Investigating a combination therapy of robot-driven rehabilitation techniques with viral delivery of brain-derived neurotrophic factor in treating adult spinal cord injury

A Thesis

Submitted to the Faculty

of

Drexel University

by

John Kim Lee

in partial fulfillment of the

requirements for the degree

of

Doctor of Philosophy

June 2016

Dedications

To Catrick Swayze.

Acknowledgements

First and foremost, I would like to express my sincerest thanks to Dr. Simon F. Giszter for his mentorship and friendship the past four years. His support and patience throughout my time in his lab has been invaluable to my growth as a scientist. I would also like to thank Dr. Veronica J. Tom, who has taught me many important lessons about being an effective researcher. This work is only possible because of her guidance and collaboration.

In addition, I am grateful to the other members of my committee. Dr. Uri Hershberg has always made himself available to me for questions. Dr. Ilya Rybak always made sure to ask the hard questions, prodding me to be a better investigator. Finally, I thank Dr. Mijail Serruya for being a role model for me in my pursuit of becoming a physician-scientist. His clinical perspective has been a driving force in my research endeavors.

I would like to take the opportunity to thank current and former colleagues in the Giszter lab: Dr. Chintan Oza, Dr. Arun Ramakrishnan, Dr. Taegyo Kim, Dr. David Logan, Dr. Qi Yang, Kendall Schmankudovich, Josephine Vanloozen's bicycle, David Wallace, Kavon Noorbehesht, and Kristina Carter. I value our friendships, as well as the time we spent together in the laboratory.

I would like to thank other members of the Department of Neurobiology & Anatomy. In particular, Michelle Klaw, Dr. Di Wu, Theresa Connors, Megan Detloff, Joy Hudson, and Robert Parker have been great friends the past four years. I would like to thank Natalia Broz in the School of Biomedical Engineering, who has put up with enough

of my nonsense and late scheduling to last a lifetime. I would also like to express my gratitude to Dr. Jane Clifford, the head of the MD/PhD program, without whom I would not even have had the opportunity to pursue this goal.

To my friends – Luke Mitchell, Bartholomew Bacak, Simon Danner, and Guillaume Caron – thanks for your support (especially with bench press) and I hope we have many more steak nights and sausage fests in our future. To my fellow MD/PhD students: there is a light at the end of the tunnel (I think)!

I would like to thank my family for all of their support throughout the years. Without my parents, sisters, brother-in-law, and nephews, I could not have accomplished any of this work.

Finally, to Lauren Finn, thank you for everything. But especially for chocolate chip cookies.

Table of Contents

List of Figures

Abstract

Investigating a combination therapy of robot-driven rehabilitation techniques with viral delivery of brain-derived neurotrophic factor in treating adult spinal cord injury

John Kim Lee
Simon F. Giszter, Ph.D

A complete spinal cord injury (SCI) disrupts the normal architecture of the central nervous system, resulting in severe and irreversible impairment of the healthy functions of the body. SCI physically interrupts the neural networks used to relay descending motor information from and ascending sensory information to supraspinal structures in the brain separating circuits in spinal cord from brain supervision and recruitment. Depending on the location of the injury, complete SCI can lead to paraplegia or quadriplegia. In the injured individual, the loss of autonomy and mobility can severely decrease quality of life, as well as negatively impact health outcomes. As a result, locomotor rehabilitation is an area of interest for research for its potential translational benefits in the clinic.

In previous work in our lab studying the rat model for SCI, we have demonstrated the efficacy of robotic technology in the rehabilitation of adult rats transected as neonates (NTX), which are unique in their ability to produce autonomous stepping after complete SCI without intervention. Using robotic assistance at the pelvis in our trunk-based rehabilitation paradigm, we have significantly improved locomotor function in such animals. Viewing the NTX model, thus, as a signpost for what is possible in recovery when using our robot in animals that can step after SCI, we have also shown that our robot can be used to drive epidural stimulation (ES) in the rat transected as an adult

(ATX) to promote stepping patterns and increase body weight support.

Recently, the use of neurotrophins, such as brain-derived neurotrophic factor (BDNF) has been investigated as a means to induce stepping and locomotor behaviors in the ATX model to varying levels of success. We believe that there are potentially synergistic benefits to combining our robot rehabilitation techniques with the use of BDNF to rehabilitate ATX animals. This thesis addresses this idea in depth.

We first investigated how BDNF and our robot-assisted treadmill training might interact in the ATX model. Next, we added robot-driven epidural stimulation to the treatment regimen to further understand how the therapies might interact in rehabilitation. Finally, to elucidate the mechanisms underlying locomotor recovery following injury, we used intracortical microstimulation (ICMS) to map the motor cortex of successfully rehabilitated animals.

Our results suggest that BDNF and robot technologies can be combined successfully to provide robust stepping patterns, characterized by body weight support and plantar stepping in the ATX model for rats. Furthermore, we show that epidural stimulation can be used to mitigate pathological sequelae that come from BDNF use. Finally, our work shows how active stepping using BDNF and robot rehabilitation in the ATX model may induce significant reorganization of the trunk motor cortex, providing more clues to the relationship between the cortex and the spinal cord in motor control and muscle synergy development.

CHAPTER 1: Introduction

"A thing there is whose voice is one;
Whose feet are four and two and three.
So mutable a thing is none
That moves in earth or sky or sea.
When on most feet this thing doth go,
Its strength is weakest and its pace most slow."
The Riddle of the Sphinx, Atheneaus

A. The Riddle of the Sphinx

For a long time in the course of human history, the concept of rehabilitation in spinal cord injury was considered a futile effort. As early as 2500 BCE, there is record in the ancient Egyptian document, the Edwin Smith Papyrus, of SCI as a disease that is "not to be treated." In Western history, Greek physicians such as Hippocrates and Paul of Aegina – twelve centuries apart – tried to improve surgical methods to improve recovery after injury. It was not until the mid-twentieth century that research in rehabilitation in SCI has seen advances, as our understanding of physical therapy techniques, robotics, imaging, and human physiology and anatomy have spurred new innovations in modern medicine. Behind all of these endeavors, from ancient history to now, the central conceit of this work has always been to bring function back where it had been lost. This concept of loss extends far beyond locomotion, too. It also relates to our distinct identity as human, where the importance of bipedal locomotion and its unique association to humankind has long been prevalent in our species' collective consciousness.

In the Ancient Greek tradition, the mythical Sphinx – with the head of a woman, the body of a lion, wings of an eagle, and a snake for a tail – was sent by the Greek gods to guard the city of Thebes, devouring any traveler who meant harm to the city. She

posed one riddle to anyone desiring to pass the city gates, asking, “Which creature has one voice and yet becomes four-footed and two-footed and three-footed?" Those who could not answer were swiftly strangled and eaten by the monstrous Sphinx. In true heroic Greek form, Oedipus – Greek for “swollen foot” – solved the riddle, answering, “Man, who crawls as a baby, then walks on two feet as an adult, and then uses a walking stick in his old age.” As the myth goes, the Sphinx subsequently threw herself off of a cliff, allowing Oedipus to enter the city of Thebes, and fulfill his destiny to kill his father and marry his mother. Even to the Greek gods, human locomotion was a unique experience and separated man from all other beasts and creatures. Thus, it is no wonder that injury to the systems that produce movement has been an area of interest in human history for a long time.

SCI is a debilitating disease that affects approximately 2.8 million people worldwide, with approximately 250,000 to 500,000 new cases every year. In the United States, approximately 276,000 Americans live with SCI, with 12,500 new cases a year. Depending on the site of injury, SCI may leave patients with severe impairment leading to immobility and/or loss of sensation below the site of injury. From a clinical perspective, SCI has a significant negative impact on the health outcomes of those injured, greatly reducing life expectancy and increasing the risk of developing secondary conditions that may also be life-threatening, such as deep vein thrombosis and pressure ulcers [1], [2].

Factors that reduce quality of life in patients include the loss of bladder and bowel function, depression, and immobility. Specifically, the inability to walk is of particular concern to physicians, as immobility can lead to life-threatening infections and

cardiovascular disease [3]. Furthermore, those who suffer from SCI are also at higher risk for bacterial infections as a result of immobility [4]. As a result, locomotor recovery is a high priority for translational medicine research in SCI.

Although Oedipus was correct in his response the Sphinx's deadly question at the time, there was no way in which he or ancient Greek storytellers might have predicted advances in robotic technology or in neuroprostheses, that comprise the modern "walking stick." Oedipus was referring to the natural progression of human locomotion as the body ages, yet his answer is more nuanced than he could have possibly imagined. In an interesting twist to this age-old puzzle, current advances in modern medicine and treatment of locomotor rehabilitation paradigms in spinal cord injury may have provided an alternative interpretation to the answer to this riddle. The following sections will provide an overview of the current state of these modern "walking sticks" that are relevant to the work in the proceeding chapters.

B. Spinal Cord Injury Results in a Divided, but Functional Central Nervous System(s)

A complete SCI disrupts the normal, healthy architecture of the central nervous system (CNS), physically separating the neural networks used to relay descending motor information from and ascending sensory information to supraspinal structures in the brain. As a result of complete SCI, instead of one anatomically and functionally contiguous nervous system, there are two autonomous networks that work independently of each other in controlling the body. Above the injury, there exists the brain and cervical and upper thoracic spinal cord, and below the injury, the lumbosacral spinal cord.

Although the nervous system below the site of injury is unable to communicate directly with the brain in the case of SCI, it still possesses the neuronal circuitry to establish and produce complex movements [5].

The lumbosacral spinal cord, separate from supraspinal influence, is an independently functioning system that is capable not only of producing movements, but also of learning. Experiments in transected cats trained to walk on treadmill showed that obstacle-stumbling response testing on the dorsum of the hind paw resulted in new locomotor patterns of the whole limb to avoid perturbations in the path of the hindlimbs [6] – [8]. This suggests that not only is the lumbosacral cord capable of producing movements, but also of receiving and integrating afferent data to produce coordinated, complex behavior.

B-1. Pantomime Horse Analogy of SCI

As a result of SCI, both systems – above and below the injury – function and develop separately. However, they are still anatomically and mechanically coupled by the musculature and structure of the body. In our lab and previous work, we liken this system to a pantomime horse, where two independent actors play the front and back halves of a horse, and must work in concert to effectively move forward. In this analogy, the actor playing the rear end of the horse is blind to the actions of the front actor, but the costume couples the two together, allowing them to integrate both halves together for coordinated movement. In the context of complete SCI, the two functional units of the separated central nervous system are coupled by trunk musculature that extends from above the site of injury to below. Both parts also partially control and share this trunk musculature.

Indeed, in spinal cord preparations studying fictive locomotion in the decerebrate cat, Takashasi *et al.* showed distinct rhythmic patterns of trunk muscle nerve activity inherent to the cord [9], revealing a connection between spinal locomotor networks and trunk musculature activity. Based on its anatomical importance, our understanding of its neural control mechanisms, and our examination of the active biomechanics of animals that do walk, we believe the data strongly supports that the trunk plays a key role in the locomotor rehabilitation in complete SCI.

C. The Trunk as a Key Mediator of Locomotion

Anatomically, the trunk is comprised of the muscles of the neck, abdomen, thorax, and pelvis, on both the dorsal and ventral aspects of the body. It is a complex amalgam of overlapping musculature that works synergistically and antagonistically to provide stability in posture, as well as to initialize movement in locomotion [10]. Although the hips and legs are the primary effectors for movement in locomotion, the trunk is essential for stability [11], [12]. Furthermore, the role of the trunk may be intertwined with the function of the hindlimbs. Studies in the development of locomotion in the neonatal rat model have shown that trunk muscle activity is coupled to the rhythmic activity of the hindlimb extensors in locomotion [13].

In humans, back musculature serves primarily to extend the dorsal column, providing stability against gravity. Additionally, dorsal muscles connect individual vertebrae, allowing for many smaller muscles to act on a larger portions of the spine. In locomotion, these muscles provide stability and stiffness to the back. The trunk muscles on the ventral aspect of the body are responsible for flexion and bending of the trunk. In

addition, they serve to allow for axial rotation of the body, which are essential for dynamic stability needed in locomotion [14]. Furthermore, ventral trunk muscles also play a role in many different systems and behaviors, including respiration, defecation, and urination.

From an evolutionary standpoint, the case can be made that the role of the trunk is not diminished or significantly changed from the bipedal human model to the quadrupedal model. Indeed, although bipedal locomotion is unique to humans across mammals, there is much evidence to suggest that axial trunk movements and control have been "conserved" from phylogenetically older locomotor systems. Indeed, Falgairolle *et al.* compared recordings of metachronally traveling waves of motor activity in lamprey spinal cord, amphibian ventral roots, neonatal rat spinal cord, and human axial trunk electromyography (EMG) to elegantly illustrate this concept [15], showing parallels and similarities of patterns across these various vertebrate groups (Fig. 1-1).

Furthermore, studies in neurodevelopment and physical rehabilitation of incomplete SCI in children suggest that as humans develop locomotor patterns from quadrupedal crawling to sitting, patterns of activation of trunk musculature is conserved [16], [17]. In a case study of a three-year old child with incomplete SCI, Behrman *et al*. demonstrated that locomotor training and rehabilitation led to similar patterns of trunk activation during crawling, stair climbing, and pedaling [16]. In physical therapy of humans, in particular, interventions that appear to activate the various trunk muscles and neural networks in locomotion seem to stimulate the control of other tasks.

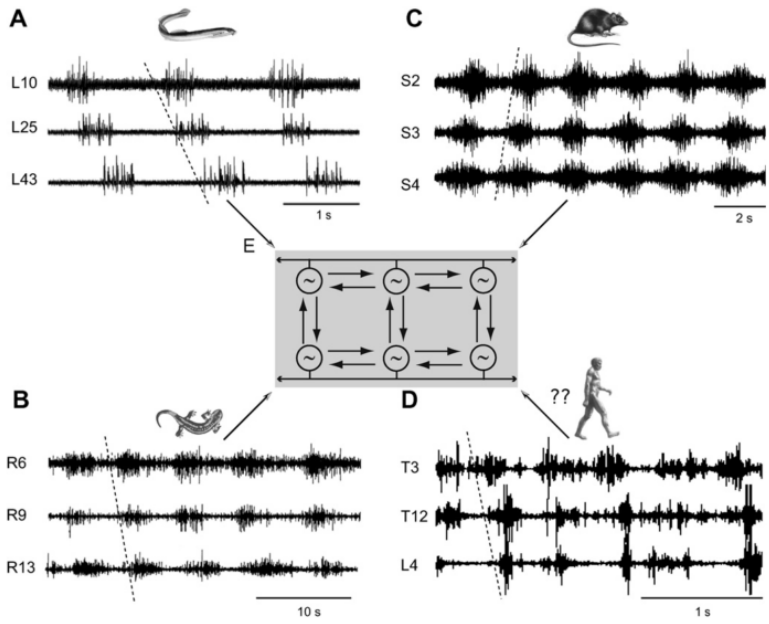


Figure 1-1. Examples of metachronally traveling waves of motor activity in (A) isolated lamprey spinal cord preparations, (B) urodele amphibian ventral motor roots, (C) newborn rat spinal cord, and (D) EMG recordings of human during locomotion. *From Falgairolle et al.*, *2006*.

As a result, based on the literature and our previous studies, we focus on the interactions of trunk in voluntary movement as the primary substrate for our robotic interventions in our own locomotor rehabilitation paradigm for SCI.

D. Fundamentals of Robot-Assisted Rehabilitation

Training is a necessary and critical component of rehabilitation in the treatment of SCI. As the damaged spinal cord is still capable of producing movements and learning, as discussed previously, training plays an important role in re-developing, creating, or reinforcing ways in which to use those movements for effective locomotion. In their study on spinalized cats, Hogson *et al.* recorded trunk EMGs, and related changes in locomotor function, weight-supported standing ability, and EMG to learning in the spinal cord [18]. In the rat model, Ichiyama *et al.* investigated how stepping training changed

both EMG and molecular expression of c-fos in the spinal cord. Their results suggest that effective training can significantly increase the efficiency of specific motor pathways [19]. Additionally, the intact neural networks in the damaged cord may be particularly primed for learning from new experiences [20]. Edgerton *et al*. provides a strong case for the plastic nature of the spinal cord in their review [21], suggesting that repetition of experiences can help to form new connections between and in groups of neural networks. Furthermore, other studies with step training in rats has shown that the spinal cord can adapt, as well as learn or create new pathways, as a result of rehabilitation [22], [23]. Thus, there is strong evidence to suggest that training can increase the efficiency of specific neural pathways, through repetition.

With regards to the nature of training, the literature advocates the use of task-specific activities to elicit specific reorganization in the neural pathways for maximally efficient recovery. That is to say, in the case of locomotor rehabilitation, specific locomotor exercises are crucial for proper reorganization, leading to effective recovery. For example, previous studies have shown that spinalized cats trained to weight-support while standing are not necessarily able to utilize those mechanisms to provide weight-supported stepping [24]. As a result, many new paradigms of rehabilitation have emerged, focusing on training animals to step and weight-support while stepping when possible.

One such paradigm has been the use of robotic interventions to interface with the anatomy of animals to help develop and reinforce patterns of locomotion. Robotics allow for more precise control of rehabilitation parameters, such as timing and distance, by virtue of the digital nature of the technology. Another additional benefit of robotics is the

ability to interface with the animal's need for rehabilitation, in real-time. This has been referred to as the "assist-as-needed" paradigm [25], to counteract the phenomenon wherein animals who are rehabilitated within specific frameworks can developed habits of "learned disuse." This is common to rehabilitation paradigms, where patients or animals become less responsive to repetitive and identical stimuli and activation [26], learning to rely on the frameworks of rehabilitation to walk, as opposed to integrating the rehabilitation itself to develop robust walking.

"Assist-as-needed" as an effective rehabilitative paradigm has been well documented in both the human and the mouse model for SCI. Cai *et al.* showed that SCI mice trained with "assist-as-needed" robot rehabilitation had significantly more consistent stepping than mice with fixed robotic intervention [27]. In the human model, there is an additional benefit of real-time feedback to the patient, where progress within training sessions and throughout the entire length of rehabilitation provides a motivating factor for patients [28]. This paradigm of recovery has also found success in many different neurological rehabilitation schemes for other motor dysfunctions, such as stroke (for a review, see Krebs and Hogan [29]). As a result, in the case of SCI, robotic technology can be used to train the animal, as-needed, and eventually wean the animal off of robot assistance as it recovers more robustly.

In our lab, we employ such a robotic intervention, focused on the rat's pelvis [30], [31]. One of the fundamental differences between a transected animal and a normal animal during treadmill training is the trunk posture during locomotion. An injured animal lacks the ability to weight-support, and its hindlimbs are dragged behind the animal as it is trained on the treadmill. Our robot is able to apply forces to the injured rat

at the pelvis to allow for a trunk posture that is nearly identical to that of a normal rat. Using an assigned equilibrium for the rat's pelvic center – defined by a three-dimension coordinate frame (x-, y-, and z-axes) – the robot can provide a uniform isotropic elastic force field to bring an injured rat's pelvis to a normal position. With training, if a rat is able to step with its hindlimbs and generate weight-support locomotion, robot forces in the z-axis are reduced as the pelvis moves closer to the height of the assigned equilibrium.

Our robot system is a PHANTOM® Premium 1.0 model (developed by SensAble Technologies, Inc.) with custom software developed in our laboratory (Fig. 1-2). It interfaces with an orthosis at the rat's pelvis through a gimbal. Our robot can apply elastic force fields to the rat's pelvis to assist with weight support during treadmill training. These forces are governed by the equation:

$$F = k(x - x_0)$$

where F is the force applied by the robot, k is the stiffness of the elastic field, x_o is the desired height of the elastic field, and x is the current height of the pelvis. The stiffness of the elastic field is predetermined to be 45 N/m. The desired height of the rat's pelvis (x_o) is set individually for each rat so as to support the rat just below a natural pelvic location for an uninjured rat at the outset.

As the animal undergoes training, our custom software calculates and adjusts the forces required to bring the animal to equilibrium at a rate of 1 kHz. Our robot system is also able to integrate with other laboratory equipment, providing synchronizing control and timing for external devices.

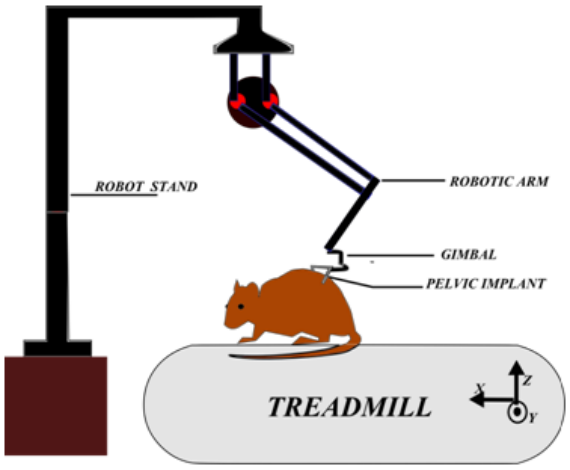


Figure 1-2. Schematic of the PHANTOM® Premium 1.0 model robot arm, as it interacts with a rat on the treadmill.

In the context of the pantomime horse model for our spinalized animals, the robot plays an important role in teaching the front half of the animal to integrate the actions and movements of the trunk, hip, and hindlimbs into normal stepping and gait patterns. This allows for more improved coordination between these motor pools, which is critical for coordinated stepping [25]. In addition, Fong *et al*. showed that robot-assisted rehabilitation can be effectively combined with pharmacological agents to increase the excitability of the spinal cord to provide even more robust recovery in spinalized mice [32]. Their work suggests that the benefits of robotic technology in rehabilitation can be best integrated and efficiently used when the spinal cord is in a "primed" state – excitable, and particularly ready to learn and reinforce motor pathways through training. One such model of an excitable spinal cord that can be effectively rehabilitated on our custom robotic intervention is the adult rat transected as a neonate (NTX), around post-natal day 5.

E. The Neonatal Model of SCI as a Signpost of Recovery

A common model for investigating SCI is the complete thoracic transection at T9/T10 in the rat. Within this model, there exists two paradigms of injury – the NTX model, and the rats injured as adults (ATX) – that we can compare and contrast to reveal important aspects of locomotion that we can use for effective rehabilitation (Fig. 1-3).

Without any intervention or additional stimuli, in the NTX model, rats are capable of spontaneous, autonomous recovery of basic, alternating hindlimb stepping in adulthood. While there is a wide variety in the stepping patterns of these animals, in approximately 20% of these animals, this stepping can be characterized by over 50% of their steps capable of weight-support [33], [34]. When we apply our unique robotic intervention at the pelvis to these animals, we can significantly increase the number of weight-supported steps these animals produce during locomotion [30] and improve their ability to body weight support, as they adapt and learn to walk with robot assistance. This is of particular interest to us as it demonstrates what is possible with robot-assisted treadmill training in animals that are able to produce their own hindlimb movement, whether it is rudimentary flexion and extension, or robust alternations. Thus, we view the NTX model as a signpost of locomotor recovery in SCI.

This lies in stark contrast with the ATX model, where animals are unable to produce their own stepping patterns without additional intervention or stimuli. As a result, they are not capable of taking advantage of the robotic interventions at the pelvis, and do not significantly recover with exercise and training. However, there are ways in which to induce stepping in the ATX model, such as pharmacological interventions and epidural stimulation. Previously in our lab, we have investigated ways in in which to

induce stepping within the framework of our robot that allow us to view the NTX model as an indication to create effective rehabilitation strategies in the ATX animal [35]. All of these stepping modalities require manipulating the remaining intact neuronal circuitry within the spinal cord network to generate rhythmic locomotor patterns that may allow for stepping. Methods that have used this approach include various therapies that focus on identifying and studying populations of neurons in the spinal cord that may be responsible for pattern generation in limb movement.

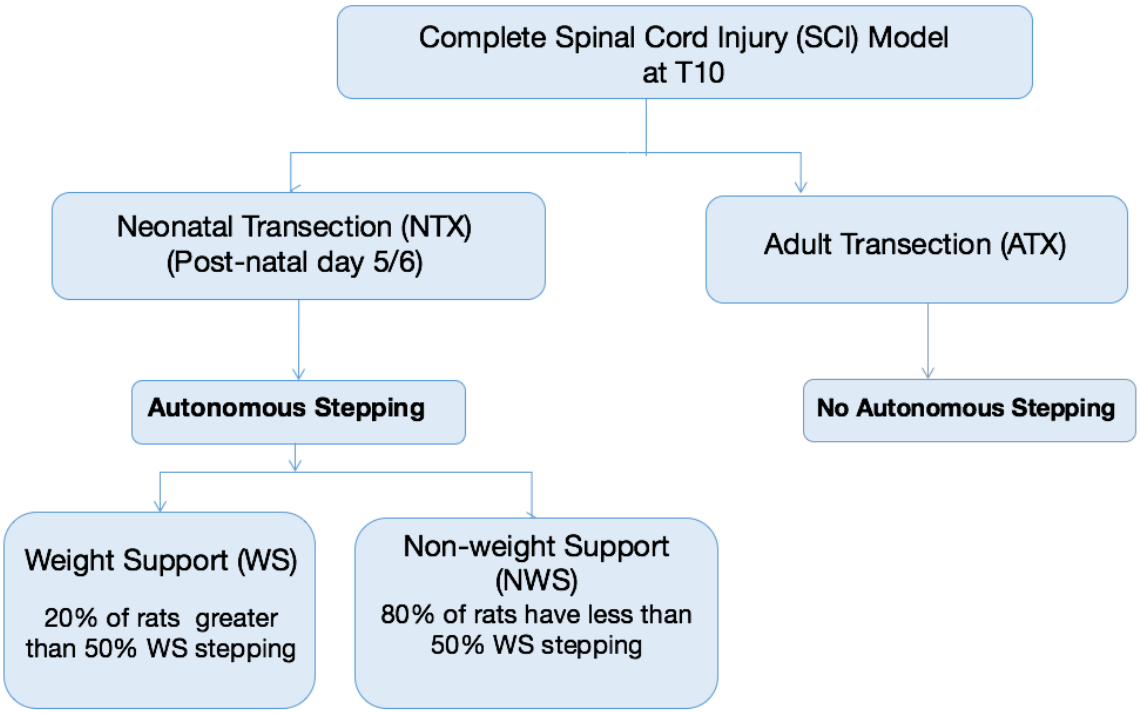


Fig. 1-3. Current paradigm of complete spinal cord injury model in the rat model used in the Giszter lab.

