(1), 37-43. doi:10.1038/sj.sc.3101101

Harvey, L. A., Byak, A. J., Ostrovskaya, M., Glinsky, J., Katte, L., & Herbert, R. D. (2003). Randomised trial of the effects of four weeks of daily stretch on extensibility of hamstring muscles in people with spinal cord injuries. *Aust J Physiother, 49*(3), 176-181. doi:10.1016/s0004-9514(14)60237-3

Harvey, L. A., Glinsky, J. A., Katalinic, O. M., & Ben, M. (2011). Contracture management for people with spinal cord injuries. *NeuroRehabilitation, 28*(1), 17-20. doi:10.3233/nre-2011-0627

Harvey, L. A., & Herbert, R. D. (2002). Muscle stretching for treatment and prevention of contracture in people with spinal cord injury. *Spinal Cord, 40*(1), 1-9. doi:10.1038/sj.sc.3101241

Harvey, L. A., McQuade, L., Hawthorne, S., & Byak, A. (2003). Quantifying the magnitude of torque physiotherapists apply when stretching the hamstring muscles of people with spinal cord injury. *Arch Phys Med Rehabil, 84*(7), 1072-1075. doi:10.1016/s0003-9993(03)00131-x

Herbert, R. (1988). The passive mechanical properties of muscle and their adaptations to altered patterns of use. *Aust J Physiother, 34*(3), 141-149. doi:10.1016/S0004-9514(14)60606-1

Herbison, G. J., Jaweed, M. M., & Ditunno, J. F. (1978). Muscle fiber atrophy after cast immobilization in the rat. *Arch Phys Med Rehabil, 59*(7), 301-305.

Hetz, S. P., Latimer, A. E., & Ginis, K. A. (2009). Activities of daily living performed by individuals with SCI: relationships with physical fitness and

leisure time physical activity. *Spinal Cord, 47*(7), 550-554. doi:10.1038/sc.2008.160

Hicks, A. L., Martin Ginis, K. A., Pelletier, C. A., Ditor, D. S., Foulon, B., & Wolfe, D. L. (2011). The effects of exercise training on physical capacity, strength, body composition and functional performance among adults with spinal cord injury: a systematic review. *Spinal Cord, 49*(11), 1103-1127. doi:10.1038/sc.2011.62

Hicks, A. L., Martin, K. A., Ditor, D. S., Latimer, A. E., Craven, C., Bugaresti, J., & McCartney, N. (2003). Long-term exercise training in persons with spinal cord injury: effects on strength, arm ergometry performance and psychological well-being. *Spinal Cord, 41*(1), 34-43. doi:10.1038/sj.sc.3101389

Hindle, K. B., Whitcomb, T. J., Briggs, W. O., & Hong, J. (2012). Proprioceptive Neuromuscular Facilitation (PNF): Its Mechanisms and Effects on Range of Motion and Muscular Function. *J Hum Kinet, 31*, 105-113. doi:10.2478/v10078-012-0011-y

Hou, S., Duale, H., & Rabchevsky, A. G. (2009). Intraspinal sprouting of unmyelinated pelvic afferents after complete spinal cord injury is correlated with autonomic dysreflexia induced by visceral pain. *Neuroscience, 159*(1), 369-379. doi:10.1016/j.neuroscience.2008.12.022

Jacobs, P. L. (2009). Effects of resistance and endurance training in persons with paraplegia. *Med Sci Sports Exerc, 41*(5), 992-997. doi:10.1249/MSS.0b013e318191757f

Jean, A. (2001). Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev, 81*(2), 929-969. doi:10.1152/physrev.2001.81.2.929

Julius, D., & Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature, 413*(6852), 203-210. doi:10.1038/35093019

Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., . . . Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev, 19*, 72-85.

Katalinic, O. M., Harvey, L. A., & Herbert, R. D. (2011). Effectiveness of stretch for the treatment and prevention of contractures in people with neurological conditions: a systematic review. *Phys Ther, 91*(1), 11-24. doi:10.2522/ptj.20100265

Katz, R. T., & Rymer, W. Z. (1989). Spastic hypertonia: mechanisms and measurement. *Arch Phys Med Rehabil, 70*(2), 144-155.