F. Central Pattern Generators

An important aspect of the spinal cord networks is the central pattern generator (CPG), a neural network that is capable of producing rhythmic outputs without any rhythmic inputs or stimuli. CPGs responsible for locomotion have been studied in many lower vertebrates and non-primate humans, but has been theorized to exist within the human model, as well [36]. They are theorized to be essential components of micturition, ejaculation, and respiration. Sherrington's work in 1910 on spinally transected dogs and cats provided the first suggestions that basic motor patterns for locomotion are the result of reflex actions mediated by a central pattern generator. This work has been expanded upon, with several different paradigms of how the CPG network might be organized [37], [38]. Recent studies suggest that CPGs may also exist within the lumbosacral region of the human spinal cord [39], [40], as well as in the mouse and rat models [41], [42]. In clinical

studies of prenatal and postnatal movements of human neonates, corroborating primitive stepping patterns and repertoires has also been identified [43], [44].

Though the exact organization of the CPG is yet unclear, it has been shown that in the case of complete SCI, the spinal CPG is still able to generate patterns for locomotion in response to sensory afferents. Work as early as 1980 by Delcomyn *et al*. showed that locomotor-like activity could be produced in fictive locomotion preparations [45], while work by Bretzner and Drew in 2005 on cats suggest that other areas in the spinal cord and brain may also affect modulation of locomotor activity [46]. Indeed, in our own lab, we discovered that stimulating trunk afferents in fictive locomotion preps in a rat can regulate the duty cycle during the extension step phase of gait, suggesting a connection between the trunk and the locomotor CPG (Udoekwere – unpublished).

In animal models, studies to understand the pharmacological control of the CPG have led to the discovery of agents that effectively activate the CPG to produce locomotor-like activity. Previous work in stimulating the noradrenergic system have revealed how L-DOPA can induce locomotion in cats [37], [47], [48]. In the rat model, many different neurotransmitters have been been studies, but serotonin or serotonin agonists have been found to be particularly effective [49] – [51]. In these models, locomotor activity in response to CPG-activating agents is characterized by plantar foot placement, weight support by the hindlimbs, and large-amplitude movements of the hindlimbs. This behavior in response to CPG activation strongly suggests a role for pharmacological intervention of the CPG in locomotor recovery. It should be noted, however, that these pharmacological interventions may be specific within the context of SCI, where injury itself changes the milieu of the spinal cord networks. SCI has shown to affect the sensitivity of the persistent

sodium current [52] and to upregulate calcium channels to sensitize neurons to be more excitable in response to serotonin. Regardless, the use of pharmacological agents to activate the CPG is a long-studied intervention in SCI rehabilitation.

G. Brain-Derived Neurotrophic Factor

In addition to the use of neurotransmitters to induce locomotion, the delivery of neurotrophins to the spinal cord has been investigated extensively for its ability to induce rudimentary stepping patterns in rats (for an extensive review, see Harvey *et al*. [53]). Though neurotrophins produce phenotypically similar motor behaviors to the use of neurotransmitters, studies on cats by Howland *et al.* [54] showed they do so by different means. Specifically, they produce very broad structural changes in the spinal cord, repairing damage from SCI by promoting an environment within the spinal cord for axonal growth with cellular transplantation. Unlike serotonin or dopamine administration, the effects of neurotrophins are long-lasting and are not fast-acting. Using transplants of fibroblasts that have been genetically modified to secrete various growth factors, including brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), Mitsui demonstrated improvements in locomotor behaviors after contusion [55]. This approach to recovery of locomotor function based on gene therapy has proven to be successful in combination with treadmill training in spinalized cats [56], though there have been some concerns with the invasive nature of the transplant surgery needed to deliver the fibroblasts.

BDNF is an excellent candidate for use in locomotor recovery as a means to bolster or initiate the regenerative ability of the spinal cord. BDNF is a neurotrophic

factor that promotes regeneration [57], [58] and induces synaptic plasticity in the adult CNS [59], [60]. It is widely expressed in the brain and spinal cord endogenously [61]. Furthermore, it has been shown to be upregulated in the spinal cord of transected animals in response to exercise and training [62], [63], perhaps to play a crucial role in learning. Recent studies have also shown that BDNF is upregulated in the spinal cord as a response to SCI alone, which suggests that it is already an essential component of recovery after injury [64] – [66].

BDNF, like many neurotrophins, is a ligand for tyrosine kinase B (TrkB) receptors [67], which are widely distributed in in the nervous system, and play a role in the formation and maintenance of neuronal circuits. In the context of injury, neurotrophins, via Tyrosine kinase receptors, promote growth and survival of cells (for a review, see Snider *et al*. [68]). Similar to its effect on BDNF concentration, damage to the spinal cord may also induce changes to trkB expression in the spinal cord. Work by Liebl *et al*. suggests that this may be to sequester BDNF availability to maximize neuronal recovery and regeneration [69]. This may suggest a role for the use of exogenous BDNF to provide more recovery in the context of SCI. Furthermore, work by Ziemlínska *et al.* has shown the downstream effects of BDNF on neurotransmission, altering the balance between excitation and inhibition in the spinal cord [70]. By increasing levels of glutaminergic and GABAergic neurotransmission and decreasing the expression of the potassium-chloride co-transporter, BDNF increases the overall excitability of the spinal cord. This was corroborated by Boyce *et al*., who showed that BDNF therapy decreases the rheobase of affected neurons [71].

Recently, work by Blits *et al*. [72] has demonstrated a novel means of delivering BDNF in the spinal cord, by the use of an adeno-associated viral (AAV5) vector encoding for the neurotrophin. Blits showed evidence that this approach to BDNF delivery was able to improve hindlimb function and locomotor activity in the ATX rat model similar to other models of BDNF delivery. This work was built upon previous studies that have investigated the most efficacious site of delivery post-injury for maximum locomotor gains [73], as well as the importance of the timing of delivery after injury [74].

More recently, in a study by Boyce and Mendell [71], adult transected rats treated with AAV5-BDNF were able to demonstrate autonomous plantar stepping after injury. When the animals were supported for balance, the viral treated rats were able to produce weight-supported plantar stepping. With histology, Boyce also proved that intraspinal injections of AAV5 specifically target and transfect interneurons within the intermediate grey and motoneurons within the ventral horn. The recovery observed in this study is consistent with studies done in cats [56], [75] and in other studies with rats both on treadmill [70], [72] and in open-field environments [76].

Unfortunately, the use of exogenous BDNF to rehabilitate animals with SCI has also shown evidence of maladaptive effects, characterized as increasing frequency of clonic movements in the hindlimbs [70]. This has been found to cause a loss of function in hindlimb locomotor ability, which has been commented on in reviews of the use of BDNF [77], [78]. This may hinder the usefulness and translational potential for this therapy in the treatment of SCI. Investigations into how the pathological effects of BDNF

can be mitigated while the locomotor benefits can be maximized is an avenue of interest, especially in the context of combination therapies.

The use of BDNF to create a milieu that promotes plasticity and regeneration after injury is an encouraging development in the treatment of SCI in the animal model, especially with the potential to create combination therapies with potentially complementary rehabilitation regimens. There is already a precedent for this in the epidural stimulation model of rehabilitation, where pharmacological interventions, such as clonidine [79] or quipazine [32], have been successfully combined with robot rehabilitation in cats and mice, respectively. Indeed, work with quipazine and robot rehabilitation in the rat model [80] has led to our own investigations into how epidural stimulation may play role in our robot-assisted rehabilitation paradigm of recovery with BDNF.

H. Epidural Stimulation

In the ATX rat model, recovery of locomotor-like activity has also been shown through non-pharmacological recruitment of the CPG. In 1983, Meisel demonstrated that ATX rats can hindlimb step and alternate in response to perineal stimulation or tail pinch [81] by manual, physical activation of the CPG. These animals were able to step, albeit with low range of motion and fluidity, and alternate their otherwise quiescent hindlimbs in response to tail pinch. Alternatively, it has been discovered that electrical stimulation (ES) of the dorsal surface of the spinal cord can also activate stepping in cats [82], [83] and in rats [84], [85]. Work by Courtine, *et al*. in rats [86] and Gerasimenko, *et al*. in cats [87], in particular, have shown that the lumbosacral spinal circuitry in adult transected

animals is capable of generating partial weight-supported stepping in response to epidural stimulation. They hypothesize that epidural stimulation activates spinal neural networks that recruit local motoneurons to allow for coordinated stepping. In humans, though initially used in pain management [88], epidural stimulation has also been demonstrated to elicit locomotor patterns in the lower limbs, as well [40], [89], [90].

H-1. Conventional Epidural Stimulation

In conventional epidural stimulation techniques, there are several parameters for which to account to produce locomotor activity in the spinal cord (for a review, see Gerasimenko *et al*., 2008 [90]). The site of stimulation has been a critical area of investigation, with small differences discovered between humans [40], [91] and rats [85] to elicit bilateral flexion and extension movements. Studies analyzing EMG patterns have converged upon the various lumbar segments as the optimal location for epidural stimulation [92], further supporting the role of the CPG in locomotion. Work by Ichiyama *et al*. in the demonstrated how epidural stimulation at different spinal segment levels from T13 to L6 in the rat model resulted in different, specific locomotor behaviors, such as bilateral stepping, unilateral stepping, flexion, and synchronous stepping [85]. In addition to the specific locomotor behavior, they also assessed the duration of rhythmic hindlimb activity elicited by stimulating spinal segment levels, observing that the greatest mean duration of bilateral stepping occurred at L2. Their work also investigated the joint kinematics and coordination of the knee and ankle in the stance and swing phase of locomotion produced by ES at the T12, L2, and L6 spinal segments. By these three measures, Ichiyama *et al.* concluded that bilateral stepping was best achieved by

stimulation at the L2 spinal level, which corroborates the many different aforementioned studies in the rat and cat models for ES.

Another critical parameter of ES has been the frequency of stimulation, with different optimal ranges discovered for cats (3-5 Hz) [93], rats (20-110 Hz) [85], and humans (30-40 Hz) [40]. Of particular note is the effect to which different frequencies affected the quality of locomotion. Jilge *et al.* demonstrated that it was possible to change movements from extension to rhythmic alternations of flexors and extensors by increasing the frequency within 5 to 15 Hz [94]. As a result, there is a wide variety of applications for epidural stimulation in locomotor rehabilitation, especially to elicit specific movements with training. This is further corroborated in the human model by Minassian *et al*. [95], who showed that epidural stimulation can elicit a wide variety of specific movements in the lower limb.

Finally, the strength of stimulation is an important parameter in the use of ES. Studies have shown that like frequency, strength of stimulus depends on the animal model [90]. In the rat model, Ichiyama *et al*. demonstrated that there was a relationship between the threshold to evoke hindlimb activity and the threshold to to induce locomotor activity [85]. The average threshold, at 40 Hz, to generate a hindlimb muscle response was significantly lower than that to stimulate bilateral hindlimb locomotor activity. Using EMG to study the activity of hindlimb muscles in rats, Ichiyama *et al*. found that optimal stimulation parameters of 40 Hz at 4V can elicit bursting activity of the hindlimb muscles.

Unfortunately, there are limitations to the intensity of stimulation to promote stepping. Higher voltages that have been used in previous studies to activate stepping

patterns caused discomfort in the animals, and resulted in the use of lower, less effective voltages to increase compliance while training. This significantly limited the quality of stepping in the early phases of rehabilitation, though animals did eventually recover significant locomotor function in the hindlimbs. Furthermore, Ichiyama *et al.* showed that the stimulus threshold required to induce locomotor activity significantly increases as a function of time after transection. After four weeks of training, they discovered that the threshold to generate bilateral hindlimb locomotor activity nearly doubled, increasing from 3.39 ± 0.2 V to 7.55 ± 1.2 V. Though this was not addressed explicitly, their work suggests a temporal limitation to the effectiveness of conventional ES techniques, as stimulus intensity cannot indefinitely be increased to match the increasing threshold.

H-2. Robot-driven epidural stimulation

In our lab, we demonstrated that the parameters used for epidural stimulation can be employed more efficiently by synchronizing the frequency of stimulation to our previously discussed robot. Unlike conventional stimulation techniques that provide a constant frequency of stimulation, we use our robot to measure pelvic height as a prompt for intermittent electrical stimulation, as needed by the rat. When an animal's pelvis is lower than a criterion height, our robot can trigger stimulation of the cord to increase locomotor activity. While the concept of intermittent stimulation is not unique to our rehabilitation paradigms [96], [97], it has shown to be effective. In our previous work with the ATX model, we showed that robot-driven epidural stimulation significantly decreases assistive body weight support by the robot, as well as increases behavioral scoring of locomotor activity that showed alternation of limbs and plantar stepping [35].

In addition to more efficiently providing stimulation, our robot-driven ES technique of intermittent stimulation further reinforces the "assist-as-needed" effect of robotic interventions. In this way, both robotic-assisted treadmill training and robot-driven epidural stimulation can function to minimize "learned disuse" [25] that may lower the efficacy of rehabilitation. Together, they also provide a weaning effect for the animal, to allow animals to discover the mechanical, anatomical, and neural controls needed for quadrupedal movement without the machine.

Another benefit of our robot-driven intermittent epidural stimulation is that it allows us to use lower voltages to elicit activity [35] to minimize noncompliance in the training animals. Previous studies by Ichiyama *et al*. [85] and Gerasimenko *et al*. [80] circumvented this obstacle to effective ES by using quipazine to initiate stepping and increasing stimulus intensity to produce more rapid stepping. They demonstrated that it is possible to combine complementary interventions to produce synergistic benefits to the rehabilitating animal and significantly increase recovery. We believe, therefore, that our robot-driven epidural stimulation technique can be combined effectively with AAV5 delivery of BDNF to produce similar, if not better, recovery.

I. Importance of Trunk Motor Cortex in SCI Rehabilitation

I-1. Muscle Synergies and Motor Primitives

Earlier, in our pantomime horse analogy for SCI, we discussed the importance of activating spinal networks below the site of injury for effective rehabilitation. As we discussed in previous sections, the spinal cord is capable of processing and producing complex movements. However, its relationship with the primary motor cortex (M1) is

essential to its function for natural movement and stability [98]. In an intact animal, the relationship between the spinal cord and the motor cortex is a complex relay of descending supraspinal control and ascending sensory information. Information from the brain and from peripheral sensory afferents converge onto the spinal cord where it is integrated, processed, and acted upon for movement, stability, and posture. There are many theories as to how the spinal cord and brain interact specifically to create movement, but all agree that motor control requires translation from abstract task-specific goals into a variety of combinations of tangible complex muscle activation patterns.

For example, a task such as opening a door is accomplished by activating a combination of muscles including supinators of the arm, latissimus dorsi, and flexors of the fingers. Studies in motor behavior suggest that each of these muscle activations is a basic unit that can be combined to form synergies, which in turn can be combined into any number of permutations to accomplish a task [99] – [102]. Furthermore, just as there is more than one way to open a door, for a given motor task, there can be many different combinations of synergies to produce motor behavior to accomplish that goal [103]. In this way, redundancies in the motor system can allow for flexibility in movement, based on specific context like environmental factors and other sensory stimuli [104]. As a result, this interpretation of muscle synergies is that they provide a way to translate tasks into combinations of simple controls, as part of a system of hierarchical control relaying commands from the cortex and task-relevant sensory information from the periphery [105].

Muscle synergies may also reflect the evolution of complex nervous systems, where more “primitive” patterns of motor coordination have been conserved from

phylogenetically older neural networks [106]. Work by Hart *et al*. in frog motor behavior supports the idea that complex movements can be decomposed into small modules that are comprised of units of premotor drives in the spinal cord and specific, associated muscular activity [107], [108]. These are low-level synergies that may present at birth, or during development, that represent optimal ways of moving limbs based on anatomical mechanics. In essence, they provide a basic foundation for movements. Through development, these synergies, or modules, can be accessed or suppressed by the CNS when needed, for specific motor behavior [109]. Indeed, based on experience and training, these synergies may be built upon or changed for different motor behaviors.

Though there lies a debate on if synergies are learned or inherent to our neurobiology, studies of infants between the ages of 4 and 5 found that set patterns of muscle activation emerge as a response to disruptions to posture [110], suggesting that there may be an innate component to muscle synergy. However, studies investigating the differences between individuals and their motor behaviors show that factors such as experience and anatomy can change and modify muscle synergies over time [111], [112], though the rate at which this occurs is yet unknown.

I-2. The Role of Cortex in Muscle Synergy Activation

In our understanding of muscle synergies, the primary motor cortex plays an essential role in the control and use of muscle synergies to produce motor behaviors. Kargo *et al.* demonstrated in the rat model that M1 is essential to early skill learning, but are particularly important to action selection and motor adaptation in response to environmental factors [113]. As a result, we believe the cortex plays a role in organizing

motor modules that exist in the spinal cord. This is of importance given the effects of SCI, where descending control from the cortex is not relayed to these spinal motor modules. Giszter *et al*. suggests that in the absence of cortical control – in an isolated system – the basic muscle synergies or modules in the spinal cord can be expressed without modification [114]. In previous work in our lab, Yang *et al.* showed that in both the stepping NTX and non-stepping ATX models, there is a similar reduced repertoire of synergies that exist, regardless of the level of rehabilitation in the animals. This suggests that there are a core collection of circuits or synergies that underlie motor behavior (Yang – unpublished) upon which modifications and adjustments can be made.

However, to complicate further the effects of injury such as stroke or SCI, these pathologies can also induce changes and reorganization of muscle synergies and modularity. Using robotic therapy devices to measure and assess the changes in smoothness of movement in stroke patients, Rohrer *et al*. showed that gradual merging of sub-movements, or synergies, may be the cause of recovery of function, though the motor behavior may look similar to before rehabilitation [115]. This has been further corroborated by Dipietro *et al*. and Hayes Cruz *et al.* [116], [117], who showed that therapy can modulate, or "fine-tune", existing synergies rather than creating new ones. This notion of injury affecting the organization of synergy has been corroborated by Cheung *et al*. in the human stroke model, where they observed three distinct ways in which synergies can be affected – preservation, merging, or fractionation [118]. They believe that studying these changes in patterns of muscle synergies can even be used as physiological markers to assess the severity of impairment in the stroke model and potentially in other diseases, such as SCI.

In addition to changing the organization of the synergies in the spinal cord, injury to the nervous system can have profound effects on the organization and structure of the sensorimotor cortex. This has been extensively observed and studied in the case of limb amputation. Specifically, peripheral nerve injuries can cause de-afferentation of specific areas in the motor cortex corresponding to injury, which, in turn, allows for expansion of adjacent areas into the dennervated areas [119], [120]. This pattern of reorganization has been observed in the rat model as early as seven days following injury [121], [122]. Unfortunately, changes to the organization of the sensorimotor cortex can also result in pathological sequelae as a result of maladaptive plasticity. In the case of peripheral nerve injuries, this can manifest most commonly into phantom limb pain. Using focal transcranial magnetic stimulation (TMS), Karl *et al.* explored the relationship between the reorganization found in the somatosensory and motor cortex in amputees with and without phantom limb, and found significant differences in cortical plasticity [123]. Using magnetic resonance imaging (MRI) and functional MRI techniques to examine the structure and behavior of the brain in intact patients and amputees, Raffin *et al*. further emphasized the effects of reorganization on the primary motor cortex in phantom limb pain sensation [124]. Fortunately, the negative effects of reorganization can mitigated or remodeled with interventions, such as replanting lost limbs [125]. In patients who had successful replantation of lost hands, Röricht *et al*. used TMS to show that replacing the limb had broad and long-term effects on motor cortex reorganization years after treatment [126].

I-3. Organization of the Sensorimotor Cortex

Sensorimotor representation in the cortex is organized topographically, in such a way that it is possible to create an approximate two-dimensional map of the body that can be overlaid onto the cortex [127], [128]. However, this does not imply a one-to-one correspondence between a site in the cortex and a muscle or muscle activation. Rather, the literature suggests that the motor cortex is structured somatotopically in such a way to take advantage of the muscle synergies that exist to create many varied combinations of motor behaviors.

One consistent observation in the motor cortex organization is the activation of the same musculature by stimulating different and noncontiguous motor cortex areas. In his review, Schieber describes this method of motor control as "convergence", where separate territories in the cortex can converge onto motor neuron pools in the spinal cord for muscle activation. The precise nature of the stimulation of noncontiguous areas has been shown in the cat model [129] and in primates [130]. In the cat model, Schneider *et al.* used ICMS to assess electromyographic (EMG) responses of up to twelve forelimbs, concluding that there are widespread and physically disparate areas of the motor cortex with common target territories in the spinal cord. Furthermore, work by Phillips *et al.* in the cat motor cortex reveals that cortical areas for different musculature can overlap [131], again allowing for synergistic and efficient control for motor behaviors. Using TMS to study the human motor cortex, Devanne *et al.* confirmed the existence of these principles of convergence and overlap in the representation of the proximal and distal musculature of the upper limb [132].

In addition to the convergence of different cortical sites to produce the same muscle activation, the literature also suggests that a single cortical site can have divergent activations of different muscle groups. This is a relatively novel perspective on the cortex – previous beliefs held that any given corticospinal neuron had a single monosynaptic connection to a motor neuron of a muscle. Using EMG of forelimb muscles in response to stimulation, work by Fetz and Cheney [133], [134] in macaque monkeys identified connections between neurons of the primary motor cortex and several different motor neuron pools. This was further demonstrated with individual hand muscles in the monkey by Buys *et al.* [135] and Lemon *et al.* [136] Furthermore, in reaching tasks performed by monkeys, McKiernan *et al*. analyzed spike-triggered averaging of rectified EMG activity to show that divergence of activation of M1 neurons were not confined to finger and wrist muscles, but also to the elbow and shoulder [137], suggesting a goal-oriented organization of musculature activation. In this way, divergence may also limit the potential repertoires available to a single motor output within the constraints of anatomy or function.

I-4. The Role of the Trunk Motor Cortex in Locomotion

Given the importance of the role of the trunk muscles in gait stability and locomotion, as previously discussed, we believe that trunk motor control at the cortical level is also an essential component to rehabilitation. Despite the interruption in the direct networks to and from the cortex, there is ample evidence to suggest that cortical representation of the trunk muscles – above and below the site of transection – plays a critical role in control and movement. In anatomical studies using intramuscular injections of horseradish

peroxidase to label motor neurons of ventral trunk musculature in cats, Miller *et al.* showed that any given level of trunk muscle is innervated by a wide variety of axial spinal segments [138]. This suggests that there may be partial, but significant cortical control of musculature below injury. This is certainly true in the case of incomplete SCI, where transection is not anatomically complete.

There are many clinical studies using techniques such as EMG analysis of trunk muscles during walking [12] and axial trunk rotation [14] to understand the role of trunk motor control. However, the critical role of the trunk motor cortex in rehabilitation is evident when studying the previously discussed NTX model of recovery, where approximately 20% of animals are capable of producing weight-supported stepping (>50% of steps). In this model of rehabilitation, the subset of animals that can weight support have significant different reorganization patterns in the motor cortex, as compared to animals that do not step. Specifically, Giszter *et al*. showed that weight-supporting rats retain representation of caudal trunk (below the site of injury) in the cortex as adults [139]. In addition, lesioning this area of the trunk motor cortex in this animals results in the loss of weight-supported stepping [140].

The importance of the trunk motor cortex in effective rehabilitation was further bolstered by previous studies in our lab. Oza *et al.* studied NTX rats who were successfully rehabilitated with robot-assisted treadmill training, and lesioned the trunk motor cortex below bregma. These previously weight-supporting NTX rats significantly lost overall locomotor ability as a result of damage to the cortex (Oza – unpublished). We believe that these lesions significantly hinder the rats' ability to integrate robot support into their motor controls for locomotion. This was accompanied by a lack of change and

reorganization in the motor cortex that are typical of rehabilitation on robot in the NTX model, further emphasizing the role of the trunk motor cortex in successful locomotor rehabilitation.

I-5. The Effect of SCI on Motor Cortex Representation

In the case of SCI, the effects of injury on the sensorimotor cortex have been well documented in the literature. Much like in peripheral nerve injuries, there is not only a somatotopic reorganization of the motor cortex [141], [142], but there are also significant changes to sensory representation [143], [144]. Additionally, it is well known that the loss of motor output as a result of nervous system damage results in de-afferentation of areas in the motor cortex corresponding to the loss. Previous studies have shown that adjacent motor areas can expand and migrate into these silenced areas [145], [146].

In our lab, we have attempted to investigate how specific paradigms of SCI and modalities of treatment – treadmill training, robot rehabilitation, and active stepping and passive non-stepping training – affect the reorganization of trunk motor cortex. When comparing between treadmill-trained and robot rehabilitated animals in the NTX model, Oza *et al.* discovered that successful rehabilitation with robot forces led to an increased expansion of caudal trunk areas in the motor cortex and an increase in overall coactivation per site. They concluded that robot rehabilitation was able to partially reverse some plastic changes that resulted from non-stepping paraplegia in SCI [147], resulting in more normal topography than seen in non-rehabilitated rats.