Kawanishi, C. Y., & Greguol, M. (2013). Physical activity, quality of life, and functional autonomy of adults with spinal cord injuries. *Adapt Phys Activ Q, 30*(4), 317-337. doi:10.1123/apaq.30.4.317

Keller, A., Rees, K., Prince, D., Morehouse, J., Shum-Siu, A., & Magnuson, D. (2017). Dynamic "Range of Motion" Hindlimb Stretching Disrupts Locomotor Function in Rats with Moderate Subacute Spinal Cord Injuries. *J Neurotrauma, 34*(12), 2086-2091. doi:10.1089/neu.2016.4951

Keller, A. V., Hainline, C., Rees, K., Krupp, S., Prince, D., Wood, B. D., . . . Magnuson, D. S. K. (2019). Nociceptor-dependent locomotor dysfunction after clinically-modeled hindlimb muscle stretching in adult rats with spinal cord injury. *Exp Neurol, 318*, 267-276. doi:10.1016/j.expneurol.2019.03.006

Keller, A. V., Rees, K. M., Seibt, E. J., Wood, B. D., Wade, A. D., Morehouse, J., . . . Magnuson, D. S. K. (2018). Electromyographic patterns of the rat hindlimb in response to muscle stretch after spinal cord injury. *Spinal Cord, 56*(6), 560-568. doi:10.1038/s41393-018-0069-z

Keller, A. V., Wainwright, G., Shum-Siu, A., Prince, D., Hoeper, A., Martin, E., & Magnuson, D. S. (2017). Disruption of Locomotion in Response to Hindlimb Muscle Stretch at Acute and Chronic Time Points after a Spinal Cord Injury in Rats. *J Neurotrauma, 34*(3), 661-670. doi:10.1089/neu.2015.4227

Khan, F., Amatya, B., Bensmail, D., & Yelnik, A. (2019). Non-pharmacological interventions for spasticity in adults: An overview of systematic reviews. *Ann Phys Rehabil Med, 62*(4), 265-273. doi:10.1016/j.rehab.2017.10.001

Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. *Annu Rev Neurosci, 29*, 279-306. doi:10.1146/annurev.neuro.29.051605.112910

Kiehn, O. (2016). Decoding the organization of spinal circuits that control locomotion. *Nat Rev Neurosci, 17*(4), 224-238. doi:10.1038/nrn.2016.9

Kirmizigil, B., Ozcaldiran, B., & Colakoglu, M. (2014). Effects of three different stretching techniques on vertical jumping performance. *J Strength Cond Res, 28*(5), 1263-1271. doi:10.1519/JSC.0000000000000268

Kniffki, K. D., Schomburg, E. D., & Steffens, H. (1981). Effects from fine muscle and cutaneous afferents on spinal locomotion in cats. *J Physiol, 319*, 543-554. doi:10.1113/jphysiol.1981.sp013925

Kraus, K. H. (1996). The pathophysiology of spinal cord injury and its clinical implications. *Semin Vet Med Surg Small Anim, 11*(4), 201-207. doi:10.1016/s1096-2867(96)80013-2

Kwon, B. K., Tetzlaff, W., Grauer, J. N., Beiner, J., & Vaccaro, A. R. (2004). Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J, 4*(4), 451-464. doi:10.1016/j.spinee.2003.07.007

Lasfargues, J. E., Custis, D., Morrone, F., Carswell, J., & Nguyen, T. (1995). A model for estimating spinal cord injury prevalence in the United States. *Paraplegia, 33*(2), 62-68. doi:10.1038/sc.1995.16

Lindan, R., Joiner, E., Freehafer, A. A., & Hazel, C. (1980). Incidence and clinical features of autonomic dysreflexia in patients with spinal cord injury. *Paraplegia, 18*(5), 285-292. doi:10.1038/sc.1980.51

Lundeberg, T. (1995). Pain physiology and principles of treatment. *Scand J Rehabil Med Suppl, 32*, 13-41.