In contrast, when exploring how rehabilitation might affect the ATX model, Oza *et al.* observed that without any active stepping – regardless of robot assistance – animals

had significant expansion of the trunk motor cortex into rostral areas coding for forelimb. This led to an increase in trunk and forelimb coupling. Interestingly, they also discovered that any type of passive rehabilitation without stepping exacerbates this migration rostral shift beyond what was observed in transection alone [148]. There is a clear effect of injury on the cortex, and further changes may be directly correlate with the level or success of rehabilitation.

The differences observed in the cortices between animals that underwent active and passive rehabilitation in these studies may be a result of cortical plasticity inducing functional and structural changes. Changes in motor representation in the cortex are hypothesized to be associated with novel skill acquisition [149], [150]. Kleim *et al.* showed in reaching studies in rats that learning new behaviors resulted in precise functional and structural changes to specific areas of the motor cortex associated with the newly acquired skill [151]. Similar work has been show in humans using TMS, with suggestions that the degree of change may even be related to the relative difficulty of the new behavior [152].

In the NTX model, transected animals successfully rehabilitated with robot assistance have essentially acquired a new skill – locomotion integrating active robot assistance with trunk musculature and hindlimb activity. This was corroborated by the changes in trunk motor cortex representation. In the ATX model, a motor skill – quadrupedal walking – was lost and a new way of moving was never successfully learned, as shown by the motor cortex maps by Oza *et al*. [148] The cortex, thus, is an important factor of locomotor rehabilitation. In the context of muscle synergies, in the NTX animals, it was perhaps needed to build representations that were not part of the

standard repertoire found in the spinal cord. By doing this, new skills were and acquired, ultimately leading to successful rehabilitation on robot, with weight-supported stepping. In the case of the ATX model without active stepping, the trunk motor cortex looked similar to that of the non-rehabilitated, injured animal.

With SCI and rehabilitation, the fundamental relationship between cortex and spinal cord networks to control locomotion has been altered, with an increased emphasis on intact spinal circuitry to drive stepping behaviors. We have discussed previously the many ways in which to activate the spinal cord without supraspinal control for locomotion. In the NTX animal, the development through adulthood and changes in afferent feedback from the periphery to the spinal cord plays a role in stepping behavior. Neurotrophins can play a similar role in the ATX animal by creating an excitable milieu for stepping. Epidural stimulation and serotonin agonists are other methods that the adult spinal cord could be engaged in rehabilitation. However, our work in both SCI models has revealed the importance of the cortex as well. It does not only provide a complementary component to the spinal cord in producing locomotion, but it is essential for successful recovery.

J. Specific Aims

From a clinical perspective, one of the most important aspects in treating spinal cord injury lies in dealing with patient immobility. This loss of locomotor ability not only severely impacts patients' physical health, but can also lead to depression and innumerable health costs to both the injured and caregivers. In recent years, new therapies have been discovered that we believe can be combined to create a more robust rehabilitation regimen for locomotor recovery.

Our long-term goal is to investigate the combination of robot-assisted rehabilitation at the pelvis with the biological therapy of viral delivery of Adeno-associated virus engineered to produce brain-derived neurotrophic factor (BDNF) in treating spinal cord injury. In this work, we study how our unique robot rehabilitation may be used in the ATX rat model when induced to step using robot-driven epidural stimulation and AAV5-BDNF. In doing so, we hope to understand the cortical changes that occur with reorganization and plasticity after SCI with active, stepping rehabilitation. **We hypothesize that our robot-assisted treadmill training will work synergistically with ATX rats induced to step with AAV5-BDNF, and that adding robot-driven epidural stimulation will serve to improve locomotor recovery. We also hypothesize that we will see significant reorganization of the trunk motor cortex with active weight-supported stepping rehabilitation.**

J-1. Specific Aim 1

Investigate the efficacy of robot-assisted treadmill training on rats transected as adults (ATX) treated with AAV5-BDNF to induce stepping. Our unique robot-

assisted rehabilitation at the pelvis provided during treadmill training has shown to be effective when animals are able to perform crude stepping patterns before starting training [30], [31], [153]. While adult rats transected as neonates (NTX) that can step with weight-support are able to benefit from training, ATX animals without additional intervention are unable to do so. **We hypothesize that ATX rats treated with AAV5-BDNF will be able to show significant recovery in locomotor ability with treadmill training, as compared to ATX rats without AAV5-BDNF.** To test this hypothesis, we will compare our AAV5-BDNF animals with rats treated with a sham AAV5 virus engineered to produce green fluorescent protein (GFP), a functionally quiescent protein in the CNS.

J-2. Specific Aim IIA

Study the locomotor recovery outcomes of a combination therapy of robot-assisted treadmill training, robot-driven epidural stimulation, and AAV5 viral delivery of BDNF in the ATX rat model. We believe that we have chosen potentially complementary therapies in our rehabilitation regimen. The use of AAV5-BDNF, though it does promote plasticity in the adult spinal cord, may cause hyperreflexia or spasticity in the hindlimbs and lower body the animals. We believe that mass robot-assisted treadmill training may ameliorate this. This may potentially allow for increased viral loads, with less pathological reflex responses. As a result, this improved excitability of the spinal cord will allow for the use of low voltage in epidural stimulation, a limiting factor in previous work in our lab. Low voltage will allow stimulation to become more intermittent, which will provide a phasic excitation that may allow for more robust

muscle synergies to form, better integrating lumbar stepping patterns into whole body motions during locomotion. Finally, activation of stepping provided by AAV5- BDNF and epidural stimulation will also provide consistent stepping frequencies that may bolster the effects of our pelvic-based robot-assisted rehabilitation. **We hypothesize that combination therapy will provide the best recovery outcomes, with the individual therapies working in synergy to activate hindlimb alternation and plantar stepping, and to increase weight supported stepping by the rat.**

J-3. Specific Aim IIB

Investigate how AAV5-BDNF treatment may affect the use of robot- driven epidural stimulation when inducing stepping in ATX rats. Previous work in epidural stimulation in the ATX model by Ichiyama *et al.* has demonstrated that rhythmic, alternating hindlimb locomotor activity can be induced in ATX rats by direct epidural electrical stimulation [85]. In addition, examiners studied threshold of stimulus intensity for generating hindlimb locomotor activity, and also showed that this threshold increased as a function of time after injury. **We hypothesize that AAV5-BDNF treatment will decrease threshold of stimulus intensity required to induce hindlimb motor activity, as compared to animals without viral treatment. In addition, we also hypothesize that AAV5-BDNF treatment will increase the overall timeline in which threshold stimulus intensity acts as a function of time post-injury.**

J-4. Specific Aim III

Examine cortical reorganization in the trunk motor cortex in ATX animals after

active rehabilitation. Work in our lab by Oza *et al.* revealed the cortical changes that occur after injury in the ATX model, demonstrating the rostral shift of the overall trunk motor representation along the cortex [148]. This work also showed how passive rehabilitation (non-weight-supported stepping training) may exacerbate this shift, as well as how new synergies can be developed between trunk and forelimb, as a response of cortical reorganization. The effects of active rehabilitation on the organization of the trunk motor cortex in the ATX model are still unknown. **We hypothesize that chronic adult SCI with *active* rehabilitation will induce significant reorganization of the trunk motor cortex different from ATX animals with passive rehabilitation and no rehabilitation.** Work by Dancause and Nudo has shown that novel skill acquisition is an important factor in cortical reorganization [154]. Our previous aims investigate methods for active rehabilitation in the ATX model, where new behaviors and skills are learned as recovery progresses for weight-supported stepping. We believe that the development of these new locomotor skills to compensate for weight-supported stepping after SCI will induce these significant changes in the trunk motor cortex.

CHAPTER 2: The effect of robot-assisted treadmill training on adult-spinalized rats induced to step with viral delivery of brain-derived neurotrophic factor

A. Introduction

In the study of locomotor recovery in the treatment for spinal cord injury (SCI), a complete thoracic spinal cord transection at vertebral level T9/T10 in the rat provides a unique perspective in rehabilitation paradigms. In the SCI model for rats transected as neonates (NTX), some animals are capable of autonomous stepping without any intervention when they are adults [140], [155]. Stepping in these animals may be as crude as rudimentary alternations of the hindlimbs, but can be as robust as up to 50% of their steps supporting their body weight (WS), as seen in approximately 20% of NTX animals that step [33]. In these WS NTX animals, it is possible to improve their stepping ability through the use of robotic intervention at the pelvis by providing isotropic elastic fields to facilitate trunk support and natural posture during locomotion [30]. Robot rehabilitation at the pelvis can exploit the stepping patterns to help injured animals rediscover the natural controls needed for quadrupedal stepping without direct neural communication.

In contrast to this paradigm of recovery, in the complete SCI model for rats injured as adults (ATX), injured animals do not develop any autonomous stepping without intervention that can be exploited for rehabilitation. However, treatments such as perineal stimulation, [81] epidural stimulation, [85], [156] or serotonin-agonist drug delivery [36], [51] can induce stepping in the ATX animal. The stepping can allow a rehabilitation to proceed. We have previously shown using epidural stimulation in the ATX model that robot rehabilitation at the pelvis can be an effective treatment tool when

combined with induced stepping patterns [35]. As a result, we view the NTX model for recovery as a signpost for what is possible in recovery with our robotic intervention when an injured animal can produce some basic flexion-extension hindlimb movements and the mechanical coupling of trunk muscles allows for communication above and below the site of injury. NTX rehabilitation demonstrates the potential for possible recovery in the ATX model, when basic stepping patterns can be induced. We believe that understanding the differences between the NTX and ATX model rats is crucial to adapting treatment modalities identified in the NTX for successful ATX rehabilitation.

In a complete spinal cord injury, though supraspinal control is lost, there exists intact neuronal circuitry below the site injury that is capable of organizing complex movements, such as hindlimb reflexes [5]. In the NTX model, this system is injured at a time when the nervous system is very plastic, which may play a large role in motor learning and adaptation to injury, possibly allowing the NTX animals to develop function after injury. In contrast, in the ATX model, after injury and loss of supraspinal control, it appears that the spinal circuitry is incapable of autonomously developing ways to compensate and restore significant function on its own. As a result, we are interested in investigating means in which to provide an environment for growth and adaptive plasticity of circuits in the injured spinal cord, below the site of transection.

Brain-derived neurotrophic factor (BDNF) is an excellent candidate to promote plasticity, as it has been used in the complete spinal cord transection model to induce stepping in injured animals, [56], [59], [60] as it is a neurotrophic factor widely expressed in the central nervous system [61]. It can promote regeneration [57], [58] and induces synaptic plasticity in the adult nervous system [59], [60]. It has also been shown to be

upregulated in the spinal cord of transected animals in response to exercise and training [63]. In addition, there are many means by which to deliver BDNF to the spinal cord [54], [55], including a method demonstrated by Boyce *et al*. via an Adeno-associated virus-5 (AAV5) viral vector in the ATX rat model [71]. Boyce *et al*. suggested a possible mechanism by which AAV5-BDNF functions to induce stepping in the ATX rat model, by lowering the rheobase of transfected motoneurons, effectively increasing their excitability, and allowing for plantar, weight-weight supported hindlimb stepping.

Unfortunately, in addition to locomotor recovery, the use of BDNF to induce stepping in open field and non-treadmill assisted rehabilitation has broader, less specific effects in the spinal cord, including increased spasticity, and sensitization to noxious heat, which has detracted from its clinical uses [56]. Recently, however, Tashiro *et al*. suggested that the production of endogenous BDNF as a result of treadmill training may lead to reduced spasticity and allodynia [157]. In addition, work by Ziemlínska *et al.* [70] suggests there is a role for exogenous BDNF and treadmill training to increase locomotor function, though this may also lead to general, non-specific pathological and detrimental sequelae from the administration of BDNF. Thus, we believe that BDNF provides a broad, basic foundation for rehabilitation, coupled with some risks, that we can use or exploit with our unique robot-assisted treadmill training for specific entrainment of locomotor function.

As a result, our goal in this study was to study the efficacy of our custom robot-assisted rehabilitation training on ATX rats induced to step using AAV5-BDNF, to examine how our robot treadmill training may interact with AAV5-BDNF effects in animals with stepping patterns induced in this way. Using the NTX model as an example

of this type of recovery, we hypothesized that ATX animals induced to step with AAV5-BDNF would recover robust stepping and locomotor function with the aid of robot interactive forces at the pelvis.

To test this, we prepared four groups of ATX animals: three experimental group receiving AAV5-BDNF, and a control group receiving a sham AAV5 virus engineered to produce green fluorescent protein (GFP), a functionally quiescent protein in the nervous system. One BDNF group received a rehabilitation regimen of robot assistance at the pelvis with treadmill training. The second BDNF group received only treadmill training, while the last was rested in cage. The GFP-treated group was rehabilitated with robot-assisted treadmill training. Using a battery of functional measures to assess quality of stepping as well as weight support, our results show that ATX animals induced to step using AAV5-BDNF significantly improve in their abilities to walk with robot-assisted treadmill training, as compared to other groups. To our knowledge, this is the first exploration into combining BDNF with robotic interventions in the rehabilitation of SCI. We also discovered that regardless of combined locomotor therapy with BDNF, there is a partial, but highly significant collapse in function that accompanies AAV5-BDNF treatment in the ATX rat. This collapse is characterized by loss of motor behavior and decreased body weight support. However, animals treated with AAV5-BDNF and trained with robot-assisted treadmill training had higher overall peak motor performances and had increased body weight support, despite eventual collapse. We believe investigating this collapse in the context of robot rehabilitation techniques might provide the basis for further avenues of study to be explored further.

B. Materials and Methods

B-1. Overview

34 intact adult female Sprague Dawley rats were used in this study, divided into four groups. At the time of surgery, each animal weighed between 250 and 280 grams. All animals received a complete spinal cord transection at spinal level T9/T10 as adults (ATX), with three groups receiving microinjections of AAV5-BDNF caudal to injury and the fourth group receiving a sham virus (AAV5-GFP). One BDNF-treated group received robot-assisted treadmill training during rehabilitation. Another BDNF-treated group was rehabilitated on the treadmill, without robot assistance. The final BDNF-treated group was rested in cage for the duration of the rehabilitation. The GFP-treated group was rehabilitated with robot-assisted treadmill training. Following post-operative recovery, all groups were trained with for approximately six weeks, according to the rehabilitation regimen used in our lab [30], [153]. During rehabilitation, we assessed locomotor recovery and compared the recovery outcome measures. All procedures were carried out with the approval and guidance of the Institutional Animal Care and Use Committee (IACUC).

B-2. Complete Spinal Cord Transection at T9/T10 in the Adult Rat (ATX)

Prior to surgery, all animals were anesthetized intraperitoneally (IP) with a 1.0-ml/kg cocktail of ketamine hydrochloride (50 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg). Anesthesia was supplemented when necessary at a concentration of 0.38 ml/kg KXA. Animals received a complete spinal cord transection at spinal level T9/T10, according to similar surgical procedures described in our previous studies [35], [148],

using a mid-thoracic dorsal incision to expose the vertebrae. A full laminectomy was performed at the arches of T9 to T11 to expose the spinal cord. Following an incision to open the dura using a 30-gauge needle, iridectomy scissors and aspiration were used to remove a full segment of spinal cord at T10. The resulting cavity was filled with gelfoam.

B-3. Microinjection of Virus Caudal to Injury

We adapted surgical techniques for microinjection of virus described in Tom *et al.* [158] and Boyce *et al*. [159] Immediately following transection, virus was injected approximately 1 mm caudal to the injury site using pulled glass pipettes and a 1 ml Hamilton syringe. Approximately 2.5×10^{10} viral particles were slowly injected into the cord, using stereotactic guidance and a micromanipulator to guide the needle into the intermediate grey of the spinal cord. The virus was injected at a depth between 400-500 μm below the pia at four different locations transversely across cord 250 μm apart, with each site receiving approximately 2 μl of prepared viral suspension. A total of 8 μl was injected per animal. Upon viral injection, the incision was closed in layers.

B-4. Pelvic Orthosis Implantation

All animals were implanted with custom-fabricated pelvic orthoses following viral injection, using methods described in previous studies (Fig. 2-1). [31], [35] Two incisions were made over the pelvis, approximately 2 mm caudal to the palpable iliac crest, on the left and right side. Blunt dissection was used to separate the superficial and deep gluteus muscles, allowing for implant cuffs to be inserted against the pelvic bones at approximately 45° to the horizontal axis. After the cuffs were placed, two ends of the

implant were fastened together about a crossbar with a screw, securing the orthosis in place. Quick-drying epoxy cement (J-B weld) was applied to reinforce the connection.

Figure 2-1. Custom-made pelvic orthosis placed on the pelvis of a rat skeleton, demonstrating how it interacts with the animal. *Used with permission from Udoekwere et al., 2014*

B-5. Post-operative care

Following surgery, all animals were allowed approximately seven to ten days of recovery, during which time prophylactic antibiotics were given daily for a week. Prior to surgery, after anesthesia was administered, animals were given slow-release buprenorphine subcutaneously (0.5 mg/kg) for analgesia. Rat bladders were expressed twice daily, during which time the animals were observed for possible health issues, including autophagia or skin lesions.

B-6. Robot-Assisted Treadmill Training

All animals in this study began robot-assisted treadmill training 7-10 days after surgery, after no significant health concerns were observed. Twenty-minute training sessions

occurred five times a week for approximately six weeks, during which time video and robot data were recorded for analysis, similar to our previously established rehabilitation regimens [30], [148], [160]. We used a PHANTOM® Premium 1.0 model (developed by SensAble Technologies, Inc.) with custom software developed in our laboratory to apply forces to the injured rat at the pelvis to allow for a trunk posture that is nearly identical to that of a normal rat. Using an assigned equilibrium for the rat's pelvic center – defined by a three-dimension coordinate frame (x-, y-, and z-axes) – the robot provides a uniform isotropic elastic force field ($k_x = k_y = k_z = 45$ N/m) to bring an injured rat's pelvis to that equilibrium. Through the training session, our custom software calculated and adjusted the forces required to bring the animal to equilibrium at a rate of 1 kHz.

B-7. Locomotor Assessment

We used three outcome measures to assess locomotor recovery of the animals within and across groups [148]. First, we used videos recorded during training were assessed using the Antri, Orsal, and Barthe bipedal stepping scale [161]. This system evaluates the hindlimb stepping of ATX rats based on right-left alternation, amplitude, body weight support, and plantar foot placement, and scores the rat on a scale of 0 to 21, divided into four levels, with increasing scores for increased ability to step. Level 1 (scores 0-1) are unable to move their hindlimbs at all, except for very weak limb jerks. Level 2 (scores 2-9) animals are able to produce some rhythmic movements without any body weight support. Level 3 (score 10) animals correspond to consistent alternation of hindlimbs with occasional body weight support. Finally, level 4 (scores 11-21) animals are able to produce rhythmic movements with body weight support and plantar foot placement.

Videos were also assessed for our second outcome measure: percent body-weighted stepping (%WSS). Using a frame-by-frame analysis of the videos recorded, we calculated the total number of steps that supported the body weight of the animal – defined where no other part of the animal's body touched the ground except for the feet – and divided this by the total number of steps an animal took. We observed each trial session for each animal and kept a record of %WSS over time. Finally, we analyzed robot data collected during training sessions to investigate the interactive force between the rat and the robot in the z-axis to investigate the changes in body weight support provided by the robot over the duration of training. The force required to maintain the pelvic height was converted to newtons (N) and normalized to the weight of the animal (g) to compare the animals and the groups. Our software recorded the robot interactive forces at a rate of 1 kHz.

B-8. Histology

At the end of the rehabilitation schedule, animals were sacrificing using an IP overdose injection of Euthasol of 3 ml. Animals were then perfused intracardially with 0.9% physiological saline, and perfused with 4% buffer paraformaldehyde (PFA) to fix the nervous tissue. The next day, the spinal cords of the animals were extracted and preserved in 4% buffered PFA for approximately a week. Before sectioning the tissue, the specimens were placed in 30% sucrose solution for up to a week and embedded in M1 embedding matrix (Thermo Scientific Shandon). Once frozen, serial, parasagittal sections of 25 μm were cut by cryostat microtome. To confirm the completeness of spinal transection, we stained the tissue for Nissl Myelin. In a previous pilot study, we prepared three animals – two with AAV5-GFP and one with saline – to replicate and demonstrate

the efficacy of microinjections into the cord. Their cords were stained with anti-GFP antibodies to confirm the presence of GFP expression (see results).

B-9. Data Analysis

We compared locomotor recovery outcome measures between the two groups of animals, at the beginning of their training and at the end of their training regimen. To examine the changes within a single group over rehabilitation, we used paired t-test to assess the significant changes. To examine the recovery patterns across groups at specific time points, we used a one-way ANOVA with post hoc Tukey Kramer corrections. For all of the statistical tests, we considered a p-value of less than 0.05 to be significant. All data analysis was done using custom-written scripts in MATLAB R2014B, Mathworks. In addition, graphs and figures were produced in Microsoft Excel 2016.

C. Results

C-1. Overview

To examine the functional recovery of the animals, we recorded video and robot forces during training sessions for each rats. Using these data to compare training rehabilitation regimens, we assessed each animal against a battery of outcome measures: the Antri, Orsal, Bartes (AOB) bipedal stepping scale, robot interactive forces, and an analysis of weight-supported stepping. Overall, in all three categories of recovery, AAV5-BDNF treatment alone had a significant effect on locomotor recovery, and this was further significantly enhanced by treadmill training and robotic intervention at the pelvis.

C-2. AOB Bipedal Stepping Scale

Using the AOB scale to assess complete SCI rats on a treadmill, we assessed the locomotor recovery of the ATX animals based on their ability to (1) alternate their hindlimbs, (2) support their body weight, and (3) plantar place their hind feet during locomotion. The AOB scale is a modified BBB assessment which evaluates completely transected rats, within the framework of hindlimb locomotion on a treadmill. None of the rats were scored prior to injury. The first day of training for all of the animals were the first days of scoring in this assessment.

For all of the groups, the start of training corresponded with a low AOB score, indicating very little to no hindlimb function. There was no significant difference between the groups at this point ($p = 0.3373$, $F(3,30) = 1.17$, one-way ANOVA with Tukey Kramer post hoc corrections). The average AOB score for the AAV5-GFP-treated group was 0.25 ± 0.16 (mean ± SEM), with six of the eight animals exhibiting a score of

0, indicating no movement at all. Of the AAV5-BDNF-treated groups, all exhibited similar AOB scores indicating little to no hindlimb function. The cage rest group had a starting score of 1 ± 0.5, with one animal with a starting score of 4, indicating occasional (<50%) right-left hindlimb alternation at a weak amplitude. The treadmill-trained group had a starting score of 0.88 ± 0.23, with the highest score of a 2, and two animals with a score of 0. Finally, the robot-trained group had a starting average AOB score of 0.6 ± 0.22.

We then compared the individual performance of the therapies from the start of training to the end. The AAV5-GPF-treated group did not exhibit statistically significant improvement in AOB score, ending with a final assessment of 0.5 ± 0.19 ($p = 0.18$, paired t-test). All of the AAV5-BDNF-treated groups showed significant improvement from the start of training to the end. The group under cage rest had a final AOB score of 5.38 ± 1.25 ($p = 0.01$, paired t-test). Treadmill-trained animals had an average final AOB score of 7.13 ± 2.47 ($p = 0.05$, paired t-test). The robot-trained group demonstrated the most improvement, with a final average AOB score 13.1 ± 1.29 ($p << 0.01$, paired t-test).

At the end of training, we discovered that the AAV5-BDNF-treated animals that underwent robot training exhibited significantly more recovery than the other groups ($p << 0.01$, $F(3,30) = 12.57$, one-way ANOVA with Tukey Kramer post hoc corrections). By the same test, GFP-treated animals had significantly less final AOB scores than the robot-trained BDNF group and the treadmill-trained BDNF group, though not the cage rest BDNF group.

Consistent with previous AOB assessments in our laboratory in both the ATX model and the NTX model, animals that showed significant recovery to be able to

provide body weight support and plantar stepping exhibited a stereotypical recovery pattern in AOB over days of training [31], [35].

C-3. Robot Interactive Force (zForce)

Using our software to record the interactive force between rats and the robot, we were able to assess the percent of the body weight support provided by the robot to maintain pelvic height (zForce) as a proxy for body weight support in the rat's recovery over time. zForce was calculated as an average of the interactive forces in the vertical (z-) axis, between the rat and the robot during a training trial, normalized for the rats' weights in grams. The AAV5-GFP-treated group and the AAV5-BDNF-treated group assigned to robot rehabilitation were recorded daily, for twenty minute intervals throughout the whole training session. The robot interactive forces of the other two groups were recorded once a week, for five minutes. This was to minimize any training effects of robot recording on the rehabilitation on these animals.

At the beginning of training, there was no significant difference between the starting normalized zForce between the groups of rats ($p = 0.71$, $F(3,30) = 0.46$, one-way ANOVA with Tukey Kramer post hoc corrections). The average starting normalized zForce for the AAV5-GPF-treated group was 0.43 ± 0.01N/g. The cage rest BDNF group had a starting average normalized zForce of 0.41 ± 0.03 N/g. The treadmill-trained BDNF group and the robot-trained BDNF group had starting average normalized zForces of 0.44 ± 0.01 and 0.43 ± 0.01, respectively.

We then compared the start and end normalized zForces to assess the impact of the individual therapies on the animals' ability to body weight support. The AAV5-GFP-

treated group had a final normalized zForce of 0.42 ± 0.02, indicating no significant change in interactive robot force as a result of rehabilitation ($p = 0.52$, paired t-test). Similarly, the cage rest BDNF group (final: 0.39 ± 0.03) also did not show a significant decrease in normalized interactive zForce ($p = 0.34$, paired t-test). However, the treadmill-trained BDNF group exhibited significant decreased in normalized zForce (final: 0.39 ± 0.02; $p = 0.01$, paired t-test). Robot-training also significantly improved the ability of the AAV5-BDNF-treated animals to support their body weight ($p << 0.01$, paired t-test). They had an average final normalized zForce of 0.27 ± 0.03.

Finally, we compared the end normalized zForces among all of the groups to determine if there was a significant difference between therapies to elicit hindlimb locomotor recovery. The AAV5-BDNF-treated group that was rehabilitated with robotic interactive forces at the pelvis during training relied significantly less on the robot to maintain body weight support at the end of training than each of the other groups ($p = 0.001$, $F(3,30) = 6.97$, one-way ANOVA with Tukey Kramer post hoc corrections). No other group was significantly different than the other groups at the end of training.

C-4. Percent Weight-Supported Stepping (%WSS)

Our final assessment of recovery in this study was to analyze the stepping patterns of each animal. Specifically, we used frame-by-frame analysis of video taken during every training session to determine the percentage of steps taken that supported an animal's body. A weight-supported step was determined as one where only the limbs and tail and no other part of the rat's body were on the treadmill.

At the beginning of training, for all animals across all groups there were no weight-supported steps. This is consistent with our finding for AOB, indicating that in almost all of the animals, the first day of training consisted of random, jerky limb movements, if anything at all. The one animal in the cage rest group that had an AOB score of 4 on its first day did not have any weight-support, as a score of 4 indicates only right-and-left hindlimb alternation, but no weight support during locomotion.

The AAV5-GPF-treated group of animals had a final %WSS of 0%, as well, consistent with their lack of significant AOB and normalized zForce improvement. The BDNF group that underwent cage rest had an average final %WSS of 4.29 ± 2.90%, with six of the eight animals having no weight-supported steps at all. This was not a significant improvement ($p = 0.18$, paired t-test). Similarly, the treadmill-trained BDNF group did not have a significant improvement in %WSS, as a result of training ($p = 0.23$, paired t-test). Its average final %WSS was 15.1 ± 11.37%. Conversely, the AAV5-BDNF-treated group that received robotic rehabilitation ended with a final %WSS of 49.89 ± 10.82%, a significant improvement from the start of training ($p = 0.0013$, paired t-test).

When evaluating the final %WSS of all of the groups, we discovered that, similar to the previous two assessment criteria, the AAV5-BDNF-treated group that was rehabilitated with robot showed significant improvement compared to the other groups ($p = 0.0005$, $F(3,30) = 7.82$, one-way ANOVA with Tukey Kramer post hoc corrections). No other group was significantly different from the others.

C-5. AAV5-BDNF Treatment Results in Collapse in Function

In our three groups of rats treated with AAV5-BDNF, we noticed that animals developed a partial, but highly significantly collapse in function after performing well in all three locomotor outcomes measures. Overall, in these AAV5-BDNF-treated animals (n = 26), we observed that 12 animals developed this functional loss (four animals per group), which manifested in clonic movements, or twitching, or a complete flexion of the hindlimbs. These collapsed animals also often had tensing of the ventral trunk muscles, which resulted in a very rigid torso. This collapse in locomotor ability is consistent with findings in other studies where viral delivery of BDNF is employed in the ATX rat model to induce stepping [70]. We investigated the twelve collapsed animals as a single group, and attempted to quantify and define this collapse in function, using our battery of locomotor recovery outcome measures.

First, we compared the AOB score of these animals at the beginning of training, at their peak, and at the end of training. We defined peak performance after training was done and selected the highest score for AOB among all of the training days. At the start of training, the mean AOB score for the collapsed animals was 1.25 ± 0.30. We determined that this was significantly lower than at the peak performance of these animals (11.75 ± 0.98) and the end of training (5.92 ± 1.03) using a one-way ANOVA with Tukey Kramer post hoc corrections ($p << 0.01$, $F(2,33) = 39.22$). At peak performance, the AOB of this group indicated that animals were highly functional, able to alternate their hindlimbs and provide plantar stepping as a group. As an indicator of collapse, the final AOB was significantly lower than the peak AOB in this group of collapsed animals.

We next compared the normalized zForce at the same time points in this group. These animals relied the most on robot for meeting the pelvic height equilibrium at the beginning of training (0.44 ± 0.01 N/g), and this was significantly higher than the peak normalized zForce (0.33 ± 0.02 N/g). The starting normalized zForce was not significantly different than at the end of training. In addition, there was no significance difference between the end normalized zForce (0.38 ± 0.02 N/g) and the peak normalized zForce. We used a one-way ANOVA with Tukey Kramer post hoc corrections to determine significance ($p = 0.0034$, $F(2,33) = 6.77$). However, when comparing the peak and end normalized zForce values without the start time point, we do find a significant increase in robot interactive forces at the end time point ($p = 0.0138$, paired t-test).

Finally, we compared the %WSS between the start, peak, and end time points in the collapsed animals. At the beginning of training, none of the rats in the group of collapsed animals had any weight-supported stepping, resulting in a mean initial %WSS of 0%. At the peak of training, the mean %WSS was 34.64 ± 9.11%, which then dropped to 7.74 ± 3.48% at the end of training. There was no significant difference between the start and end %WSS values, but the peak %WSS was significantly higher than those time points ($p = 0.0003$, $F(2,33) = 10.41$, one-way ANOVA with Tukey Kramer post hoc corrections).

C-6. Effect on rehabilitation despite collapse

We then compared how collapse affected the performance of all of the AAV5-BDNF-treated groups at the peak and end time points for these groups to assess how different

rehabilitation modalities may have affected the partial, but highly significant collapse in locomotor function.

First, we evaluated the peak AOB performance among the BDNF groups. The highest AOB score was found in the group trained with robotic intervention at the pelvis, with a mean AOB of 16.1 ± 0.80, indicating a high degree of function, with alternation of the hindlimbs, body weight support, and plantar foot placement during stepping. The treadmill-trained group that did not receive robotic intervention had the second highest peak AOB at 11.5 ± 1.90, and the lowest peak AOB was found in the cage rest group, which had a mean peak AOB of 8.0 ± 0.89. We found a significant difference between the mean peak AOB between the robot-trained group and the cage rest group ($p = 0.0007$, $F(2,23) = 10.16$, one-way ANOVA with Tukey Kramer post hoc corrections).

In addition, we compared the mean peak AOB scores and the mean end AOB scores within the groups themselves. In all groups, we used paired t-tests to determine that there was a significant decrease in AOB score from the peak to end of training (robot-trained: $p = 0.0355$; treadmill-trained: $p = 0.009$; cage rest: $p = 0.049$).

Next, we assessed the mean normalized zForce at the peak of locomotor performance between groups. The highest average normalized zForce was used by the cage rest group, approximately 0.34 ± 0.03 N/g, to maintain pelvic height. The second highest average was in the treadmill-trained group (0.32 ± 0.03 N/g), with the least mean found in the robot-trained group (0.24 ± 0.03 N/g). There was no significant difference in normalized zForce at the peak of performance ($p = 0.058$, one-way ANOVA with Tukey Kramer post hoc corrections).

We then compared the peak and end mean normalized zForce within groups, to evaluate how rehabilitation techniques may have affected collapse in locomotor function. As a result of robotic intervention at the pelvis, the BDNF group that received robot training had no significant change in mean normalized zForce between the peak and end of training ($p = 0.13$, paired t-test). This contrasts with the treadmill-trained and cage rest groups, which both had significant increases in mean normalized zForce from peak to end of training (treadmill-trained: $p = 0.04$; cage rest: $p = 0.03$).

Finally, we compared the mean %WSS at the peak of performance between the AAV5-BDNF-treated groups of animals. At their peak, the robot-trained group had the highest %WSS value, approximately $72.42 \pm 5.96\%$. We determined this was significantly higher than the peak values for the treadmill-trained ($31.06 \pm 14.24\%$) and the cage rest ($10.12 \pm 3.99\%$) groups ($p = 0.0007$, $F(2,23) = 10.21$, one-way ANOVA with Tukey Kramer post hoc corrections). From the same evaluation, we discovered no significant difference between the treadmill-trained and cage rest groups.

We also compared the mean %WSS values at the peak of performance and the end of training, similar to the other two outcome measures presented above. For all three groups, using paired t-tests, we did not find any significant changes in %WSS between the peak and end time points.

C-7. Effect of Rehabilitation in Collapsed Animals

We then examined the outcome measures in the collapsed animals to examine how the three specific rehabilitation techniques might interact with collapse. There were only twelve animals total – four in each group (robot-assisted treadmill training, treadmill

only, and cage rest). As such, we only make comments on the trends we observe, without making any claims to statistical significance.

In general, BDNF-treated animals that received robot assistance at the pelvis had the best peak and end performances among all BDNF-treated animals, based on the three outcome measures we used. Their mean peak AOB score (16.25) represented frequent alternations of the hindlimbs, with frequent body weight support and plantar placement of the hindlimbs. In addition, after collapse, their mean end locomotor behavior score (9.5) was higher than animals without robot, represented by consistent right-and-left hindlimb alternation with a wide range of motion, though they did lose body weight support and plantar stepping.

In all groups, the mean normalized zForce at peak performance was lower than at the end of training. However, the group trained with robot assistance had the lowest peak mean normalized robot interactive force. This same trend was observed when evaluating %WSS in the animals, with the highest mean peak performance found in the robot-trained animals. All of these trends are consistent with the assessment of the different groups of animals with the collapsed animals included.

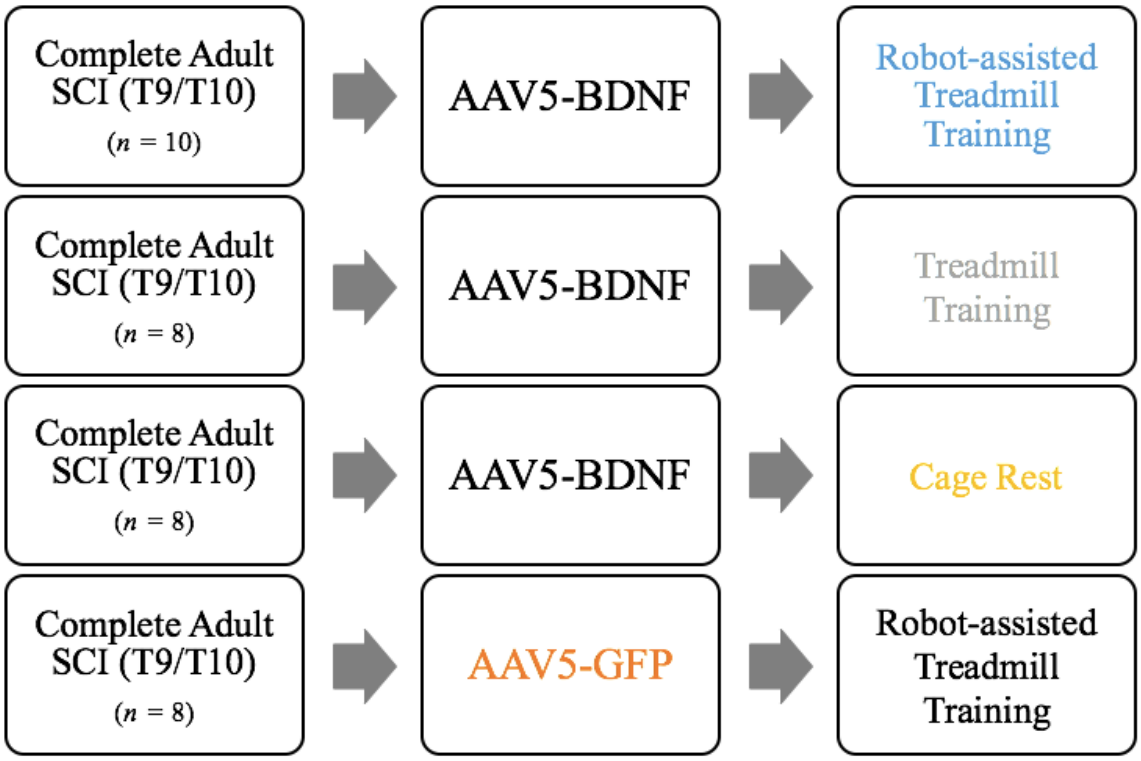


Figure 2-2. Overview of the experimental design describing the various differences between groups.

TABLE 1. Motor performance assessments in this study, using the 'adapted motor score'

Motor performance Level Score	Movement type Movement	R–L alternation	Amplitude	Body wieght support	Plantar foot placement
Level 1					
0	No	–	–	–	–
1	Weak limbs jerks	–	–	–	–
Level 2 (Rhythmic movements/dorsal foot placement)					
2	Yes	No	Weak	–	–
3	Yes	No	Large	–	–
4	Yes	Occasional	Weak	–	–
5	Yes	Occasional	Large	–	–
6	Yes	Frequent	Weak	–	–
7	Yes	Frequent	Large	–	–
8	Yes	Consistent	Weak	–	–
9	Yes	Consistent	Large	–	–
Level 3 (Large alternating movements/dorsal foot placement/occasional body weight support)					
10	Yes	Yes	Large	Occasional	–
Level 4 (Plantar foot placement)					
11	Yes	Occasional	Large	No	Occasional
12	Yes	Frequent	Large	No	Occasional
13	Yes	Frequent	Large	Occasional	Occasional
14	Yes	Consistent	Large	No	Occasional
15	Yes	Consistent	Large	Occasional	Occasional
16	Yes	Frequent	Large	Frequent	Frequent
17	Yes	Frequent	Large	Frequent	Consistent
18	Yes	Frequent	Large	Consistent	Frequent
19	Yes	Frequent	Large	Consistent	Consistent
20	Yes	Consistent	Large	Consistent	Occasional
21	Yes	Consistent	Large	Consistent	Frequent
22	Yes	Consistent	Large	Consistent	Consistent

R–L, right–left; –, not present; consistent, observed in >95% of the step cycles; frequent, 51–94%; occasional, <50%; No, 0%.

Figure 2-3. A locomotor behavior scoring scale, modified from the Basso, Beattie, Bresnahan (BBB) scale. Adapted by Antri *et al.* [161] for complete spinal cord injury and hindlimb walking on a treadmill.

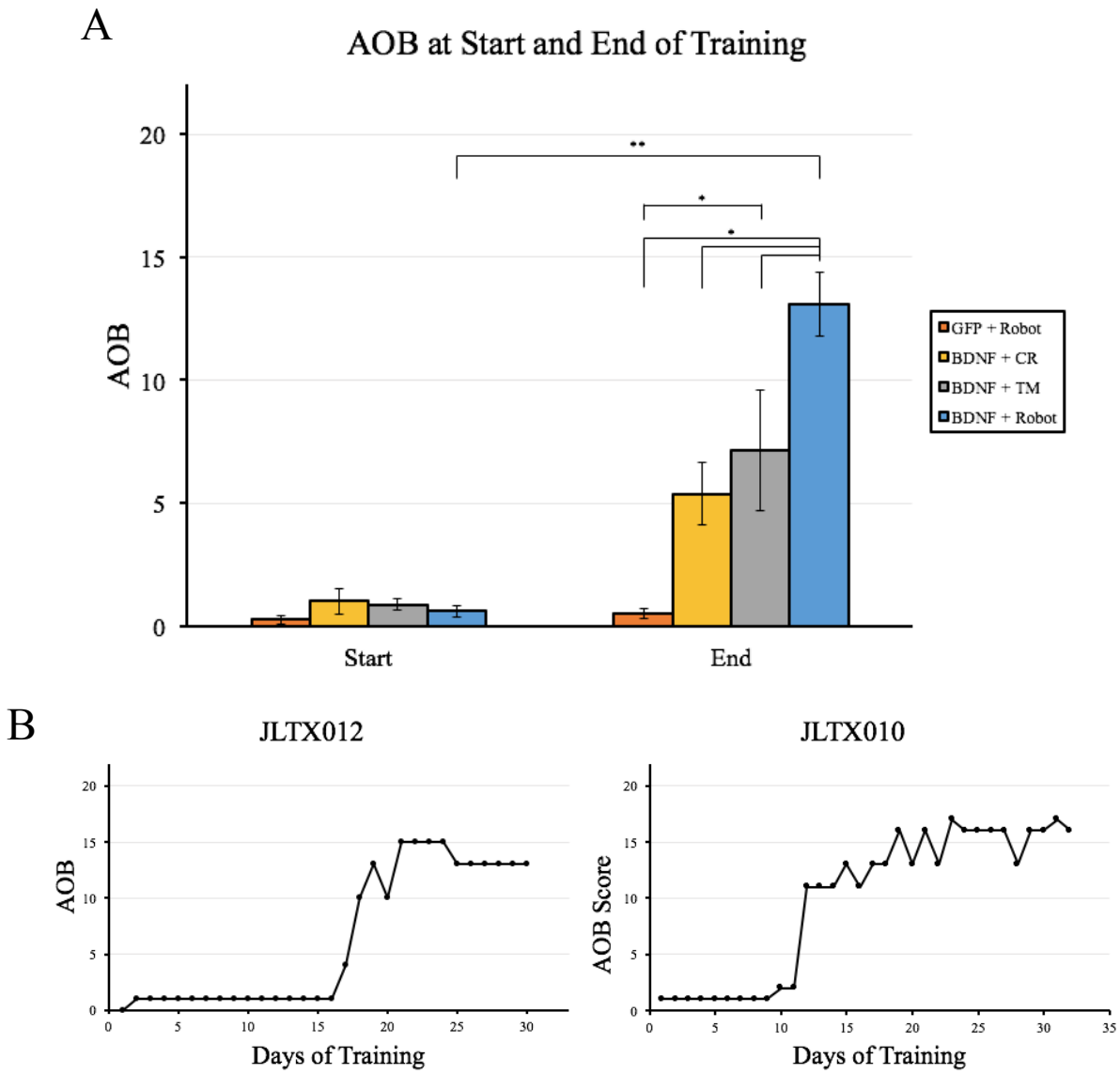


Figure 2-4. (A) Comparison of the mean AOB scores at the beginning and end of training among the groups. All groups treated with AAV5-BDNF showed significant improvement from the start to end, even though only the robot-trained group has significance bars (mean ± SEM). Animals rehabilitated with both BDNF and robot showed the most significant recovery at the end of training (* $p << 0.01$, $F(3,30) = 12.57$, one-way ANOVA with Tukey Kramer post hoc corrections; ** $p << 0.01$, paired t-test). CR – cage rest, TM – treadmill-trained, Robot – robot-trained. (B) AOB recovery as a function of day of training in two animals showing stereotypical sigmoidal recovery patterns.

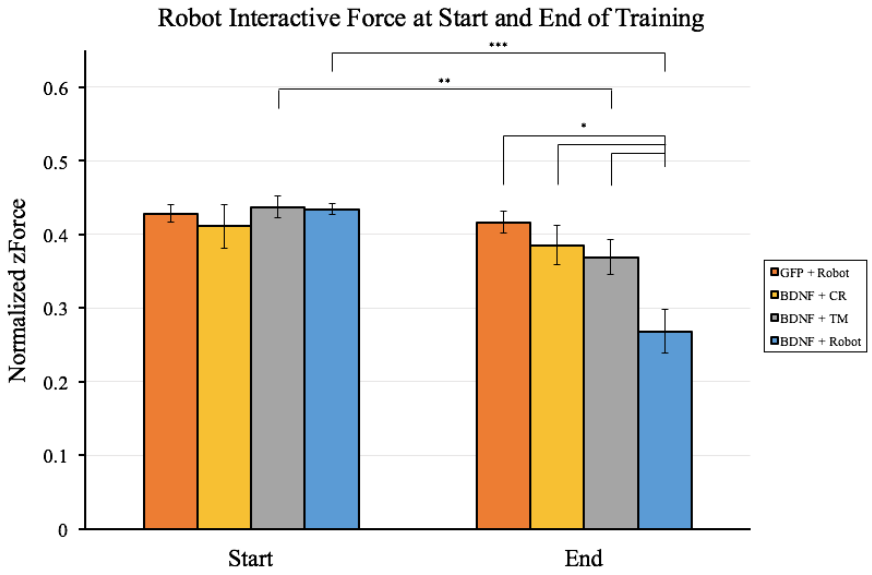


Figure 2-5. Comparison of the mean normalized zForce at the beginning and end of training. Normalized zForce is measured in Newtons/gram. There were no significant dfferences among groups at the start of training. Animals treated with both robot assistance at the pelvis and AAV5-BDNF showed the most significant improvement at the end of training ($p = 0.001$, $F(3,30) = 6.97$, one-way ANOVA with Tukey Kramer post hoc corrections). Both the treadmill-trained and robot-trained groups had significant improvement from the beginning of training (treadmill: $p = 0.01$, paired t-test; robot: $p << 0.01$, paired t-test).

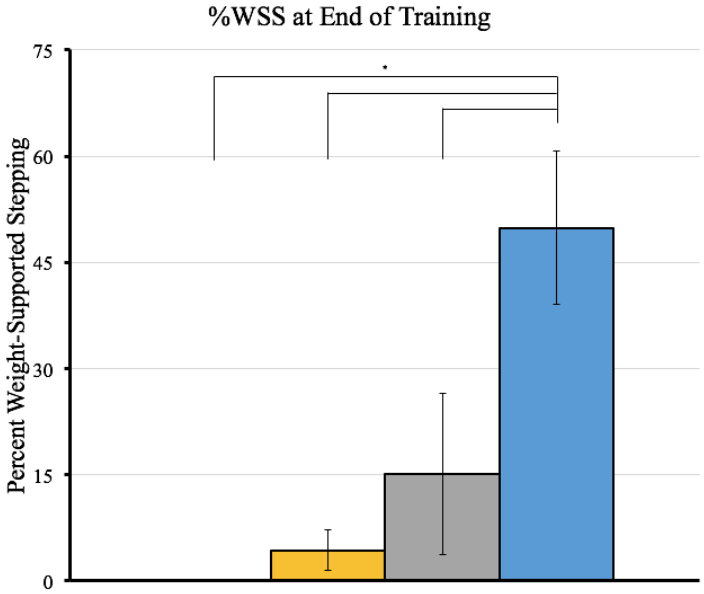


Figure 2-6. Percent weight-supported stepping all of the groups at the end of training. The GFP-treated group had 0 %WSS. (* $p = 0.0005$, $F(3,30) = 7.82$, one-way ANOVA with Tukey Kramer post hoc corrections).

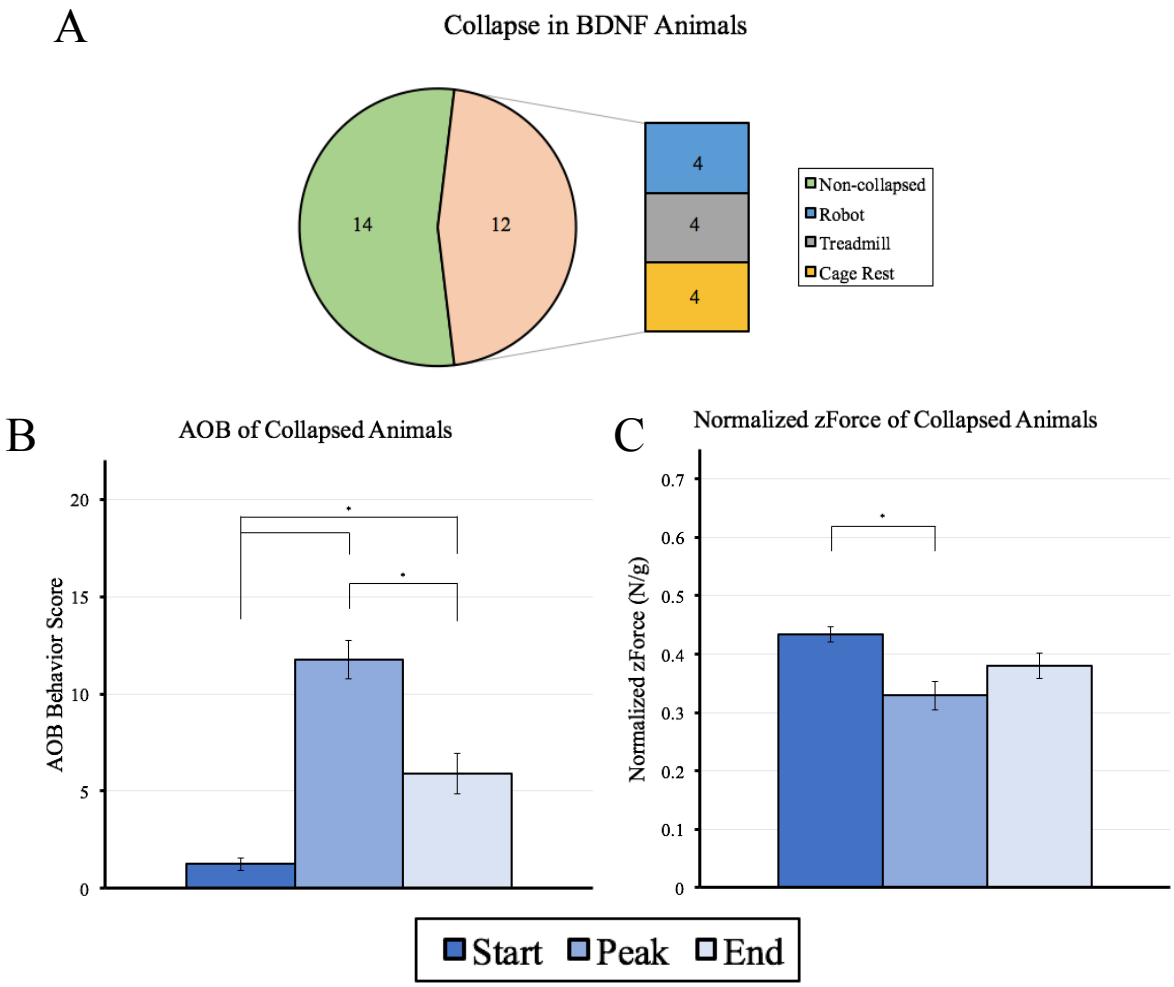


Figure 2-7. (A) Overview of collapse observed in animals treated with AAV5-BDNF. 12 of 26 had this decline in function, spread evenly throughout all treatment paradigms. (B) Comparison of AOB scores of collapsed animals at start, peak, and end of training (* $p << 0.01$, $F(2,33) = 39.22$, one-way ANOVA with Tukey Kramer post hoc comparisons). (C) Normalized zForce (N/g) of collapsed animals at start, peak, and end of training ($p = 0.0034$, $F(2,33) = 6.77$, one-way ANOVA with Tukey Kramer post hoc comparisons). (D) Peak and end comparisons of %WSS. Start data was not plotted as it was 0 %WSS. (* $p = 0.0003$, $F(2,33) = 10.41$, one-way ANOVA with Tukey Kramer post hoc corrections). All data shown in is mean ± SEM.