Marchand, F., Perretti, M., & McMahon, S. B. (2005). Role of the immune system in chronic pain. *Nat Rev Neurosci, 6*(7), 521-532. doi:10.1038/nrn1700

Maynard, F. M., Karunas, R. S., & Waring, W. P. (1990). Epidemiology of spasticity following traumatic spinal cord injury. *Arch Phys Med Rehabil, 71*(8), 566-569.

McCrea, D. A., & Rybak, I. A. (2007). Modeling the mammalian locomotor CPG: insights from mistakes and perturbations. *Prog Brain Res, 165*, 235-253. doi:10.1016/S0079-6123(06)65015-2

McCrea, D. A., & Rybak, I. A. (2008). Organization of mammalian locomotor rhythm and pattern generation. *Brain Res Rev, 57*(1), 134-146. doi:10.1016/j.brainresrev.2007.08.006

McKenna, M. J., & Hargreaves, M. (2008). Resolving fatigue mechanisms determining exercise performance: integrative physiology at its finest! *J Appl Physiol (1985), 104*(1), 286-287. doi:10.1152/japplphysiol.01139.2007

McMahon, S. B., La Russa, F., & Bennett, D. L. (2015). Crosstalk between the nociceptive and immune systems in host defence and disease. *Nat Rev Neurosci, 16*(7), 389-402. doi:10.1038/nrn3946

Meeusen, R., Watson, P., Hasegawa, H., Roelands, B., & Piacentini, M. F. (2006). Central fatigue: the serotonin hypothesis and beyond. *Sports Med, 36*(10), 881-909. doi:10.2165/00007256-200636100-00006

Morey-Holton, E., Globus, R. K., Kaplansky, A., & Durnova, G. (2005). The hindlimb unloading rat model: literature overview, technique update and comparison with space flight data. *Adv Space Biol Med, 10*, 7-40. doi:10.1016/s1569-2574(05)10002-1

Moriyama, H., Tobimatsu, Y., Ozawa, J., Kito, N., & Tanaka, R. (2013). Amount of torque and duration of stretching affects correction of knee contracture in a rat model of spinal cord injury. *Clin Orthop Relat Res, 471*(11), 3626-3636. doi:10.1007/s11999-013-3196-z

Murase, S., Terazawa, E., Hirate, K., Yamanaka, H., Kanda, H., Noguchi, K., . . . Mizumura, K. (2013). Upregulated glial cell line-derived neurotrophic factor through cyclooxygenase-2 activation in the muscle is required for mechanical hyperalgesia after exercise in rats. *J Physiol, 591*(12), 3035-3048. doi:10.1113/jphysiol.2012.249235

Murinson, B. B., & Griffin, J. W. (2004). C-fiber structure varies with location in peripheral nerve. *J Neuropathol Exp Neurol, 63*(3), 246-254. doi:10.1093/jnen/63.3.246

Nees, T. A., Tappe-Theodor, A., Sliwinski, C., Motsch, M., Rupp, R., Kuner, R., . . . Blesch, A. (2016). Early-onset treadmill training reduces mechanical allodynia and modulates calcitonin gene-related peptide fiber density in lamina III/IV in a mouse model of spinal cord contusion injury. *Pain, 157*(3), 687-697. doi:10.1097/j.pain.0000000000000422

Nemoto, A., & Goyagi, T. (2021). Tail suspension is useful as a sarcopenia model in rats. *Lab Anim Res, 37*(1), 7. doi:10.1186/s42826-020-00083-9

NSCISC. (2020). National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance.

Ogura, Y., Miyahara, Y., Naito, H., Katamoto, S., & Aoki, J. (2007). Duration of static stretching influences muscle force production in hamstring muscles. *J Strength Cond Res, 21*(3), 788-792. doi:10.1519/R-18785.1

Patrick, J. H., Farmer, S. E., & Bromwich, W. (2002). Muscle stretching for treatment and prevention of contracture in people with spinal cord injury. *Spinal Cord, 40*(8), 421-422; author reply 423. doi:10.1038/sj.sc.3101340

Pearson, K. G., & Rossignol, S. (1991). Fictive motor patterns in chronic spinal cats. *J Neurophysiol, 66*(6), 1874-1887. doi:10.1152/jn.1991.66.6.1874

Pope, R., Herbert, R., & Kirwan, J. (1998). Effects of ankle dorsiflexion range and pre-exercise calf muscle stretching on injury risk in Army recruits. *Aust J Physiother, 44*(3), 165-172. doi:10.1016/s0004-9514(14)60376-7

Pope, R. P., Herbert, R. D., Kirwan, J. D., & Graham, B. J. (2000). A randomized trial of preexercise stretching for prevention of lower-limb injury. *Med Sci Sports Exerc, 32*(2), 271-277. doi:10.1097/00005768-200002000-00004

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev, 88*(4), 1243-1276. doi:10.1152/physrev.00031.2007

Rabchevsky, A. G., & Kitzman, P. H. (2011). Latest approaches for the treatment of spasticity and autonomic dysreflexia in chronic spinal cord injury. *Neurotherapeutics, 8*(2), 274-282. doi:10.1007/s13311-011-0025-5

Rattray, B., Argus, C., Martin, K., Northey, J., & Driller, M. (2015). Is it time to turn our attention toward central mechanisms for post-exertional recovery

strategies and performance? *Front Physiol, 6*, 79. doi:10.3389/fphys.2015.00079

Robbins, J. W., & Scheuermann, B. W. (2008). Varying amounts of acute static stretching and its effect on vertical jump performance. *J Strength Cond Res, 22*(3), 781-786. doi:10.1519/JSC.0b013e31816a59a9

Roberts, T. T., Leonard, G. R., & Cepela, D. J. (2017). Classifications In Brief: American Spinal Injury Association (ASIA) Impairment Scale. *Clin Orthop Relat Res, 475*(5), 1499-1504. doi:10.1007/s11999-016-5133-4

Rossignol, S., & Frigon, A. (2011). Recovery of locomotion after spinal cord injury: some facts and mechanisms. *Annu Rev Neurosci, 34*, 413-440. doi:10.1146/annurev-neuro-061010-113746

Roy, R. R., & Edgerton, V. R. (2012). Neurobiological perspective of spasticity as occurs after a spinal cord injury. *Exp Neurol, 235*(1), 116-122. doi:10.1016/j.expneurol.2012.01.017

Rybak, I. A., Dougherty, K. J., & Shevtsova, N. A. (2015). Organization of the Mammalian Locomotor CPG: Review of Computational Model and Circuit Architectures Based on Genetically Identified Spinal Interneurons(1,2,3). *eNeuro, 2*(5). doi:10.1523/ENEURO.0069-15.2015

Schmalbruch, H. (1986). Fiber composition of the rat sciatic nerve. *Anat Rec, 215*(1), 71-81. doi:10.1002/ar.1092150111

Schomburg, E. D., Steffens, H., & Wada, N. (2001). Parallel nociceptive reflex pathways with negative and positive feedback functions to foot extensors

in the cat. *J Physiol, 536*(Pt 2), 605-613. doi:10.1111/j.1469-7793.2001.0605c.xd

Sekhon, L. H., & Fehlings, M. G. (2001). Epidemiology, demographics, and pathophysiology of acute spinal cord injury. *Spine (Phila Pa 1976), 26*(24 Suppl), S2-12. doi:10.1097/00007632-200112151-00002

Sezer, N., Akkuş, S., & Uğurlu, F. G. (2015). Chronic complications of spinal cord injury. *World J Orthop, 6*(1), 24-33. doi:10.5312/wjo.v6.i1.24

Sidhu, S. K., Weavil, J. C., Mangum, T. S., Jessop, J. E., Richardson, R. S., Morgan, D. E., & Amann, M. (2017). Group III/IV locomotor muscle afferents alter motor cortical and corticospinal excitability and promote central fatigue during cycling exercise. *Clin Neurophysiol, 128*(1), 44-55. doi:10.1016/j.clinph.2016.10.008

Smith, L. L. (1992). Causes of Delayed Onset Muscle Soreness and the Impact on Athletic Performance:A Review. In (Vol. 6, pp. 135-141). Journal of Strength and Conditioning Research.