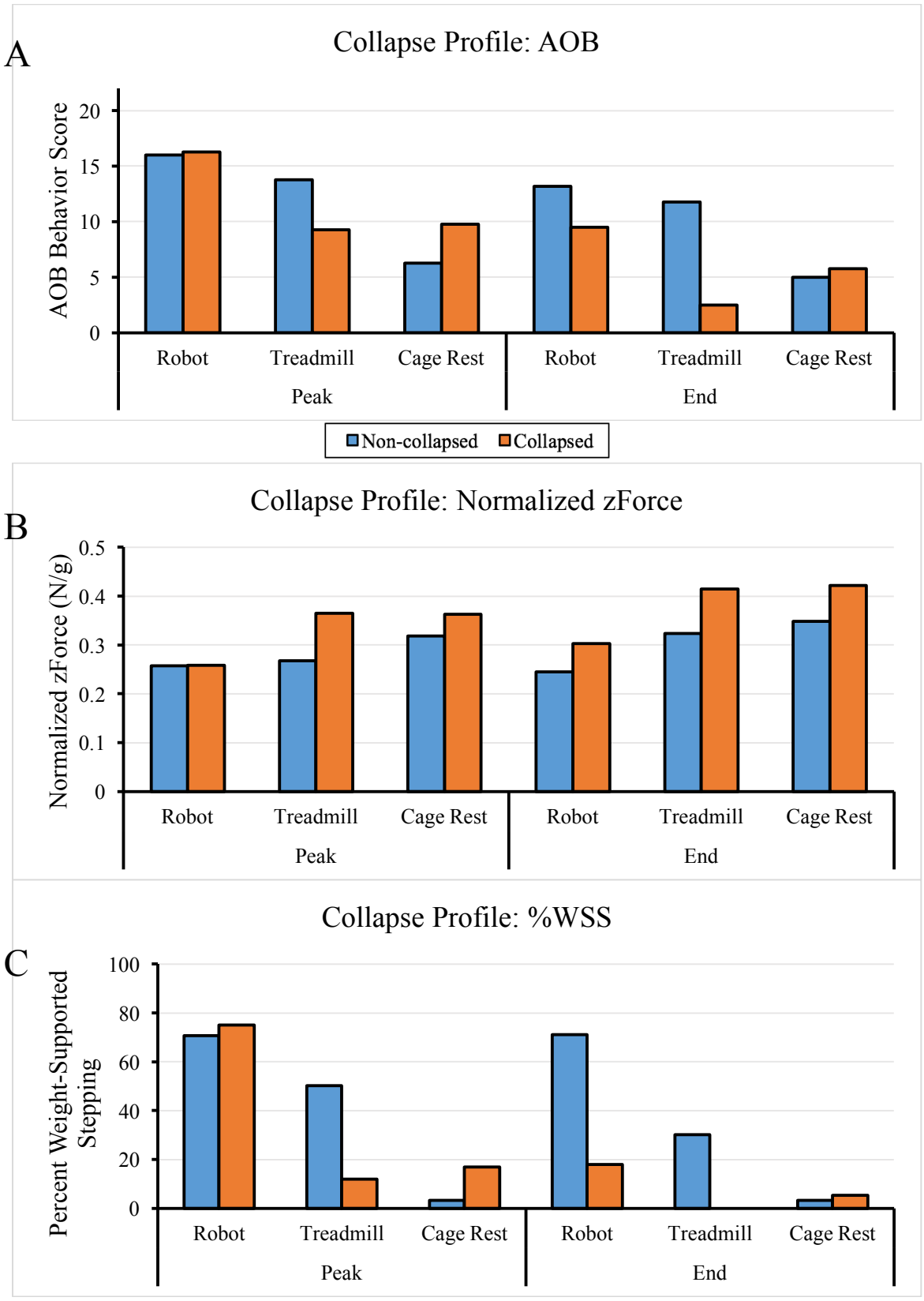


Figure 2-8. Comparison of BDNF animals that have collapsed (orange) and have not collapsed (blue). No statistical significances were determined.

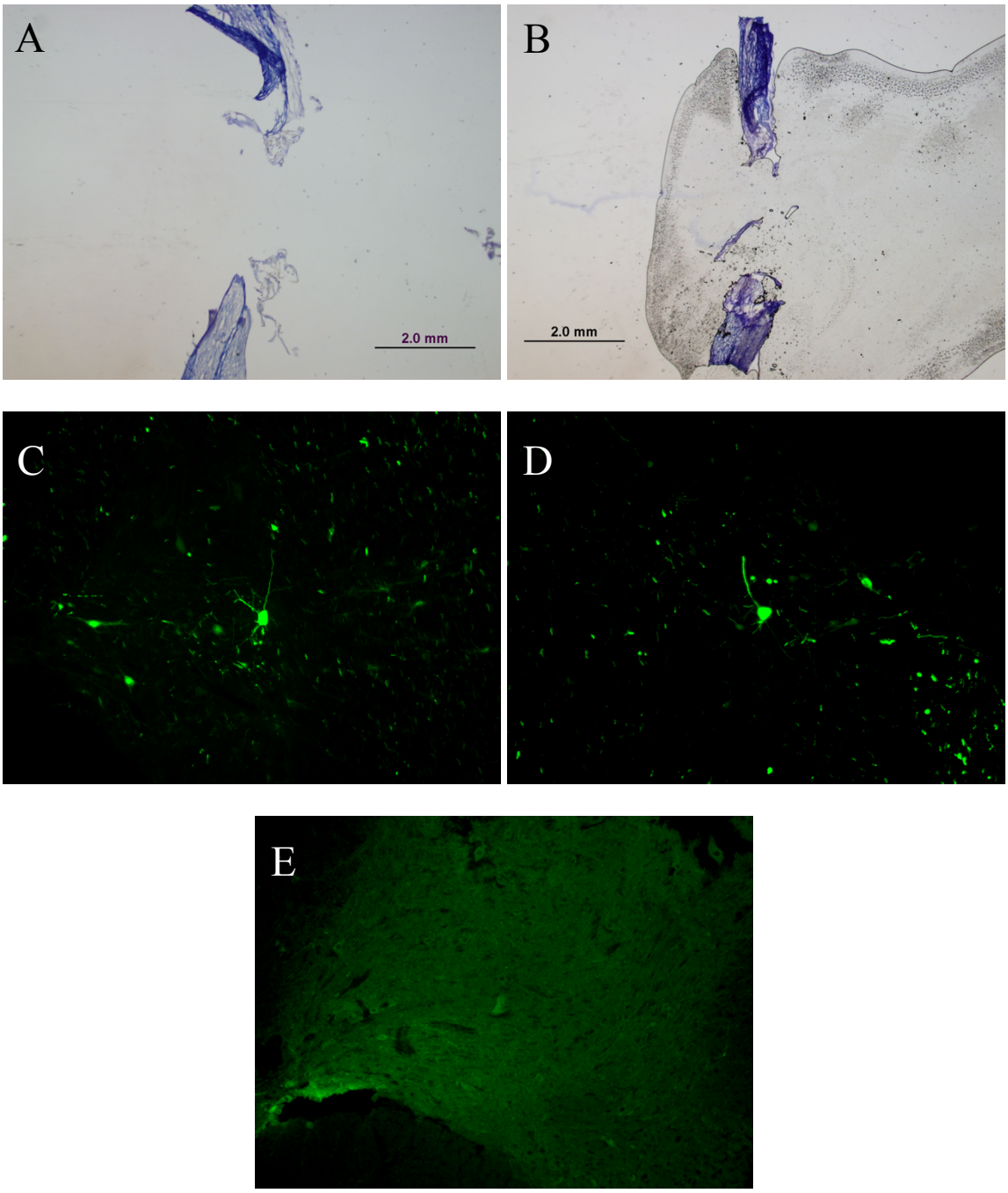


Figure 2-9. (A) and (B): Nissl myelin stains of parasagittal cuts of the spinal cord of two rats chosen at random in this study, confirming transection. (C) and (D): Fluorescent immunohistochemistry (IHC) staining of spinal cord of animals treated with AAV5-GFP to confirm the efficacy of microinjections of viral delivery of AAV5. (E) Fluorescent IHC of spinal cord of control animal injected with saline instead of AAV5-GFP.

D. Discussion

In this study, we have demonstrated the efficacy of our unique robot rehabilitation treatment modality for complete SCI in the adult transection (ATX) model induced to step with an Adeno-associated virus to deliver BDNF caudal to the site of injury. The use of neurotrophins, such as BDNF, is a relatively new approach to SCI rehabilitation, but has been studied extensively for its potential in locomotor recovery [53], [60]. BDNF, in particular, has been assessed for its ability to promote weight-supported stepping in completely transected rats [71] and cats [56], [75]. Our method of delivering microinjections of AAV5-BDNF caudal to the transection site at the time of injury has been shown to not only have localized effects directly caudal to the injury [162], but also downstream to the lumbar spinal cord [163]. Previous studies have addressed this question of where to deliver BDNF for maximal therapeutic value [73]. As a result of AAV5-BDNF treatment at the lumbar spinal cord, the balance between excitability and inhibition is altered [70], creating a milieu of affected motor neurons that are more excitable with lower thresholds for depolarization [71], as well as more plastic [164]. From a behavioral perspective, this increased excitability results in completely transected animals that are able to produce alternation of the limbs below injury, in some cases even leading to plantar stepping and weight-support during locomotion on treadmill [70], [71] or during open-field assessment [76].

Our interest in this study stemmed from this observation of these AAV5-BDNF-treated ATX animals, whose ability to produce autonomous weight-supported stepping closely resembled the locomotor ability of the SCI model of adult rats injured as neonates (NTX). In the NTX model, even in the absence of therapeutic treatment, animals can

produce stepping patterns in the open field or on treadmill [33]. Approximately 20% of these animals are able to develop weight-supported stepping (>50% of their steps are weight-supporting) without direct supraspinal control [153]. In previous studies in our lab, we have discovered that we can significantly improve the locomotor ability of these stepping NTX rats using our unique robotic rehabilitation focused at the rat's pelvis [31]. As a result, we view the NTX model as a signpost of potential recovery when transected animals are able to produce autonomous stepping patterns, whether they are rudimentary flexion-extension movements, or alternating steps. We believe combining our unique robotic rehabilitation paradigm with the use of AAV5-BDNF can promote weight-supported stepping and improved locomotor recovery.

Our robotic intervention at the pelvis is a key component of our rehabilitation paradigm, underscoring the importance of the trunk in locomotor recovery in SCI. The role of the trunk in stability and propulsion in movement has been observed both in our previous work [140], [147], [148], [153] and in the literature [165]. Indeed, there are currently several rehabilitation strategies targeted at the trunk in rats [31], [166] and in humans [167], [168]. In addition, our previous work has studied the role of the trunk motor cortex in locomotion, and we have shown it to be crucial for rehabilitation in NTX rats, both to develop weight-supported stepping [140] and to maintain it [147]. Our present work further supports this treatment paradigm, emphasizing trunk-based rehabilitation in animals that are able to produce autonomous stepping patterns to further improve locomotor recovery.

We confirmed the completeness of transection by direct visual confirmation at the time of surgery, and after each animal was sacrificed and the spinal cords were extracted.

The histology also verified that the injury was complete. In addition, we placed Gelfoam in the cavity between the rostral and caudal ends of the transected spinal cord, further providing a barrier of potential communication within the cord. This is an area of concern as BDNF has been shown to produce axonal sprouting through various delivery methods [169], [170]. We are confident based on our histology that this was not a contributing factor to the locomotor improvement in our animals.

In addition, by using a sham virus (AAV5-GFP) for a control group of animals, we have eliminated any possible changes in locomotor ability or excitability of the cord as a result of microinjections into the spinal cord caudal to injury. Furthermore, the AAV5-GFP demonstrated the efficacy of an Adeno-associated virus as a means to deliver BDNF to the transected spinal cord. Finally, we used the same viral titer for all of the groups of animals, eliminating the chance that improved locomotor ability was a result of increased or decreased AAV5-BDNF delivery.

We used three outcomes measures (AOB bipedal stepping scale, robot interactive forces, and %WSS) to provide a holistic evaluation of an animal's performance, each taking into account aspects of locomotion that may not be fully understood by one measure alone. For example, the AOB stepping scale takes into account body weight support and plantar foot placement, but divides functionality or improvement into large-scale divisions (occasional: between 0 and 50%, frequent: between 50% and 95%, and consistent: >95%). Our measure of robot interactive forces takes into account exactly how much of a rat's weight is supported by the rat to maintain pelvis height, providing an objective analysis of how much weight is supported, not just how often it is supported during training. In addition, our analysis of weight-supported stepping allows us to

evaluate smaller changes in improvement or loss of function on a daily basis that would not be understood within the large-scale divisions of the AOB.

In this study, we have further advanced our understanding of how robotic intervention at the pelvis combined with treadmill training can significantly improve function in chronic SCI locomotor rehabilitation. When evaluating the changes in AOB bipedal stepping [161] within and between groups, we observe that the viral delivery of BDNF alone results in significant locomotor recovery, as demonstrated by the cage rest group of animals, which had the least improvement of all of the AAV5-BDNF-treated animals. This is consistent with previous studies investigating the efficacy of BDNF treatment to improve function in ATX rats [62], [70] – [72], [163], [171], [172]. Though there was no significant difference between the GFP control group and the BDNF cage rest group at the end time point in a quantitative evaluation of the stepping scale, there was a distinct difference in the quality of improvement. Control animals, on average, were unable to move their hindlimbs, whereas the cage rest animals were able to provide right and left hindlimb alternations with varying degrees of range of motion. In addition, we observed that though they had no significant difference in mean %WSS at the end of training and compared to the other groups, the fact that they had any steps at all further emphasizes the potential role AAV5-BDNF can play and has been suggested to play [71] in broadly activating spinal networks to induce some form of functionality, no matter how little or inconsistent.

In addition, we observed that BDNF treatment could be combined with other therapies to improve function. While the idea of training and task-based rehabilitation to improve locomotion in SCI is not new [18], [31], [35], our current work further

emphasizes the importance of a specific training context (robotic intervention at the pelvis to improve treadmill training) to a broadly acting recovery strategy, such as viral delivery of BDNF to lumbar spinal cord. Animals trained with robot performed significantly better than animals trained on treadmill and in cage, while animals trained on treadmill performed better, on average, than their cage rest counterparts. These animals were able to frequently (between 50 and 95%) alternate their hindlimbs with a large amplitude, occasionally body weight support (between 0 and 50%), and occasionally plantar place their feet. In contrast, on average, AAV5-BDNF-treated animals that were only trained on treadmill were unable to weight support or plantar place their feet. On average, their hindlimb movements were limited to right and left alternations that varied in amplitude and in consistency.

The benefits of exercise and training were particularly emphasized when assessing the robot interactive forces, as both the treadmill-trained and robot-trained groups showed significant improvement in the normalized zForce from the beginning of training to the end of training. This is consistent with the literature that suggests a strong role for exercise training post-SCI to promote rehabilitaition [173]. We also confirmed that our robot strategy was able to increase %WSS, which we showed to be a significant result in the NTX model of animals [30], [31], [153]. As a result, we are confident that our robot rehabilitation strategy is an effective tool to improve locomotor recovery in the AAV5-BDNF-treated animals, and significantly better than treadmill training alone.

In addition to the extent of recovery of locomotion observed in animals treated with AAV5-BDNF and on various rehabilitation regimens, we also noted the specific recovery patterns of these animals. In previous studies in our lab, we have observed a

stereotypical sigmoidal recovery pattern in AOB score, as a function of time of training on robot-assisted treadmill training. Both in the NTX model [30], [31] and an ATX model where rats were induced to step with robot-driven epidural stimulation [160], individual animals showed that AOB scores typically began low, then increased at a high rate until reaching a plateau of peak function. We believe that this may be a result of the scaling of the AOB scoring system [161], but when we compare this with animals that were rehabilitated on treadmill only or on cage rest, we see that this trend does not hold. This further advances our understanding of how robot interactive forces may play a role in rehabilitation on treadmill. Specifically, this may address how long it may take a spinalized animal that can step or is induced to step to integrate pelvic assistance and trunk control in hindlimb motion with forelimb coordination and movement.

Next, we observed that the delivery method of BDNF required time for therapeutic and observable effects on the treadmill. It has been posited that the effects of exogenous BDNF expression in the spinal cord after injury has downstream effects, which are responsible for the changes in the balance between excitability and inhibition in the spinal cord [70], [72], [76]. In these studies, too, exogenous BDNF delivery required time before improvement in locomotion was observed. The relationship between BDNF and time to recovery is of particular interest to us as it provides some insight into the relationship between BDNF and rehabilitation training – specifically, as exogenous BDNF is believed to drive locomotion in this case, and endogenous BDNF is upregulated as a result of voluntary exercise [63]. In the context of robot-assisted treadmill training, we may be using the exogenous BDNF as a tool to induce stepping, but it may require further study to efficiently maximize the locomotor recovery.

This becomes an issue in the context of the partial, but highly significant collapse of function that we observed in all of the AAV5-BDNF-treated groups of SCI rats. This is an observed phenomenon in previous studies where the use of exogenous BDNF led to early improvements in motor functions [70], [71], which was later mitigated by an increased frequency of clonic movements in the hindlimbs in some of the animals. We observed the same phenomenon in a subset of our animals (approximately 46.15%), with some animals developing hyperreflexia that hindered any locomotor movement. In addition, some of our collapsed animals also developed strong activation of their trunk muscles, leading to taught abdomens and difficulty with bladder expression. In general, these animals had far worse health outcomes than their non-collapsed counterparts, with increased scalding of their skin and high recurrence of bladder infections.

Blits *et al*. observed this phenomenon as far as out as sixteen weeks post-injection in their animals, and suggested that this collapse in function may be a result of chronic BDNF overexpression [72]. This hypothesis is further bolstered by studies that have suggested that deterioration in gait ability might be a result of TrkB desensitization [174], [175], as BDNF is a ligand for TrkB tyrosine kinase receptors [67], [176]. The distribution and function of BDNF TrkB receptors in the spinal neural networks have been shown to play important roles in the development of motor neuron functionality [59]. In addition, Ziemlínska *et al.* suggested that the effects BDNF has on expression of excitatory and inhibitory neurotransmission (such as glutamate, glycine, and GABA), as well as on KCC2 co-transporters, further emphasizes the detrimental effects of BDNF overexpresson on gait quality.

While we were unable to prevent or mitigate the occurrence of this phenomenon with our different training regimens, our present study offers insight into how collapse specifically affects locomotor recovery, or gait. Specifically, we are the first study to quanitatively analyze the changes in function from peak of function after treatment to after collapse due to the effects of BDNF. In particular, we observe that this collapse in function as a result of exogenous BDNF production results in a significant loss of AOB, where animals lose the ability to weight support and plantar place their hind feet on the treadmill during walking. In addition, we can quantify the extent to which weight-supporting animals that collapse lose this ability. Animals that collapse return to their pre-training reliance on the robot to maintain their pelvic height, even after reaching a significant decrease in normalized zForce at the peak of training. Though we did not find this to be significant in our one-way ANOVA with Tukey Kramer post hoc corrections when accounting for the beginning of training, when did find a significant difference between peak and end time points for normalized zForce. Though these animals behave differently than at start – they are able to produce alternations and have an increased frequency of clonic hindlimb movements – they are unable to incorporate those movements to weight support. This provides an interesting window into how our robot interacts with animals that can step previous to training. In the NTX model, the ATX epidural stimulation model, and in non-collapsed ATX animals (all animals that can step to some degree on their own), our robot can effectively decrease reliance on the robot and increase body weight support by the animal, as well as increase %WSS. In the context of the collapsed ATX animals, however, it appears that the robot is unable to take advantage of these animals as well as in the other models, perhaps as a result of the general

activation of the trunk and tensing of the abdominal musculature we observed previously. We believe this further emphasizes the role of the trunk and the effects of trunk activation in an animal's ability to develop weight-supported stepping, and sustain it.

In general, as discussed above, robot rehabilitation does provide significantly better locomotor recovery than treadmill and BDNF, and AAV5-BDNF alone. In line with our work in previous studies, it significantly increases AOB, body weight support, and %WSS, especially at peak of function, before any potential collapse. As a result, we are interested in pursuing the temporal effect of training – when it might be best suited to apply robot forces, how long robot forces should be applied – as it applies to reducing or preventing collapse. In addition, we are interested in investigating how we might prevent collapse at the peak of function with robot, perhaps with other combined therapies.

From a clinical assessment, our present work provides a unique perspective into combining biological and bionic therapies to in the rehabilitation of SCI. Much like in the NTX model, we have taken a broadly acting therapy (AAV5-BDNF in this study, autonomous stepping in the NTX) and used a specific therapy (robot rehabilitation) to guide and focus the training for significant benefits. We see much potential in this for translational work in humans, where motor incomplete SCI and certain models of neurological diseases, such as multiple sclerosis, may be analogous or similar enough to provide this basic stepping with which we can apply similar robot therapy focused at the trunk for gains in locomotion.

E. Conclusion

This present work has demonstrated the efficacy of robotic intervention at the pelvis in the ATX rat model induced to step with viral delivery of BDNF caudal to the lumbar cord. To our knowledge, this is the first study investigating how exogenous BDNF delivery can be combined with robotic therapies to improve locomotor rehabilitation following SCI. We have shown that our robot can provide significant recovery benefits to ATX animals induced to step with AAV5-BDNF, even in the scenario of collapse of function.

Furthermore, our study has characterized the phenomenon of collapse, as it relates to loss of body weight support and behavioral scoring. We have demonstrated that functional collapse as a result of exogenous BDNF occurs regardless of combined therapy, and must be considered in the use of BDNF as a long-term rehabilitation therapy.

From a larger perspective, we have further advanced our understanding of the importance of the trunk in developing and maintaining weight-supported stepping in SCI. This could have potentially impactful translational implications, as well as ramifications on our future directions in trunk-based robot rehabilitation. We plan to investigate further means to reduce or prevent collapse in BDNF animals, to maintain their peak performance on robot-assisted treadmill training.

CHAPTER 3: Robot-driven epidural stimulation prevents collapse in function found after brain-derived neurotrophic factor treatment of adult spinal cord injury.

A. Introduction

Although a complete spinal cord injury (SCI) interrupts descending supraspinal control of locomotion, there exists intact neuronal circuitry below the site of injury that can function to produce rhythmic motor activity without voluntary input from the brain. This network of neurons, referred to as central pattern generators (CPGs), can be manipulated and activated by external means in the SCI model to produce locomotor activity in humans [40] and in rats [85].

Previously, we demonstrated this in the complete SCI model for rats transected as neonates (NTX), where autonomous recovery is possible without intervention [153]. In these animals, approximately 20% of rats are able to produce body weight-supporting (WS) steps, without supraspinal control. In some cases, more than 50% of their steps are WS steps. Using our unique robot-assisted treadmill training focused at the pelvis [30], we have shown that it is possible to take advantage of the intact spinal circuitry below the site of injury, along with mechanical coupling of the trunk muscles above and below injury, to rehabilitate NTX animals and significantly improve their locomotor function and increasing the percentage of WS steps they are able to take. We believe it allows rehabilitating rats to learn how to efficiently integrate trunk and hindlimb muscles in new motor patterns while walking on the treadmill.

In the treatment of SCI in animal models, another external method to activate the neuronal system below the site of injury has been the use of pharmacological agents to activate the CPG. Studies to understand this pharmacological control of the CPG have led

to the discovery of drugs that effectively produce locomotor-like activity, such as L-DOPA in cats [36], and serotonin or serotonin agonists in rats [81], [161], [177], [178]. In these models, locomotor activity in response to CPG-activating agents is characterized by plantar foot placement, weight support by the hindlimbs, and large-amplitude movements of the hindlimbs [36]. This behavior in response to CPG activation strongly suggests a role for pharmacological intervention in promoting function of the CPG in locomotor recovery.