Squair, J. W., DeVeau, K. M., Harman, K. A., Poormasjedi-Meibod, M. S., Hayes, B., Liu, J., . . . West, C. R. (2018). Spinal Cord Injury Causes Systolic Dysfunction and Cardiomyocyte Atrophy. *J Neurotrauma, 35*(3), 424-434. doi:10.1089/neu.2017.4984

Squair, J. W., West, C. R., Popok, D., Assinck, P., Liu, J., Tetzlaff, W., & Krassioukov, A. V. (2017). High Thoracic Contusion Model for the Investigation of Cardiovascular Function after Spinal Cord Injury. *J Neurotrauma, 34*(3), 671-684. doi:10.1089/neu.2016.4518

Stacey, M. J. (1969). Free nerve endings in skeletal muscle of the cat. *J Anat, 105*(Pt 2), 231-254.

States, G. J. R., Keller, A., Shum-Siu, A., Petruska, J. C., & Magnuson, D. S. K. (2022). Broad opioid antagonism amplifies disruption of locomotor function following therapy-like hindlimb stretching in spinal cord injured rats. *Spinal Cord, 60*(4), 312-319. doi:10.1038/s41393-021-00705-6

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again. *Med Sci Sports Exerc, 48*(11), 2294-2306. doi:10.1249/MSS.0000000000000923

Taylor-Schroeder, S., LaBarbera, J., McDowell, S., Zanca, J. M., Natale, A., Mumma, S., . . . Backus, D. (2011). The SCIRehab project: treatment time spent in SCI rehabilitation. Physical therapy treatment time during inpatient spinal cord injury rehabilitation. *J Spinal Cord Med, 34*(2), 149-161. doi:10.1179/107902611x12971826988057

Teasell, R. W., Arnold, J. M., Krassioukov, A., & Delaney, G. A. (2000). Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury. *Arch Phys Med Rehabil, 81*(4), 506-516. doi:10.1053/mr.2000.3848

Tomiya, S., Tamura, Y., Kouzaki, K., Kotani, T., Wakabayashi, Y., Noda, M., & Nakazato, K. (2019). Cast immobilization of hindlimb upregulates sarcolipin expression in atrophied skeletal muscles and increases

thermogenesis in C57BL/6J mice. *Am J Physiol Regul Integr Comp Physiol, 317*(5), R649-R661. doi:10.1152/ajpregu.00118.2019

Tornero-Aguilera, J. F., Jimenez-Morcillo, J., Rubio-Zarapuz, A., & Clemente-Suárez, V. J. (2022). Central and Peripheral Fatigue in Physical Exercise Explained: A Narrative Review. *Int J Environ Res Public Health, 19*(7). doi:10.3390/ijerph19073909

Trimble, M. H., Behrman, A. L., Flynn, S. M., Thigpen, M. T., & Thompson, F. J. (2001). Acute effects of locomotor training on overground walking speed and H-reflex modulation in individuals with incomplete spinal cord injury. *J Spinal Cord Med, 24*(2), 74-80. doi:10.1080/10790268.2001.11753558

van Gorp, S., Deumens, R., Leerink, M., Nguyen, S., Joosten, E. A., & Marsala, M. (2014). Translation of the rat thoracic contusion model; part 1-supraspinally versus spinally mediated pain-like responses and spasticity. *Spinal Cord, 52*(7), 524-528. doi:10.1038/sc.2014.72

Wahlgren, C., Levi, R., Amezcua, S., Thorell, O., & Thordstein, M. (2021). Prevalence of discomplete sensorimotor spinal cord injury as evidenced by neurophysiological methods: A cross-sectional study. *J Rehabil Med, 53*(2), jrm00156. doi:10.2340/16501977-2774

Warren, G. L., Hayes, D. A., Lowe, D. A., Prior, B. M., & Armstrong, R. B. (1993). Materials fatigue initiates eccentric contraction-induced injury in rat soleus muscle. *J Physiol, 464*, 477-489. doi:10.1113/jphysiol.1993.sp019646

Weppler, C. H., & Magnusson, S. P. (2010). Increasing muscle extensibility: a matter of increasing length or modifying sensation? *Phys Ther, 90*(3), 438-449. doi:10.2522/ptj.20090012

West, C. R., Crawford, M. A., Laher, I., Ramer, M. S., & Krassioukov, A. V. (2016). Passive Hind-Limb Cycling Reduces the Severity of Autonomic Dysreflexia After Experimental Spinal Cord Injury. *Neurorehabil Neural Repair, 30*(4), 317-327. doi:10.1177/1545968315593807

West, C. R., Squair, J. W., McCracken, L., Currie, K. D., Somvanshi, R., Yuen, V., . . . Krassioukov, A. V. (2016). Cardiac Consequences of Autonomic Dysreflexia in Spinal Cord Injury. *Hypertension, 68*(5), 1281-1289. doi:10.1161/hypertensionaha.116.07919

Williams, P. E. (1988). Effect of intermittent stretch on immobilised muscle. *Ann Rheum Dis, 47*(12), 1014-1016. doi:10.1136/ard.47.12.1014

Williams, P. E. (1990). Use of intermittent stretch in the prevention of serial sarcomere loss in immobilised muscle. *Ann Rheum Dis, 49*(5), 316-317. doi:10.1136/ard.49.5.316

Wolman, L. (1965). THE DISTURBANCE OF CIRCULATION IN TRAUMATIC PARAPLEGIA IN ACUTE AND LATE STAGES: A PATHOLOGICAL STUDY. *Paraplegia, 2*, 213-226. doi:10.1038/sc.1964.39

Wu, Z., Yang, Q., Crook, R. J., O'Neil, R. G., & Walters, E. T. (2013). TRPV1 channels make major contributions to behavioral hypersensitivity and spontaneous activity in nociceptors after spinal cord injury. *Pain, 154*(10), 2130-2141. doi:10.1016/j.pain.2013.06.040

Yarkony, G. M., Bass, L. M., Keenan, V., & Meyer, P. R. (1985). Contractures complicating spinal cord injury: incidence and comparison between spinal cord centre and general hospital acute care. *Paraplegia, 23*(5), 265-271. doi:10.1038/sc.1985.43

Zając, A., Chalimoniuk, M., Maszczyk, A., Gołaś, A., & Lngfort, J. (2015). Central and Peripheral Fatigue During Resistance Exercise - A Critical Review. *J Hum Kinet, 49*, 159-169. doi:10.1515/hukin-2015-0118

CURRICULUM VITAE

Greta Cesarz

PhD Candidate
Clinical Assistant Professor
University of Louisville

EDUCATION

University of Louisville Health Science Campus, School of Medicine
Expected Graduation: December 2022
Ph.D. Physiology
Dissertation: Defining the Impact of Clinically-Modeled Hindlimb Stretching, Exercise, & Inactivity on Functional Recovery & Central Afferent Plasticity After Spinal Cord Injury

University of Louisville Health Science Campus, School of Medicine May 2020
M.S. Physiology

University of Louisville, Department of Health and Sport Sciences
M.S. Exercise Physiology May 2017
Concentration: Strength and Conditioning

University of Louisville, Department of Health and Sport Sciences
B.S. Health and Human Performance May 2015
Concentration: Exercise Science
Minor: Psychology

TEACHING EXPERIENCE & TRAINING

Clinical Assistant Professor August 2022-present
University of Louisville
Courses Taught: Fitness Assessment & Prescription, Graduate Human Physiology

Instructor – Department of Health and Sport Sciences January 2022 – May 2022
University of Louisville
Course Taught: Fitness Assessment & Prescription