In Chapter 2, we used an AAV5-viral delivery of brain-derived neurotrophic factor (BDNF), following prior work of Boyce et al. Ziemlinska et al. , to induce locomotor activity in rats transected as adults (ATX). BDNF not only promotes regeneration [57], [58] and induces plasticity [59], [60] in the damaged nervous system, but it is believed to lower the rheobase of transfected motoneurons, effectively increasing their excitability [56]. We showed in our study that AAV5 delivery of BDNF resulted in significant locomotor recovery on the treadmill, with even more recovery in response to robot-assistance during training. Unfortunately, we also showed that in a subset of those animals (approximately 37%), the rats developed a partial, but highly significant "collapse" in function, resulting in hyperreflexia in the lower limbs, and a decrease in locomotor ability.

Outside of pharmacological intervention, electrical epidural stimulation (EES) has been shown to be an effective means by which to produce robust, locomotor recovery in the the motor complete transected human models [89], [179] and even weight-supporting stepping in the completely spinally transected rat model [40], [85], [86]. In our lab, we have designed and employed a unique robot-driven EES strategy [160], in contrast to

conventional EES, for rehabilitation. Our robot-driven EES is characterized by stimulation timing determined by the interaction with our robot and the consequent height of a rat's pelvis, unlike conventional EES, which provides constant stimulation throughout training. The benefit of our technique is two-fold: we have discovered it allows us to use lower voltages to elicit locomotion in ATX rats, and by the robot determining stimulation, it allows for more intermittent stimulation patterns. Using lower voltages is beneficial as it causes less discomfort in animals, and more compliance during training as a result. Intermittent stimulation is beneficial as it may provide a weaning effect for the animal, allowing the specific and "as-needed" stimulation. This as-needed stimulation may provide a more autonomous route to recovery for injured animals discovering their neural controls for quadrupedal stepping. Indeed, various different methods of intermittent stimulation are currently being investigated for their benefits to locomotor recovery in SCI [96], [97].

The use of these individual therapies in the animal model has produced encouraging results for locomotor recovery after SCI in the ATX rat. Robot-driven epidural stimulation, and AAV5-viral delivery of BDNF have been shown to individually promote stepping behavior, with varying degrees of weight support, when coupled with robot-assisted treadmill training. Having studied separately the effects of AAV5-BDNF treatment and robot-driven EES in the ATX rat model, we believed that there might be a possibly synergistic interaction between the therapies that may result in significantly improved recovery. Alternatively, the epidural stimulation could exacerbate the collapses observed with BDNF alone. To test these alternatives, we designed a combination experiment. As a result, we next examined a rehabilitation regimen for the ATX rat

model of SCI that combines robot-assisted rehabilitation and robot-driven epidural stimulation with AAV5 viral delivery of BDNF to the spinal cord post- injury.

We hypothesized that robot-assisted treadmill training, robot-driven EES, and AAV5-delivery of BDNF will provide potentially complementary effects on each other, allowing for more robust locomotor recovery. By virtue of its effect on rheobase, BDNF creates a more excitable spinal cord that requires less stimulation to elicit locomotor activity than one that is not treated with BDNF. We speculated that this will complement our robot-driven EES, which already relies on less stimulation than constant EES to produce locomotion. In addition, our pelvis-oriented robot-assisted treadmill training has been shown to increase weight-supported stepping in NTX rats. Finally, our robot-driven EES treatment, coupled with lower voltages from the interaction with BDNF, may further allow for better integration of those muscles through intermittent excitation of lumbar spinal cord. However, as noted above, it was also conceivable that epidural stimulation might exacerbate the negative effects of BDNF in some rats, causing more extensive and prevalent functional collapses than seen with AAV5-BDNF and robot training.

To test our hypothesis, we prepared 35 rats in four groups to study the interactions between BDNF, robot-assisted treadmill training, and epidural stimulation to investigate our hypothesis. One group (n = 9) of rats received the full combination of therapies. A control group (n = 8) received a sham AAV5 virus to produce green fluorescent protein (GFP), which is functionally quiescent in the nervous system. A third group (n = 10) received AAV5-BDNF and robot-assisted treadmill training, but not epidural stimulation. Finally, a fourth group (n = 8) received AAV5-BDNF, constant epidural stimulation, and treadmill training, without robot. Based on a functional measures used to assess the

quality of stepping and the level of weight support, we discovered that animals that received the full combination of therapies recovered significantly as a result of rehabilitation, although as a cohort, they did not recover significantly better than the group that only received robot rehabilitation. Both groups, however, recovered significantly better than the other groups.

We also discovered that BDNF had a quantifiable and tangible effect on robot-driven epidural stimulation. All groups that received BDNF and epidural stimulation resulted in significantly lower stimulation intensity required to elicit locomotor activity, and maintaining this throughout rehabilitation.

Finally, we believe that epidural stimulation may play a role in relieving or disrupting collapse in function that we have shown to be associated with AAV5-BDNF treatment. Our data show that Providing electrical stimulation to the spinal cord at sites corresponding to hindlimb flexors and extensors in animals that have spastic limbs may help to disrupt unwanted activation of limbs that lead them stuck in flexion or extension, which is characteristic of collapse following AAV5-BDNF treatment. In our group of animals that received the full combination of therapies, we did not have a single animal that collapsed in function, which we believe to be a significant side effect of the combination therapy.

B. Materials and Methods

B-1. Overview

We prepared 35 intact adult female Sprague Dawley in this study separated over four different groups, with twenty-seven receiving AAV5-BDNF and eight receiving a sham

virus of AAV5-GFP. All of the animals in the study received a complete transection of the spinal cord at spinal level T9/T10. In the same surgery, animals received four microinjections of virus caudal to the transection site into the spinal cord. Epidural stimulation wires were also implanted into the rats, above spinal level L2/L3 and S1/S3 at the time of surgery. Finally, all animals were implanted with pelvic orthoses following transection injury, virus injection, and implantation of wires. Upon recovery, all of the animals were trained on our treadmill for approximately six weeks, each receiving a combination of therapies outlined below, based on grouping. Video and robot data collected during training sessions were used to assess locomotor recovery. All surgical procedures and training regimens were carried out with the approval and guidance of Drexel University College of Medicine's Institutional Animal Care and Use Committee (IACUC).

B-2. Complete Transection of the Spinal at T9/T10 in the Adult Rat (ATX)

Animals were anesthetized intraperitoneally (IP) with a 1.0 ml/kg combination of ketamine hydrochloride (50 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg) before surgery. Prior to surgery, after anesthesia was administered, animals were given slow-release buprenorphine subcutaneously (0.5 mg/kg) for post-operative analgesia. Through the surgery, animals were given supplemental anesthesia, as needed, at a concentration of 0.38 ml/kg. We used a mid-thoracic dorsal incision to expose the vertebrae above vertebral level T9 and T10, and performed a full laminectomy of the T9 to T10 arches, and a partial caudal laminectomy of the T11 arch to expose the spinal cord, providing a large window for transection and for viral injection. To gain access to

the cord through the dura, we used a 30-gauge needle to make sagittal incisions in the dura above the spinal cord, parallel to the length of the cord. We then used iridectomy scissors and aspiration to remove a full segment of spinal cord at T10, filling the resulting cavity with gelfoam.

B-3. Microinjection of AAV5 Virus Caudal to Transection Site

Immediately following the transection, we injected prepared virus approximately 1 mm caudal to the transection, using pulled glass pipettes and a 1 ml Hamilton syringe. We adapted procedures and methods for microinjection of virus described in Tom *et al*., and Boyce *et al.* to use stereotactic guidance to inject four different sites transversely across the cord, at a depth between 400-500 μm. Each site received 2 μl of virus, with a total of approximately 2.5×10^{10} viral particles delivered into the intermediate gray of the spinal cord.

B-4. Implantation of Epidural Stimulation Wires

For animals in the cohort receiving epidural stimulation, using techniques similar to Hsieh *et al*., [35] during the same surgery, an additional incision was made transversely along the muscles above spinal level T13. Bone rangeurs were used to expose the spinous processes, and iridectomy scissors were used to cut the ligamentum flavum between T13 and L1, allowing access to the spinal column. Using this window, epidural stimulation wires (0.003" Teflon insulated stainless steel with 0.5 mm exposed at the implanted end) were placed at segmental levels L2/L3 and S1/S2. The emerging leads were sutured to the vertebral spine and reflected back subcutaneously toward the pelvis for fixing onto

the pelvic orthosis. Following implantation of the wires, the transection injury site was closed in layers.

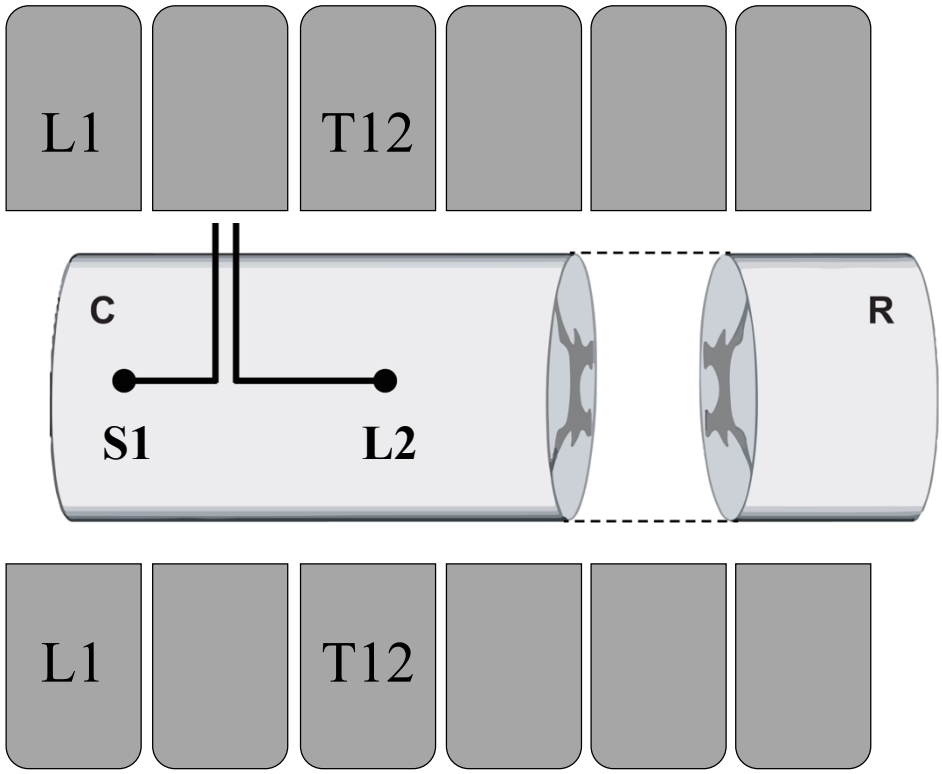


Figure 3-1. Schematic of implantation of epidural stimulation wires through the vertebrae and onto the dorsal surface of the spinal cord.

B-5. Pelvic Orthosis Implantation

Using our methods described in previous studies, following viral injection, all animals were implanted with custom-made pelvic orthoses. On the dorsal surface of the trunk, we made two incisions in the skin, approximately 2 mm caudal to the iliac crest on either side, allowing access to the gluteus muscles. We used blunt dissection to separate the superficial and deep gluteus muscles, allowing for the cuffs of the pelvic implants to be inserted against the pelvic bones on both sides. After cuff placement, the two ends of the implant were fastened together around a crossbar with screws on both ends, to secure the

orthosis in place. We used quick-drying epoxy cement (J-B Kwik) to weld the orthosis pieces together.

B-6. Connecting the Epidural Stimulation Wires

After implantation of the orthosis, the stimulation wire leads were tunneled subcutaneously through the back skin to an incision for the pelvic orthosis. They were subsequently glued around an implant arm to prevent chaffing of the skin at the wire exit points. Leads were then soldered to miniature pin connectors (Omnetics Corp.). The connectors were attached to front of the pelvic implant crossbar with JB fast epoxy.

B-7. Post-operative care

After surgery, animals were allowed to recuperate for seven to ten days, during which time they received prophylactic antibiotics. In addition, we expressed the bladders of the animals twice daily. This also allowed us to examine the animals for health issues, including autophagia, skin lesions, or opening of wounds.

B-8. Robot-Assisted Treadmill Training

Using our previously establish rehabilitation regimens, we trained our animals for twenty-minute sessions, five times a week, for approximately five to six weeks. During training, we recorded video and robot data for analysis.

Our robot is a PHANTOM® Premium 1.0 model (developed by SensAble Technologies, Inc.), which we use with custom software to apply uniform isotropic elastic forces to the pelvis of a transected rat to allow for a trunk position that is nearly

identical to that of a normal rat. We assign an equilibrium center for the rat's pelvis – defined by a three-dimensional coordinate frame (x-, y-, and z-aces) – that the robot uses to center a rat's pelvis. Throughout a training session, our robot calculates and adjusts the forces required to bring the animal's pelvis to the equilibrium center at a rate of 1 kHz, in real time. The forces delivered by the robot are governed by the equation:

$$F = k(z - z_o)$$

where F is the force applied by the robot, k is the stiffness of the elastic field, z_o is the desired height of the pelvis, and z is the current height of the pelvis. The stiffness is predetermined to be 80 N/m.

B-9. Robot-driven Epidural Stimulation

Conventional epidural stimulation provides constant stimulation to the spinal cord at a fixed frequency, with specific parameters (inter-pulse period: 0.25×10^{-1} s, pulse duration: 0.2×10^{-3} s). Our robot-driven epidural stimulation is unique in that the position of the robot determines the administration of stimulation. Stimulation is only applied when the animal's pelvis is below the given equilibrium height for the rat.

Our custom robot software is able to monitor the rat's pelvis height at a rate of 1 kHz. We use an algorithm that monitors this pelvic height to deliver stimulus to the spinal cord. Stimulation intensity (V) was measured and recorded with each training session.

B-10. Locomotor Assessment

Animal recovery was measured against a battery of outcome measures previously used in our laboratory to assess locomotor recovery in completely transected rats. First, using

video recorded from training sessions, we assessed an animal's hindlimb motor activity using the Antri, Orsal, and Barthe (AOB) bipedal stepping scale. His scale assesses motor performance in animals with complete SCI in the context of treadmill training, and evaluates locomotor function based on right-left hindlimb alternation, range of motion, body weight support, and plantar foot placement during stepping. Based on these parameters, rats are assigned a score on a scale of 0 to 22, with increasing scores indicating increased recovery.

Videos from the training sessions were also assessed for the percentage of steps that were taken that were weight-supporting (%WSS). We analyzed the videos frame-by-frame, and defined a weight-supported step as one where no other part of the animal's body touched the treadmill except for the limbs and tail.

Finally, using robot data that recorded robot interactive forces throughout training sessions, at a rate of 1 kHz, we assessed the force in the vertical axis (zForce) required to maintain the pelvic height. This force was normalized to the weight of the animal, to compare the animals within and across groups.

B-11. Histology

Upon completion of the rehabilitation regimen, animals were sacrificed with an intraperitoneal injection of Euthasol. Some animals were chosen at random to be perfused intracardially with 0.9% physiological saline, followed by 4% buffer paraformaldehyde (PFA). The following day, the spinal cord was extracted and preserved in 4% buffered PFA, and kept until sectioning, when they were placed in a 30% sucrose solution. Sagittal

sections were prepared from the tissue and stained for Nissl myelin, to confirm the completeness of transection.

B-12. Data Analysis

To compare locomotor recovery outcome measures between the groups of animals and within the groups of animals, we used a one-way ANOVA with Tukey Kramer post hoc corrections to compare within groups at specific time points. When assessing individual groups of animals across rehabilitation time points, we used paired t-tests to assess statistical significance. Finally, when analyzing the change in stimulus intensity in individual groups of animals treated with epidural stimulation, we used linear regression to measure differences in voltage as a function of time. For all statistical tests, a p-value of less than 0.05 was considered significant. All statistical analysis was done using custom-coded scripts in MATLAB R2014b. All graphs and charts were produced in Microsoft Excel 2016.

C. Results

C-1. Overview

Video and robot data were recorded for all animals during training sessions. From the video and robot data, we analyzed each animal against a series of outcome measures, including AOB bipedal stepping scores, robot interactive forces, and percentage of steps that supported body weight. We also kept a daily record of the stimulus intensity (V) required to elicit hindlimb locomotor activity in the groups of rats that received either robot-driven or conventional epidural stimulation (ES), and compared each group at weekly intervals.

C-2. AOB Bipedal Stepping Scale

The foundation of the 22-point AOB bipedal stepping scale is based on three criteria of the hindlimbs: (1) right-and-left alternation, (2) body weight support, and (3) plantar foot placement during locomotion. We did not score the animals before injury, starting each animal at the first day of training, approximately 7-10 days after surgery.

At the start of training, the AAV5-BDNF-treated group that received only robotic intervention (n = 10) at the pelvis had the lowest average AOB score of the groups, at approximately 0.6 ± 0.22 (mean ± SEM). Of the ten animals, the highest score was a two at the start of training, and the median score was 0. All other groups demonstrated some activity on the first day of training, indicative of the immediate effects of epidural stimulation. The second lowest AOB (2.88 ± 0.13) was found in the AAV5-GFP-treated group (n = 8) that received both robotic intervention and robot-driven epidural stimulation. This average score indicated that most animals had no alternations of the

hindlimbs, but had large amplitude movement. Both AAV5-BDNF-treated groups that received robotic intervention and epidural stimulation (both conventional and robot-driven) had higher average starting AOB scores at 3.12 ± 0.99 and 3.44 ± 0.84, respectively. In the latter group, one animal had a starting AOB score of 10, indicating occasional body weight support (<50%), but no plantar foot placement during stepping. Comparing across groups at the start of training, both of the BDNF groups with epidural stimulation had significantly higher starting scores than the BDNF-treated group on robot without any epidural stimulation ($p = 0.0097$, $F(3,31) = 4.51$, one-way ANOVA with Tukey Kramer post hoc corrections), though they were not significantly different from one another.

To assess the efficacy of the various treatment regimens, we examined the change in AOB from start of training to the end. Among all groups, there was significant increase in AOB from the beginning to the final day. The GFP group that received robotic intervention and robot-driven epidural stimulation had a final AOB score of 8.25 ± 0.80, consistent with our findings that in ATX model, robot-driven epidural stimulation alone can significantly improve function ($p << 0.01$, paired t-test)[1]. On average, this score indicates consistent (>95%) alternation of the hindlimbs, with weak amplitude or small range of motion. The AAV5-BDNF-treated group that received conventional epidural stimulation ($n = 8$), but no robotic intervention, had a final average AOB score of 9.75 ± 0.16, showing significant improvement ($p << 0.01$, paired t-test) with consistent (>95%) large-amplitude alternations of the hindlimbs. The AAV5-BDNF-treated group that only received robotic intervention ($n = 9$) had a significantly higher final average AOB score of 13.1 ± 1.30 ($p << 0.01$, paired t-test). On average, this score indicated that the animals

had frequent alternations of the hindlimbs (between 51 and 95%), with occasional body weight support and occasional plantar foot placement. Finally, the group of animals that received the full complement of therapies (AAV5-BDNF, robotic intervention, robot-drive epidural stimulation) had an average final score of 13.33 ± 0.60, a significant improvement from the start of training ($p << 0.01$, paired t-test).

Finally, we compared the AOB scores on the final day of training of the four groups to examine the efficacy of each training regimen against the others. The group that received the full combination of therapies was significantly different from both the BDNF group that received constant epidural stimulation and no robotic intervention, and the GFP group ($p = 0.0005$, $F(3,31) = 7.89$, one-way ANOVA with Tukey Kramer post hoc corrections). In addition, the BDNF group that received only robotic intervention was significantly different from the GFP-treated group as well ($p = 0.0006$, $F(3,31) = 7.89$, one-way ANOVA with Tukey Kramer post hoc corrections).

C-3. Robot Interactive Forces (zForce)

Our custom robot software records the interactive elastic forces between an individual animal and the robot to maintain the rat's pelvis at an equilibrium point in the x-, y-, and z-axes. We use the force in the z-axis (zForce) in Newtons as a proxy for body-weight support, normalized to the weight of the animal in grams. Animals that received robotic rehabilitation at the pelvis had robot interactive forces recorded every day of training. The animals that did not receive robotic rehabilitation at the pelvis as part of their training regimen were placed on the robot for short five-minute intervals at weekly

intervals, to assess their body weight support, and allow us to compare robot data across groups.

On the first day of training, there was no significant difference between groups for the normalized zForce to maintain the pelvic height at equilibrium ($p = 0.11$, $F(3,31) = 2.19$, one-way ANOVA with Tukey Kramer post hoc corrections). The AAV5-BDNF-treated group with only robot intervention had the lowest initial average normalized zForce of 0.43 ± 0.01 N/g. The next lowest average initial normalized zForce was found in the group of rats that received the full combination of therapies (0.46 ± 0.01 N/g). Finally, the GFP group and the BDNF group with constant epidural stimulation had approximately identical average initial starting normalized zForces, at 0.47 ± 0.01 N/g.

Next, we assessed the normalized zForces of the groups across the full training period, comparing the initial averages with the final averages, on the last days of training. In all treatment modalities, we observed significant decreases in normalized zForce from the beginning of training to the final day. The GFP-treated group had a final average normalized zForce of 0.41 ± 0.02, indicating a significant decrease in zForce ($p = 0.02$, paired t-test). This corresponded to approximately a 12.01% decrease in robot elastic force. The BDNF group that received constant epidural without robotic intervention had the next lowest net decrease in normalized zForce (16.95%), with an average final normalized zForce of 0.39 ± 0.03 N/g. We found this decrease to be significant ($p = 0.03$, paired t-test). The BDNF group that only received robotic intervention had a final average normalized zForce of 0.27 ± 0.03 N/g, a significant decrease of approximately 38.24% ($p << 0.01$, paired t-test). Finally, the BDNF group that received the full

combination of therapies had the largest percentage decrease in normalized zForce (41.2%) at 0.27 ± 0.01 N/g.

Finally, we compared the final average normalized zForces among the groups to find significant differences between the treatment modalities. Of the four therapies, we found that the BDNF group that received robot-driven epidural stimulation and robotic intervention at the pelvis had a significantly lower average final normalized zForce than the GFP-treated group and the BDNF group that received conventional epidural stimulation ($p = 0.0002$, $F(3,31) = 9.14$, one-way ANOVA with Tukey Kramer post hoc corrections). Similarly, the BDNF group that received only robotic intervention also had a significantly lower average final normalized zForce than the GFP-treated group and the BDNF group that received conventional epidural stimulation ($p = 0.0002$, $F(3,31) = 9.14$, one-way ANOVA with Tukey Kramer post hoc corrections). These two groups were not significantly different from each other.

C-4. Percent Weight-Supported Stepping (%WSS)

In addition to the previous two outcome measures, we assessed the percentage of steps that an individual animal took that were able to support the animal's body weight, using a frame-by-frame analysis of the video taken during every training session. We determined a weight-supporting step as one where only the rat's limbs and tail were on the treadmill during a step.

At the beginning of training, three of the four groups (GFP, BDNF with robot, BDNF with constant epidural stimulation) had an average start %WSS of 0, consistent with their starting AOB scores. The group that had received the full combination of

therapies had an individual rat whose initial %WSS (24.13%) was consistent with its starting AOB score of 10. As a result, the average starting %WSS for that group was 2.45 ± 2.45%. However, across groups at the start of training, there was no significant difference in average %WSS ($p = 0.42$, $F(3,31) = 0.96$, one-way ANOVA) with Tukey Kramer post hoc corrections.

We then assessed the change in %WSS across the completion of training in each group, to analyze the efficacy of treatment in increasing %WSS. The AAV5-GFP-treated did not show significant improvement in %WSS, ending with a final %WSS of 11.82 ± 5.78% ($p = 0.08$, paired t-test). The BDNF group that received conventional epidural stimulation without robotic intervention had a final average %WSS of 28.09 ± 8.29%, a significant improvement from the first day of training ($p = 0.01$, paired t-test). The BDNF group that received only robotic intervention had a similar significant improvement in %WSS, having a final average of 49.89 ± 10.82% ($p = 0.01$, paired t-test). Finally, the BDNF group that received the full combination of therapies had a final average %WSS of 51.19 ± 6.31%, which was also found to be a significant improvement from the beginning of training ($p << 0.01$, paired t-test).

We then compared the average %WSS on the final days of all the groups. We found that the BDNF group that received the full combination of therapies had a significantly higher %WSS than the AAV5-GFP-treated group that received robot-driven epidural stimulation ($p = 0.006$, $F(3,31) = 4.96$, one-way ANOVA with Tukey Kramer post hoc corrections). In addition, the BDNF group that received only robotic intervention at the pelvis had a significantly higher %WSS than the GFP group ($p =$

0.006, F(3,31) = 4.96, one-way ANOVA with Tukey Kramer post hoc corrections). No other groups were significantly different from another.

C-5. Stimulus Intensity to Elicit Hindlimb Locomotor Activity

To assess the effect of AAV5 viral delivery of BDNF on epidural stimulation in the ATX model for SCI, we recorded the stimulus intensity, in volts, required to elicit hindlimb locomotor activity in the three groups of animals that received epidural stimulation, both robot-driven and conventional epidural stimulation. We compared these values at the start of training, and at weekly intervals thereafter until the final day of training, after approximately five-six weeks of training.

On the first day of training, the mean stimulus intensity in the BDNF group that received the full treatment of therapies was 0.66 ± 0.10 V. The BDNF group that received constant epidural stimulation and no robotic intervention had an average stimulus intensity of 0.69 ± 0.06 V on the first day. Finally, the AAV5-GFP-treated group that received robot-driven epidural stimulation had a mean initial stimulus intensity of 1.53 ± 0.12 V. Comparing across groups on the initial day, the GFP group had a significantly higher mean initial stimulus intensity than the two BDNF groups ($p << 0.01$, $F(2,22) = 25.2$, one-way ANOVA with Tukey Kramer post hoc corrections).

Over the course of training, GFP group required significantly higher voltages to elicit hindlimb locomotor activity ($p << 0.01$, paired t-test), consistent with findings in our previous work in robot-driven epidural stimulation[1] and in the literature for constant epidural stimulation[2]. On the final day of training, the average required stimulus intensity

in this group was 4.23 ± 0.33 V. Using linear regression, we modeled a linear relationship between weeks of training and stimulus intensity ($R^2 = 0.98$).

In the BDNF group that received constant epidural stimulation without robotic intervention at the pelvis, the final mean required stimulus intensity was 1.51 ± 0.05 V, a significant increase ($p << 0.01$, paired t-test). As compared to the GFP group, the slope of linear increase in stimulus intensity was not as high, but was an accurate model of the relationship between weeks of training and stimulus intensity ($R^2 = 0.96$).

Finally, the BDNF group that received the full combination of therapies had a final mean stimulus intensity at 0.74 ± 0.1 V. This was not a significant change from the first day of training ($p = 0.18$, paired t-test). The slope of the linear fit to this line indicates a relatively miniscule change in stimulus intensity ($R^2 = 0.84$) as a result of weeks of training.

On the final day of training, we compared the mean stimulus intensities of each group to evaluate the differences between treatment modalities in the effect that BDNF treatment had on epidural stimulation in the ATX model. We found that all of the groups were significantly different from one another ($p << 0.01$, $F(2,22) = 87.01$, one-way ANOVA with Tukey Kramer post hoc corrections).

	Robot-assisted Treadmill Training	Robot-driven Epidural Stimulation	Virus
Group 1 *n* = 9	✕	✕	BDNF
Group 2 *n* = 8	✕	✕	GFP
Group 3 *n* = 10	✕		BDNF
Group 4 *n* = 8		✕	BDNF

Figure 3-2. Overview of the experimental design in this chapter.

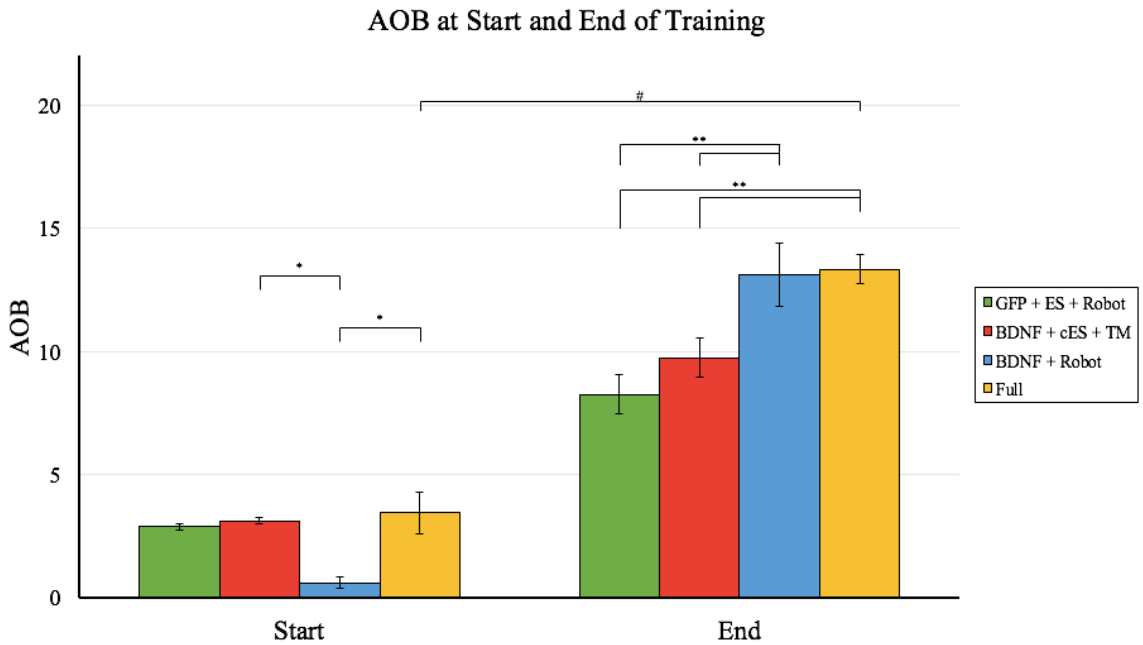


Figure 3-3. Comparison of the AOB hindlimb locomotor behavioral score among groups at the beginning and end of training. All groups showed statistically significant improvement in AOB as a result of training (using paired t-tests). Only the animals treated with the full combination of therapies have their improvement highlighted. There was no significant different at the beginning of training between the groups of animals treated with any form of epidural stimulation. There was no significant difference between animals that received the full combination of therapies and the BDNF-treated animals rehabilitated with robot assistance at the pelvis (* $p = 0.0097$, $F(3,31) = 4.51$, one-way ANOVA with Tukey Kramer post hoc corrections; ** $p = 0.0005$, $F(3,31) = 7.89$, one-way ANOVA with Tukey Kramer post hoc corrections; # $p << 0.01$, paired t-test).

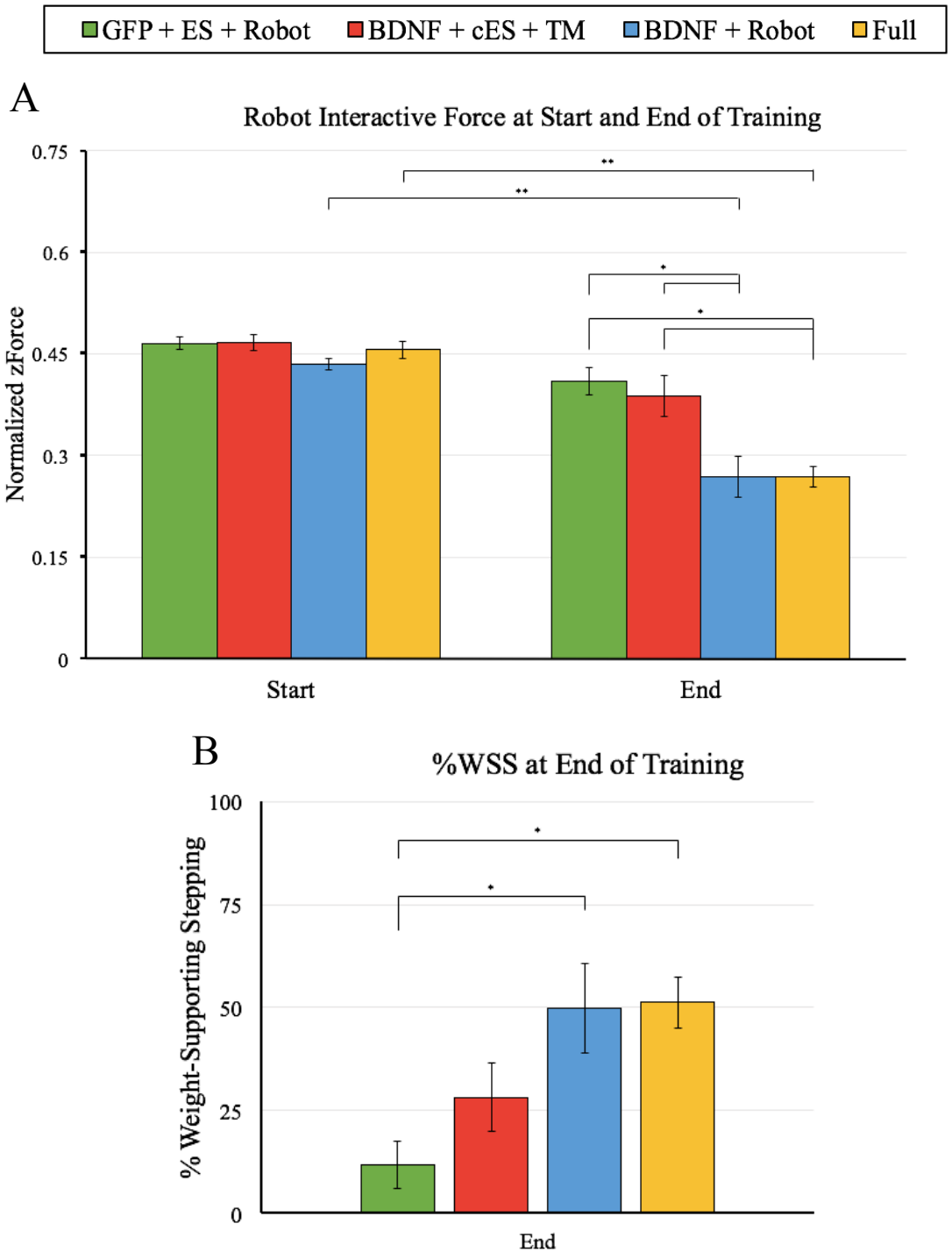
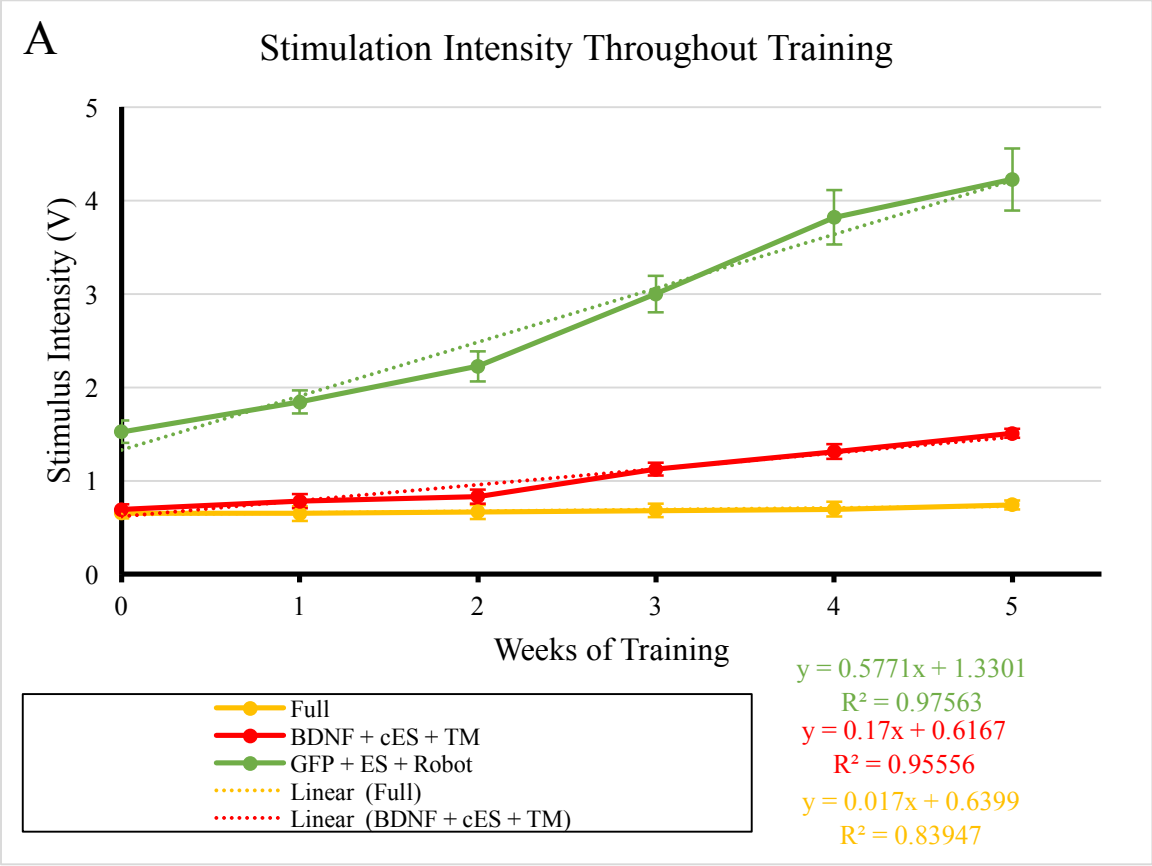


Figure 3-4. (A) Changes in normalized zForce (N/g) at the start and end of training across all groups. At the start of training, there was no significant difference in normalized zForce between all groups. (* $p = 0.0002$, $F(3,31) = 9.14$, one-way ANOVA with Tukey Kramer post hoc corrections; ** $p << 0.01$, paired t-test). (B) %WSS at the start of training was not shown as all groups of animals had mean start %WSS of 0. (* $p = 0.006$, $F(3,31) = 4.96$, one-way ANOVA with Tukey Kramer post hoc corrections).



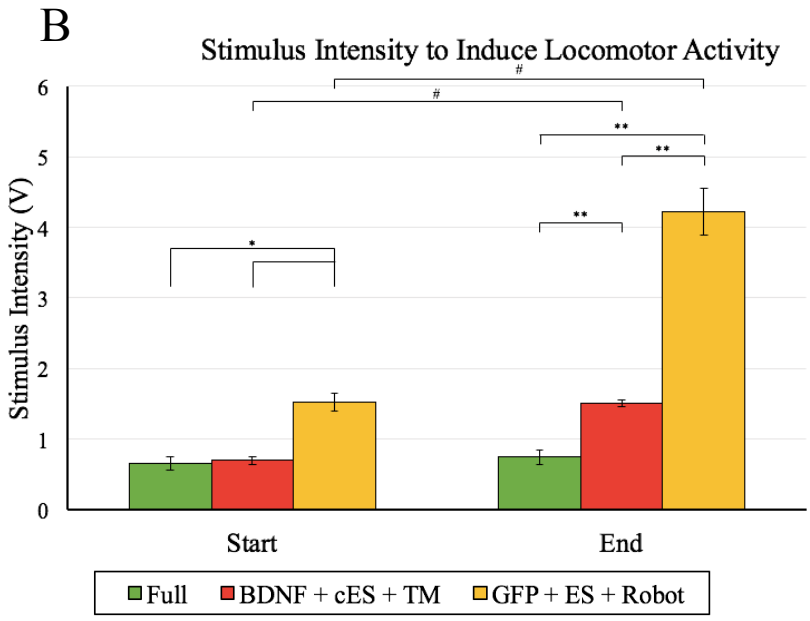


Figure 3-5. (A) Stimulus intensity (V) to elicit hindlimb motor activity as a function of weeks of training. (B) Statistical comparison between the stimulus intensity at the start of training and the end of training. (* $p << 0.01$, $F(2,22) = 25.2$, one-way ANOVA with Tukey Kramer post hoc corrections; ** $p << 0.01$, $F(2,22) = 87.01$, one-way ANOVA with TukeyKramer post hoc corrections; # $p < 0.05$, paired t-test).

D. Discussion

In SCI, despite the loss of descending supraspinal control, there exists intact neuronal circuitry in the spinal cord below the site of injury that is capable of producing locomotion [180]. In our present study, we have demonstrated the efficacy and synergistic benefits of combining the different treatment modalities of robot-driven and biological interventions at this intact circuitry in the locomotor rehabilitation of SCI. Our work further advances many previous studies that show this to be an effective target for locomotor recovery in the human and animal model for SCI [36], [181]. In particular, this work with our unique paradigm of robotic intervention at the pelvis during treadmill training builds upon previous work from our lab in robot-driven epidural stimulation (ES), combining Adeno-associated viral delivery of BDNF to the lumbar spinal cord in rats transected as adults (ATX) and robot assistance to significantly improve locomotor function.

The use of BDNF to induce locomotor activity in spinalized animals is not a novel technique, but its potential in combined therapy to promote rehabilitation has not been fully realized. As a singular treatment, BDNF has been shown to promote weight-supported stepping and functional improvement in the cat [56], [75] and the rat models for SCI [70] – [72], [76]. Many studies have investigated the process through which BDNF improves functionality of the hindlimbs, but of particular interest to our current work is how BDNF affects the balance between excitatory and inhibitory neurotransmission in the spinal cord [70], effectively creating a more excitable spinal cord. In our previous study, we demonstrated that viral delivery of BDNF could work in concert with robot-assisted treadmill training to improve function, when measured

against a battery of outcome measures, including the AOB bipedal stepping scale [161], percentage of weight-supported stepping, and interactive robot forces as a proxy for weight support. This was, to our knowledge, the first case of combining BDNF and robotic elements to increase function in SCI.

Our previous work had also demonstrated that robot interactive forces at the pelvis results in significant improvement of locomotor function in the ATX model of induced stepping. This is the foundation of our rehabilitation paradigm – that therapies aimed at the integrating and bolstering trunk during treadmill training is an essential component of therapy [30], [31], [153], [155]. In addition, we have discovered that the trunk motor cortex [140], [147], [148] is essential to produce weight-supported stepping after SCI, further supporting the utility of robot-assisted treadmill training in the rehabilitation of SCI. We also believe that our robot promotes a more natural context of locomotion, providing a framework for quadrupedal movement. We have shown that robot assistance at the pelvis also is able to increase weight-supported stepping in the model of adult rats transected as neonates (NTX), where animals had some ability to step before rehabilitation [31], and in the ATX model induced to step with viral delivery of BDNF.

In addition to providing interactive forces to engage the trunk in the injured animal, our robot is the foundation of our unique technique of robot-driven epidural stimulation (ES), which we have shown to be an effective means of locomotor rehabilitation in SCI [35]. The principles of our robot-driven ES are based on conventional methods of ES – providing electrical activation of the intact neuronal circuitry in the spinal cord to elicit motion. This is concept has been well documented and

studied rigorously in humans [40], [90], [182], cats [82], [83], and in rats [84] – [86], [183], although its therapeutic use in humans has also extended to areas such as pain management [88]. Unlike conventional methods of ES, our robot-driven technique does not use a constant frequency of stimulation, but rather, is determined by the height of the rat's pelvis, which is monitored by our robot. In this way, the animals only receive intermittent stimulation as their locomotor patterns require it, allowing for better integration of trunk and hindlimbs in a more natural pattern with weight-supported stepping. The concept of intermittent stimulation is not a completely new perspective for epidural stimulation [96], [97]. We believe this provides a weaning effect for injured animals, as well, allowing them to develop the quadrupedal controls needed to increase their own body weight support without the use of robotic interventions. With all other epidural stimulation parameters, such as inter-pulse period, pulse duration, and burst width, we used settings given in the literature from other groups working with conventional ES [85], [92], [184].

Another potential synergy that we hypothesized might exist between the use of ES and BDNF is the ability to manipulate the intact neuronal circuitry to provide specific hindlimb movements. The use of BDNF in the lumbar spinal cord in the rat model has been shown to have very specific effects at the ankle extensor [71], and we have seen in our previous study that there are other possible stereotypical hindlimb movements. The idea that individual and precise movements can be induced with therapies has also been show in epidural stimulation, in both the human [40], [91] and rat models [85]. Various studies have shown that it is possible to stimulate specific sites in the spinal cord to elicit flexion movements and extension movements at the various joints in the hindlimb [95].

Ichiyama *et al.* specifically showed how various spinal levels can be stimulated to produce bilateral stepping, unilateral stepping, flexion movements, and synchronous movements in the hindlimbs. In addition, they also examined the kinematics of movements, suggest that stimulation of the L2 segment would produce the most robust stepping. The placement of our electrodes in our study is a direct result of these studies in the rat models for ES. We hoped to elicit bilateral and alternating stepping patterns with a broad therapy such as viral delivery of BDNF, that we could fine-tune with both robot-assistance at the pelvis and specific flexion and extension electrical epidural stimulation of the spinal cord.

As such, in our present study, we have shown that combining robot-driven epidural stimulation along with AAV5-BDNF can significantly improve locomotor function in the ATX model. In agreement with our hypothesis, the animals the received the full combination of therapies performed better with regards to AOB, body weight support (normalized zForce), and percent weight-supported stepping, as compared to animals the received robot-driven ES alone and those that received BDNF and conventional ES. Unlike these two groups, the animals that received the full combination of therapies were able to support their body weight at times, and could even plantar place their hind feet while stepping. When comparing the conventional and robot-driven ES groups, we can appreciate the role of the robot in providing both intermittent stimulation and a specific framework for incorporating trunk and hindlimb in locomotion after injury. Consistent with our previous studies in robot-assisted treadmill training in the NTX model without ES, the robot is able to significantly increase body weight support, body

weight support (i.e., decrease reliance on robot interactive forces to maintain pelvic height), and %WSS [31], [153].

When we compare the outcome measures between the rats that received the full combination therapy and those that received the sham virus along with robot-associated therapies, we find that the viral delivery of BDNF does play a crucial role in significant recovery. Our interest in combining robot-driven ES and AAV5-BDNF was due to the potentially synergistic effects we hypothesized the two would provide each other when working in concert. There is a precedent in combining epidural stimulation techniques with biological therapies: Gerasimenko *et al*. used quipazine in conjunction with ES to promote weight-supported stepping in the ATX rat model [80]. Indeed, this technique is now adopted in many studies of SCI rehabilitation involving epidural stimulation [86]. We were interested in studying the effects of BDNF with epidural stimulation because exogenous BDNF decreases the rheobase of affected motor neurons [71], and increases the overall excitability of the spinal cord [70]. We believe that this works in concert with epidural stimulation, lowering the threshold needed for stimulation of hindlimb motor activity. Indeed, we showed that combining BDNF and robot-driven ES was an effective means to promote locomotor recovery in the ATX model, considering that GFP-treated animals were unable to produce any significant body weight support or %WSS as a result of therapy.

Next, when we compared all of the epidural stimulation groups, we discovered that exogenous BDNF had a significant effect on the stimulus intensity required to elicit hindlimb locomotor activity, consistent with the possible mechanism of action posited by the aforementioned work by Ziemlínska *et al.* and Boyce *et al*. First, we discovered that

on the first day of training for each animal (between seven and ten days, post-injection and post-injury), the two BDNF-treated groups had lower stimulus intensities required to produce alternating hindlimb movements as compared to the GFP group. In addition, the animals in those groups also had significantly higher mean starting AOB scores, indicating a functional benefit from combining BDNF and epidural stimulation. Our findings are consistent with previous studies using BDNF to induce stepping, where the time between injection and evidence of BDNF activity is approximately a week [70], [71], [185]. This significance was maintained throughout training, measured and recorded at weekly intervals, which is supported by studies that show that BDNF expression as a result of viral delivery can extend to up to sixteen weeks, post-injection [72]. We also observed that on the final day, there was a significant difference between the two BDNF groups that received either conventional or robot-driven ES. We believe this may be a result of the benefits of the specific framework of robot assistance that aided these animals to learn new methods of walking. This is contrast to the BDNF rats that received conventional ES, who did not have the robotic assistance that may have served to better integrate their trunk muscles into stepping.

Our interest in studying stimulation threshold has potential translational applications in the clinic. One of our major concerns for epidural stimulation is the application of high stimulus intensity, which we have observed affects performance in the rat model. Higher stimulus amplitudes may produce somewhat more robust activity up to a point, but it can cause discomfort in the animal, which may result in noncompliance during training. In addition, longitudinal studies of epidural stimulation in the rat model has shown that animals require larger and larger stimulus amplitudes over time, as they

acclimate to therapy [85]. Our combination of BDNF and epidural stimulation, regardless of technique, demonstrates that it is possible to produce the same or improved hindlimb activity, while using significantly lower or the same level of stimulus. This has many possible ramifications in the therapeutic use of epidural stimulation in the humans, where ES is used not just for locomotion therapy, but also in bladder, bowel, and sexual function.

There are many other paradigms of epidural stimulation, such as frequency-dependent stimulation that does not purport to change stimulus intensity. Indeed, these studies suggest that it is also possible to stimulate specific types of movements – rhythmic flexion-extension alternations, extension only – by changing the frequency of stimulus provided [94]. Different methods such as these are of interest to us in the context of robot-driven epidural stimulation, and we are developing ways in which to incorporate BDNF into these rehabilitative strategies.

We discovered one unexpected outcome when comparing BDNF animals that received robot-assisted treadmill training alone and those that received robot-driven epidural stimulation. We discovered no significant difference in function between the two groups when using all three outcome measures. There was a slight increase in overall mean AOB score in the group that received the full combination of therapies, but the functionality in both groups were characterized by frequent (between 50 and 95%) right-and-left hindlimb alternations, occasional (<50% of the time) body weight support, and occasional (<50% of the time) plantar stepping. We believe that though, on average, the two groups had similar recovery, there is a significant and stark contrast between the two groups.

In our previous study examining the effects of viral delivery of BDNF with robot-assisted treadmill training alone, we discovered that a significant subset of treated animals developed a partial, but highly significant collapse in locomotor function. This is consistent with the literature, where an increase in frequency of clonic movements in the hindlimbs inhibited further recovery, and in some cases, severely hindered function [70], [71]. Animals that collapsed were characterized by a significant gain and then significant loss of locomotion – specifically the ability to alternate their hindlimbs and support their body weight, in addition to being unable to retain a high percentage of weight-supported stepping. In addition, we observed that collapse animals were at risk for more severe negative health outcomes, as a result of difficulty of care due to the pathology of collapse. There are many theories as to why this might occur as a result of exogenous BDNF, but the prevailing hypothesis suggests that it may be a result of BDNF overexpression [70], [72], which may lead to a desensitization of TrkB receptors [174], [175], for which BDNF is the ligand [67]. Regardless of the cause, collapse is a major concern for the use of exogenous BDNF in the rehabilitation of SCI, though our robotic intervention at the pelvis has suggested that before collapse, BDNF animals trained on robot may reach higher levels of improvement over those trained on treadmill or in cage rest.