STEM Graduate Teaching Academy 2019
University of Louisville
Delphi Center for Teaching & Learning

Graduate Teaching Academy 2018
University of Louisville
Delphi Center for Teaching & Learning

Instructor – Department of Health and Sport Sciences 2015-2017
University of Louisville
Courses Taught: Introduction to Gymnastics, Fitness Walking

Teaching Assistant – Department of Health and Sport Sciences 2015-2017
University of Louisville
Courses: Human Anatomy and Physiology, Lab Methods in Fitness Evaluation (undergraduate), Biomechanics, Lab Methods in Exercise Physiology (graduate)

ACADEMIC SERVICE

Exercise Physiology Graduate Program Coordinator
University of Louisville
Department of Health and Sport Sciences

Faculty-Student Mentor
University of Louisville
Department of Health and Sport Sciences

POSTERS & PRESENTATIONS

Graduate Teaching Academy Evaluation
Presentation December 2018
Title: *Cardiovascular Dysfunction After Spinal Cord Injury*

Kentucky Spinal Cord Injury Research Center Journal Club Series
Presentation January 2019
Title: *Exercise-Induced Changes to the Macrophage Response in the Dorsal Root Ganglia Prevent Neuropathic Pain after Spinal Cord Injury*

International Symposium on Neural Regeneration
Poster January 2020
Title: *Hindlimb Muscle Stretch vs. Spontaneous In-cage Activity After Thoracic Spinal Cord Injury in Rats*

International Symposium on Neural Regeneration
Poster (co-first author) January 2020
Title: *Gain or Loss: The Effects of Hindlimb Stretching after T2 Spinal Contusion*

Kentucky Spinal Cord Injury Research Center
PhD Proposal Defense Presentation July 2020
Title: *The Influence of Clinically-Modeled Stretching on Functional Recovery After SCI*

Kentucky Spinal Cord Injury Research Center Seminar Series
Presentation August 2020
Title: *Defining the Impact of Clinically-Modeled Stretching on Functional Recovery & Plasticity After SCI*

Kentucky Spinal Cord Injury Research Center Seminar Series
Presentation June 2021
Title: *Evaluating Spontaneous Activity & Applied Exercise as Modulators of Stretch-Induced Functional Deficits After SCI in Rats*

University of Louisville Exercise Physiology Seminar Series September 2022
Presentation
Title: *Physical Therapy & Exercise as Modulators of Functional Deficits After SCI in Preclinical Models of Spinal Cord Injury*

PUBLICATIONS

Parmar P.J., Perry R.A., **Cesarz G.,** Roberts A., Hardman H., and Caruso J.F.
"Physiological effects of spaceflight/microgravity conditions and the mitigating effects of flywheel-based resistive exercise."
Gravitational and Space Research 2016

Martin J.L., Perry R.A., Baptista R.A., McArtor J.D., Clutter L.B., Symons T.B., Terson de Paleville D., Roberts A., **Cesarz G**., and Caruso J.F.
"Workload¹s impact on perceived gender-based differences in delta blood lactate values from supramaximal exercise."
Isokinetics and Exercise Science 2016

Perry R.A., Martin J.L., Vickers S.D., **Cesarz G.M.,** Bai L., Selimovic E.A., Muntis F., Parmar P.J., and Caruso J.F.
"Lower leg anthropometry as a correlate to performance and metabolism during dynamic exercise."
Gravitational and Space Research 2017

Cesarz G.M., Roberts A.H., Walden A.J., Symons T.B., Bai L., Selimovic E., West J.O., Bouchet A., and Caruso J.F.
"The addition of electrolytes to a carbohydrate-based sport drink: effect on aerobic exercise performance."
Kentucky Alliance for Health, Physical Education, Recreation, and Dance Journal 2017

Cesarz G., Wiest, M.J., Patsakos E., Newton E., Coleman A., Craven B.C., Magnuson D.S.K.M.
"*Evaluating Parathyroid Hormone as a Determinant of Bone Health in Individuals with Spinal Cord Injury: A Scoping Review"* (In Preparation)

Cesarz, G., Sharp, M., Shum-Siu, A., Magnuson, D.S.K.M
"*Spontaneous Activity & Applied Exercise as Modulators of Stretch-Induced Functional Deficits After Spinal Cord Injury in Rats" (*In Preparation)

Cesarz, G., Sharp, M., Shum-Siu, A., Magnuson, D.S.K.M
"Effects of Clinically-Modeled Hindlimb Stretching on Cardiovascular and Locomotor Function following High Thoracic Spinal Cord Injury" (In Preparation)

AWARDS & HONORS

Graduate Teaching Academy Certification	2018
Graduate Teaching Academy STEM Certification	2019
Graduate Student Council Travel Award	2020
International Symposium on Neural Regeneration Top 6 Poster Award	2020
Sharp, M., **Cesarz, G**., Shum-Siu, A., Wood, D., Magnuson, D.	
Title: "*Gain or Loss: The Effects of Hindlimb Stretching after T2 Spinal Contusion"*	
Dissertation Completion Scholarship Award	2022

PROFESSIONAL MEMBERSHIPS & ACTIVITIES

National Strength & Conditioning Association	2015-present
American College of Sports Medicine	2015-present
American Physiological Society	2017-present
Society for Neuroscience	2019-present
National Neurotrauma Society	2019-present
Society for Neuroscience, Louisville Chapter	2019-present
Kentucky Physiological Society	2020-present
Science Policy Outreach Group, University of Louisville	2020-present
KSCHIRT Symposium Keynote Speaker Organizing Committee	2021
Graduate Student Advisory Panel for IPIBS Orientation	2021

MENTORED STUDENTS

Caroline Lynch, Undergraduate Bioengineering Student Co-op 2018-2019

- Currently pursuing Master of Science in Physician Assistant Studies, University of Kentucky (anticipated graduation May 2022)

Jessey Manison, Visiting Undergraduate Animal Science Student 2018

- Completed Bachelor of Science in Animal Science, Colorado State University (May 2019)
- Completed Master of Science in Medical Sciences, University of Kentucky (May 2020)

Megan Brutscher (Snodgress), Undergraduate Bioengineering Student Co-op 2018-2019

- Completed Master of Engineering Management, University of Louisville (May 2021)

- Current employment: Senior Fulfillment Manager,
  GE Appliances – Louisville, KY

Anna Goestenkors, Undergraduate Bioengineering Student Co-op 2018-2020

- Currently pursuing PhD Biomedical Engineering,
  Washington University (anticipated graduation May 2026)

Alexandra Mitchell, Undergraduate Bioengineering Student Co-op 2018-2020

- Currently pursuing Doctor of Medicine,
  University of Kentucky (anticipated graduation May 2024)

Jenna Wilkerson, Undergraduate Bioengineering Student Co-op 2020-present

- Currently pursuing Bachelor of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2022)
- Accepted to Master of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2023)
- Accepted to School of Medicine,
  University of Louisville (anticipated graduation May 2027)

Amelia Franxman, Undergraduate Bioengineering Student Co-op 2020-2021

- Currently pursuing Bachelor of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2022)
- Accepted to School of Medicine,
  University of Louisville (anticipated graduation May 2027)

Randi House, Undergraduate Bioengineering Student Co-op 2020-2022

- Currently pursuing Bachelor of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2022)
- Accepted to Master of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2023)

McKenna Butler, Undergraduate Neuroscience Student. 2021-present

- Currently pursuing Bachelor of Science in Neuroscience,
  University of Louisville (anticipated graduation May 2022)
- Accepted to PhD Anatomical Science & Neurobiology,
  University of Louisville (anticipated graduation May 2027)

Savannah Jordan, Undergraduate Bioengineering Student Co-op 2021-present

- Currently pursuing Bachelor of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2023)
- Accepted to Master of Science in Bioengineering,
  University of Louisville (anticipated graduation May 2024)