Here, we have discovered a means to mitigate, and possibly eliminate, collapse in the ATX animals treated with AAV5-BDNF to induce stepping, which has many possible ramifications in the future use of BDNF in the animal model for SCI. In our previous study, we observed that out of 26 adult ATX rats treated with AAV5-BDNF, 12 (approximately 46.15%) of these animals had a collapse in function, which resulted in

significant loss of locomotor recovery after peak performance. We observed this phenomenon in three different treatment modalities: cage rest, treadmill training, and robot-assisted treadmill training. Here, in our present study, we discovered that out of 17 ATX animals treated with AAV5-BDNF and rehabilitated with epidural stimulation (both conventional and robot-driven), there was no incident of collapse. Using binomial probability, we find this lack of collapse to be highly significant. Our work suggests that it is possible to prevent collapse in function due to BDNF overexpression, which has far reaching consequences in the use of BDNF for therapeutic benefit. We have demonstrated the efficacy and possible therapeutic benefits of combining the different modalities of treatment of biological and robotic interventions to provide synergistic effects in the rehabilitation of SCI.

In some regards, our results are not entirely unexpected, considering how epidural stimulation alone in the context of training and rehabilitation has been show to modulate the physiological state of spinal circuitry [90]. Our current work further emphasizes the role for ES in SCI, harnessing this ability in specific ways to not only improve locomotor function, but to reduce and prevent pathology that results from a broadly-acting therapeutic agent such as exogenous BDNF. If this is the case, the locomotor patterns develop in the BDNF rats with robot-driven epidural stimulation may be developed from different mechanisms than those of BDNF rats trained with robot alone. There is precedent of this, as task-based rehabilitation not only significantly improves function [186], [187], but induces reorganization, as well [19], [188], [189]. This may explain the wide variability of function in the BDNF animals trained with robot alone, as compared

to the small variance in function in the BDNF animals trained with robot-driven epidural stimulation.

From a clinical perspective, the prevention of collapse may have much more therapeutic benefits outside of locomotor rehabilitation. In our previous study, we observed that collapse in function led to negative health outcomes, such recurring bladder infections, skin lesions, and atrophy of hindlimb musculature. There were also difficulties with animal care as a result of collapse. By preventing collapse with the use of epidural stimulation (conventional or robot-driven), we may be able to prevent these pathological sequelae of collapse, as well. Clinicians interested in therapeutic benefits of exogenous BDNF to promote locomotion may find epidural stimulation of interest to reduce the risk of similar negative health outcomes in patients.

Using the three outcome measures that we did to assess locomotor activity, we hoped to develop a complete and holistic picture of the recovery in our animals, where each measure corroborated or added to the recovery profile of each animal. AOB is not a precise tool to measure recovery, as its criteria for “occasional”, “frequent”, and “consistent” encompass large bins – between 0 and 50%, between 51% and 95%, and above 95%, respectively. However, by using measurements of robotic interactive force, and by analyzing high-definition video in a frame-by-frame manner, we attempted to provide a more exact account of recovery in our animals. In addition, we verified the completeness of transection in all animals by visual inspection when each animal was sacrificed and by histology. We also confirmed the placement of our epidural stimulation electrodes when we extracted spinal cord from our animals for histological analysis.

E. Conclusion

In summary, our present work presents a novel approach to locomotor rehabilitation in the adult SCI model, combining bionic and biological interventions to significantly improve function. We showed that it is possible to combine robot-driven epidural stimulation along with Adeno-associated viral delivery of BDNF to produce robust, weight-supported stepping with plantar foot placement in SCI animals. The use of BDNF worked in concert with epidural stimulation, lowering stimulus thresholds to induce stepping and locomotor activity. This happened at the beginning of training, and persisted throughout all of rehabilitation. This is of particular interest to clinical use of epidural stimulation, where stimulating electrodes can lose efficacy over time.

We did not see any significant differences or improved function when comparing against ATX animals treated with BDNF and robot-assisted treadmill training. However, we discovered that the use of epidural stimulation prevented the occurrence of collapse in function that has been widely observed in the use of exogenous BDNF to induce stepping patterns in SCI rats. This has very important implications to potential translational applications of exogenous BDNF use and epidural stimulation in rehabilitation. We believe we have built a foundation for future investigations into how robotic interventions and viral delivery of BDNF can be combined more effectively and efficiently to produce more robust and improved locomotion in the ATX model for SCI.

CHAPTER 4: Active stepping rehabilitation induces significant reorganization of the trunk motor cortex in the adult spinalized rat

A. Introduction

Injuries to and diseases of the nervous system can lead to somatotopic reorganization of the brain sensorimotor cortex, in response to changes in function that accompany injury. This has been observed and well established in stroke [149], [190], limb amputation [191], [192], and in spinal cord injury (SCI) [141], [148]. In response to loss of motor output and sensory input, the plasticity of the nervous system can lead to undesired pathological sequelae, such as phantom limb sensation [124], allodynia [193], orspasticity [194]. These plastic changes may occur on different timescales, with rapid [195] and slower alterations [196] both playing a role in recovery and pathology. As a result, it is important to understand the patterns and trends in somatotopic reorganization that may accompany specific injuries to the nervous system.

In SCI, the normal, healthy architecture of the central nervous system is disrupted, interrupting descending supraspinal control from and ascending sensory information to the brain. Depending on the location and severity of the injury, SCI can lead to irreversible impairment of the body, such as paraplegia and quadriplegia. This loss of autonomy and mobility can severely decrease quality of life, as well as negatively impact health outcomes [197], [198]. As a result, locomotor rehabilitation is of utmost importance in the study of SCI.

In both the human and the rat model for locomotion, trunk muscles, and therefore trunk motor control, play crucial roles in locomotion, providing postural stability [140], as well as providing biomechanics for forward momentum [165]. In addition, in the rat

model for SCI, the trunk motor cortex is essential for adult rats transected as neonates (NTX) to develop autonomous weight-supported stepping (WSS) [140]. NTX rats are a unique paradigm for locomotor rehabilitation in SCI, as 20% of these animals are able to produce autonomous WSS (up to 50%) without intervention by the time they reach adulthood [33], [34]. However, NTX rats with lesions to their trunk motor cortex are unable to develop WSS [140], while previously weight-supporting NTX rats lose their ability to weight-support when their trunk motor cortex is lesioned (C. S. Oza, and S. F. Giszter, unpublished observations). Thus, we believe there is a crucial relationship between the intact neuronal spinal circuitry below the site of injury and the cortical control at the brain above, coupled mechanically via trunk muscles that span the length above and below injury, working together to provide stability and control for locomotion in the SCI model for weight-supported stepping rats.

In addition to revealing the importance of trunk motor control in locomotion after SCI, Oza *et al.* detailed specific changes to the trunk motor cortex and its relationship to other controls in the motor cortex. In the SCI model for rats completely transected as adults (ATX), unlike the NTX model, animals are unable to step without intervention. From a cortical perspective, SCI leads to a significant rostral shift of the trunk representation in the motor cortex, as compared to normal rats [147]. This shift is exacerbated by passive locomotor rehabilitation, where the hindlimbs are unable to step. Additionally, there are significant changes in the number of co-activated muscles per site in the cortex, as well as significant changes in the synergies between trunk muscles and forelimb control.

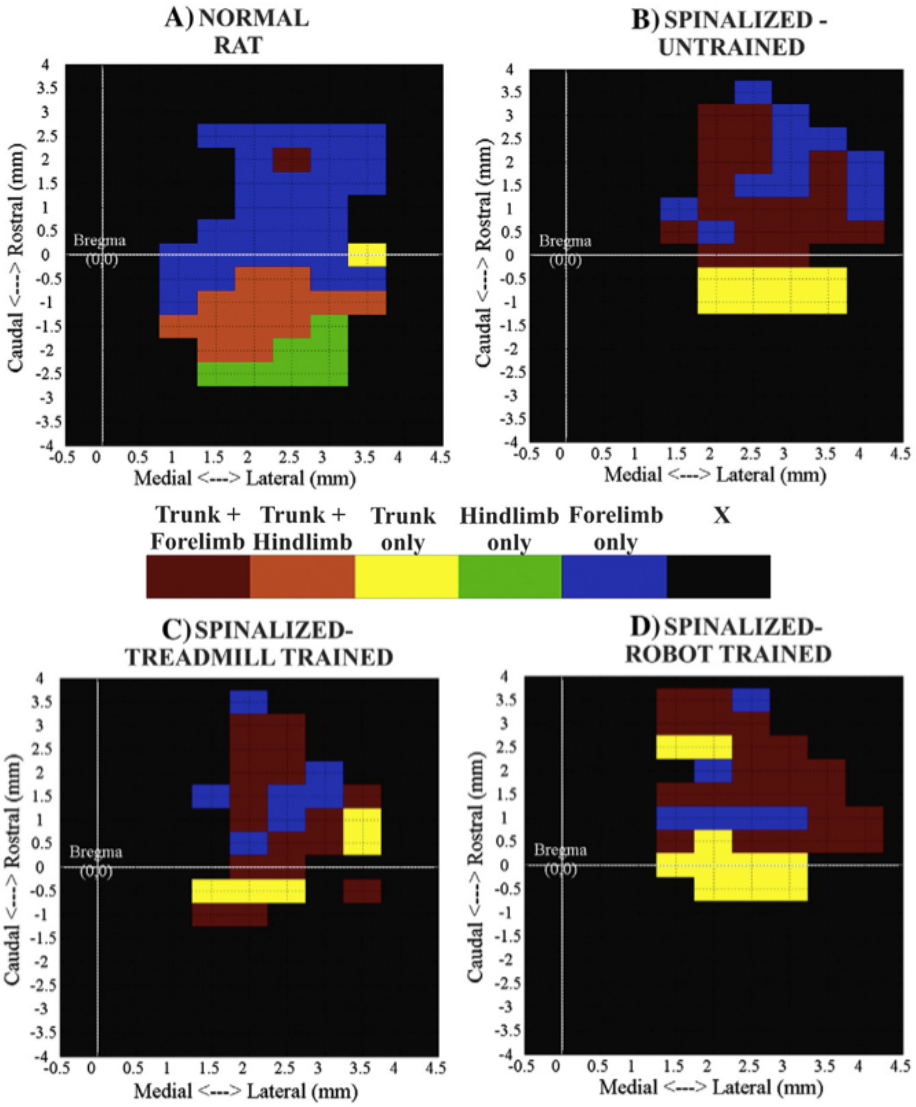


Figure 4-1. Representative trunk motor cortical maps in the rat model created from ICMS techniques. (A) Motor cortex map of the normal, intact rat. (B) (C) Motor cortex maps of ATX rats that do not step. (D) Motor map of the cortex in the ATX rat rehabilitated with robot assistance at the pelvis. All of these rats did not step during or after recovery. In all ATX rats, there is a loss of hindlimb activation sites (green) and a shift of the representation rostrally. *Used with permission from Oza et al., 2014*

In the NTX model of rats that can step with weight support, there is also an increased co-activation of of trunk motor sites in the cortex [148]. However, unlike the ATX rats that do not step, the trunk motor representation in the stepping NTX model does not have a significantly more rostral shift, though it does lose normal topography as

compared to the motor cortex of intact animals. In both the NTX model and the ATX model, there is a significant loss of low-trunk and lumbar representation in the motor cortex that is accompanied by SCI – a direct result of the loss of supraspinal control [147], [148].

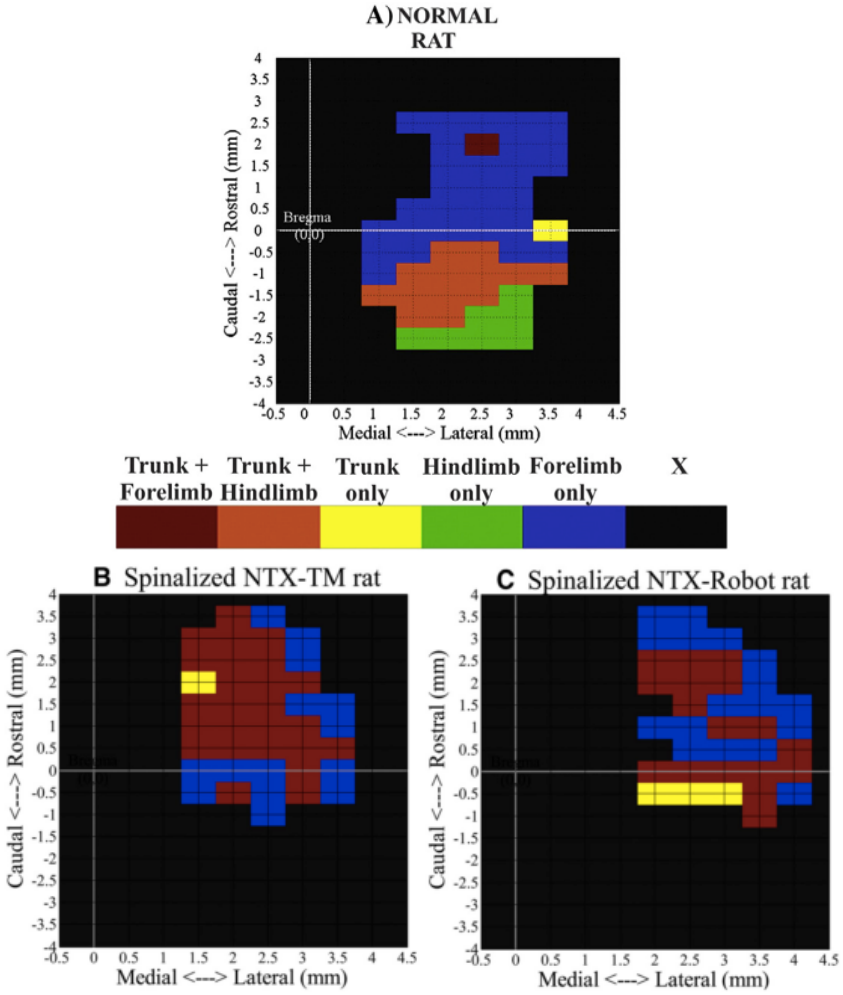


Figure 4-2. Representative maps of the motor cortex in various rats obtained by ICMS techniques. (A) Normal, intact rat motor cortex. (B) Map of the motor cortex in an NTX rat rehabilitated with treadmill training only. (C) Map of the motor cortex in an NTX rat rehabilitated with robot-assisted treadmill training. In both NTX maps, there is a significant rostral shift of the overall representation. However, in the robot-trained animal (C), there is a more normal topography of the trunk motor cortex (yellow). *Used with permission from Oza et al., 2015*

With locomotor recovery, the NTX model provides a signpost for potential recovery and function with rehabilitation for ATX animals that are induced to step using various interventions, such as viral delivery of brain-derived neurotrophic factor (BDNF) [56]*, epidural stimulation [85], [92], [160], or a combination of these therapies*. With these various therapies, ATX animals can be induced to step and weight support, like the NTX rat. The process of novel skill acquisition leads to changes in cortical organization [151], [154]. There is a gap, therefore, in our knowledge of the changes to the trunk motor cortex with the ATX rat that can step and support its own weight.

The NTX rats provide an excellent model for comparison in this regard, providing an impetus to understand how the trunk motor cortex of a weight-supported stepping ATX animal may reorganize in response to injury and then rehabilitation.

To examine this further, we prepared a cohort of six ATX animals and induced them to step and weight-support, using an Adeno-associated virus (AAV5) to deliver BDNF to the spinal cord caudal to the site of injury. BDNF promotes plasticity in the nervous system [185], [199], and it shown to increase the excitability of the spinal cord [70]. Phenotypically, as a response to BDNF treatment, ATX rats behave like NTX rats, with some spasticity, rudimentary flexion-extension movements in the hindlimbs, and occasional weight-supported stepping [30], [153].

In half of the rats, we also used epidural stimulation at the lumbar spinal cord to induce stepping, in tandem with AAV5-BDNF. This was done as a test to study the efficacy of combined therapies, and it resulted in both cohorts of animals recovering similarly against a battery of locomotor outcome measures. Both groups of animals were treated as one group of weight-supporting ATX animals, as the target of their therapies

were below the site of injury, and thus, not directly interacting with cortical mechanisms. In addition, the animals all performed similarly after rehabilitation, both in body weight support and in stepping patterns.

We hypothesized that the effects of BDNF caudal to the site of injury lead to increased spinal control of the low thoracic and lumbar trunk muscles, as demonstrated by increased locomotor recovery. We believe that this would then reveal insight into the nature of the relationship between the cortex and spinal networks in locomotion in the SCI model, as they negotiate working control of the trunk for locomotion.

We used intracortical microstimulation (ICMS) to create a binary response map of the motor cortex of all of the animals, using electromyography for trunk musculature and visual observation for limb movement. These maps were then compared with those of normal, intact animals, as well as with the ATX and NTX models.

Our work show that active stepping rehabilitation in the ATX model as a result of BDNF expression increases spinal control of trunk muscles in locomotion. However, like the NTX model, the active stepping ATX model shows an increase in low trunk and lumbar control in the cortex. Finally, we show that active rehabilitation in the ATX model further attenuates the rostral shift seen in the passive rehabilitation ATX model that is mitigated in the NTX model for recovery.

B. Materials and Methods

B-1. Overview

All surgical and experiment procedures were performed in accordance with the Drexel University College of Medicine's Institutional Animal Care and Use Committee

(IACUC). We prepared six intact adult female Sprague Dawley rats, all from the studies in the previous chapters. All animals received a complete spinal cord transection at vertebral level T9/T10, with microinjections of AAV5-BDNF caudal to the transection site, immediately following injury. In the same procedure, epidural stimulation wires were implanted at spinal level L2/L3 and at S1/S2. In addition, all animals were implanted with custom-made pelvic orthoses at the end of surgery. After recovery, all animals were trained for six weeks with robot assistance at the pelvis. Three of the animals also received robot-driven epidural stimulation. Video and robot data were collected during training for assessment and analysis of locomotor recovery during training. At the end of rehabilitation, animals underwent an intra-cortical microstimulation procedure to map their motor cortex.

For a more thorough review of the transection surgery, viral microinjection, epidural stimulation wire implantation, pelvic orthosis implantation, and robot rehabilitation techniques, please refer to the methods sections in the previous chapters.

B-2. Intracortical Microstimulation Techniques

We adapted intracortical microstimulation procedures from Oza *et al*. [147], [148] and *Giszter* et al. [140] to map the motor cortex of all of the animals after a rehabilitation training regimen. Thirty minutes to an hour prior to surgery, we delivered Dexamethasone (5 mg/kg) via an intraperitoneal injection. Animals were anesthetized for ICMS mapping using the same cocktail and dosage of KXA used in their transection surgery. Supplemental ketamine hydrochloride (0.24 ml/kg) was administered via intraperitoneal injection, as necessary. Throughout surgery, each rat was placed on a

heating pad to retain core body heat. In addition, we placed a heat lamp close to the animal to maintain a core body temperature of 37° F. Warm physiological saline was also delivered subcutaneously as needed.

To place electrodes on the ventral musculature of the animal's trunk, we made an incision in the skin covering the abdomen, and blunt dissected the skin away from the abdominal muscles. Using veterinary adhesive, we placed nine bipolar patch electrodes along the length of the abdomen, covering the right and left external oblique muscles and the rectus abdominus. The sites of the electrodes were placed to cover three levels of each muscle – mid-thoracic (corresponding to T5-T7), low thoracic (T12-T13), and lumbar (L2-L3) – to assess cortical control above, at, and below the site of injury, respectively. On the dorsal surface of the animal, we made a parasagittal incision along the midline of the skin and blunt dissected to create window to place six polar patch electrodes. The dorsal electrodes were placed in parallel along the parasagittal plane of the right and left longissimus muscles. They were placed again at mid-thoracic, low thoracic, and lumbar levels, similar to the placement used on the ventral surface of the animal. When the animal was not being actively mapped, we covered the exposed musculature with gauze wetted slightly with saline.

After the electrodes had been implanted, we placed the head of the rat in a stereotaxic frame and made an incision on the dorsal surface of the skin on the skull. Using blunt dissection, we exposed the skull and measured and recorded two suture landmarks: bregma and lambda. We then carefully removed a 1 cm by 1 cm window of the skull centered around bregma, and used a 30-gauge needle and iridectomy scissors to carefully remove the dura above the brain at the motor cortex. When the animal was not

actively mapped, we used saline and gauze to cover the exposed brain and clear away any blood that may have pooled.

We used stainless steel electrodes (~ 10 MΩ, initial impedance at 1 kHz, diameter 125 μm, and tip < 1 μm diameter, exposed tip ~ 5 μm^2 FHC) to deliver stimulus current to the motor cortex. Using the stereotaxic frame and manipulator, and lambda as a reference point, we created a grid of the motor cortex centered around bregma as the origin, with a resolution of 0.5 mm in the x- and y- axes. Using the electrode to vertically penetrate the brain at depths of 1.5 mm, we applied a constant current biphasic pulse of 75 μA with cathodal current leading to each site, applied as 0.2 ms total duration at 333 Hz in trains of 300 ms duration.

Responses in the forelimbs and hindlimbs were observed grossly and noted, with movements in the musculature or flexion of the limbs indicating a response to cortical stimulation. Trunk responses were measured using the EMG data from the previously placed path electrodes, using differential amplifiers and an A/D data acquisition system (Molecular devices: Digidata 1320). Signals from the patch electrodes were amplified with a gain of 1000, sampled at a rate of 2 kHz. We recorded any changes in EMG as a result of stimulus. We also recorded trunk EMG any time the animal responded to stimulus, to assess changes later.

We noted limb movement or trunk EMG changes as binary responses to stimulus, and created a map from the grid of the motor cortex. We distinguished sites that had no responses or a combination of forelimb, hindlimb, or trunk responses in our maps. Prior to stimulation, we fully extended forelimbs to be able to visualize small movements elicited by ICMS.

During active mapping of cortical sites, we turned off the heating pad directly below the animal to reduce electrical noise. When the animal was rested, the heating pad was turned back on. The heating lamp was not turned off for the duration of the surgery. In addition to the heating lamp and the heating pad, when the animal was rested, we placed gauze wetted with warm saline on the animal to maintain body heat. We also injected warm saline into the animal through the surgery for hydration and for heat.

B-3. Data Analysis

EMG data was assessed both during and after the surgery for responses to stimuli. We used custom-written scripts in MATLAB R2014b, Mathworks, to decode the data into visual representations for analysis, which were then compiled in an array format in Microsoft Excel, 2106. Representative motor maps were produced in MATLAB using custom-written scripts produced from the Microsoft Excel spreadsheets.

We used previous data in our lab acquired by Chintan Oza to compare our weight-supporting BDNF-treated animals with both the ATX animals and NTX animals rehabilitated with robot-assistance and with normal, intact animals. To compare across groups, we used one-way ANOVA with Tukey Kramer post hoc comparisons to test for statistical significance. We considered a p-value of less than 0.05 to be statistically significant. All statistical tests were done in MATLAB R2014b, Mathworks. Graphs and figures were produced in Microsoft Excel 2016.

C. Results

C-1. Overview

We used intracortical microstimulation (ICMS) mapping techniques to produce motor cortex representations of ATX rats induced step with AAV5 viral delivery of BDNF (n = 6) and treadmill-trained with robotic intervention at the pelvis. Half of the animals received robot-driven epidural stimulation along with training. At the end of training, all animals were able to produce robust alternations of the right and left hindlimbs. In addition, all animals were able to plantar place their feet on the treadmill at least occasionally (33.33% of the time), as well as body weight support. One more criterion was to evaluate rats that did not have a collapse in function, as a result of AAV5-BDNF treatment. These animals were all weight-supporting BDNF animals (wsBDNF), trained on robot. We mapped each animal approximately 7-8 weeks after injury, after a full training regimen. We used ICMS parameters that we found to be ideal for motor cortex mapping in our previous work [140], [147], [148]. We compared the maps of these animals with maps from our previous work on intact animals, non-stepping ATX animals trained passively with robot rehabilitation, and stepping NTX animals trained on robot.

C-2. Locomotor Recovery in wsBDNF Rats Trained with Robot Intervention at the Pelvis

We assessed the locomotor recovery of all of the animals following their rehabilitation regimens, using three outcome measures: AOB bipedal stepping scale, robot interactive forces, and percentage weight-supported stepping (%WSS). Video and robot data were collected during every day of training to assess these outcome measures per individual

rat, and as a group. As a group, the AAV5-BDNF-treated animals that weight-supported (wsBDNF) had a significant improvement in their AOB scores, starting at an average score of 1.17 ± 0.40, and ending with a mean score of 15.33 ± 2.01 ($p << 0.01$, paired t-test). Their final average AOB indicated that these animals were able to consistently alternate their hindlimbs (>95%), while occasionally (<50%) maintaining body weight and plantar placing their hind feet. We compared this group of animals with data from previous studies in our laboratory. The group of ATX rats that received passive rehabilitation while on robot during treadmill training (ATX-R) did not have significant improvement over weeks of training, with most scores of 0 and 1, and at most 3 [148]. In contrast, like the wsBDNF animals, the NTX animals trained with robotic intervention at the pelvis (NTX-R) had significant improvement in their AOB scores, as a result of training [147].

When comparing the interactive force between an animal and the robot to maintain the pelvic height of the rat (normalized zForce), we found similar trends. The wsBDNF group began with a mean normalized zForce of 0.45 ± 0.02 N/g. Through five to six weeks of training, these rats relied significantly less on the robot, ending with a mean normalized zForce of 0.27 ± 0.04 N/g ($p << 0.01$, paired t-test). Similar to the pattern of recovery in AOB scores, the ATX-R animals did not show any significant improvement in body weight support, while the NTX-R animals did have a significant increase in their body weight support.

Finally, we saw the same trends in the %WSS for all of the groups, as in the previous two measures. We define %WSS as the number of steps that support the weight of the animal, divided by the total number of steps that an animal takes. Using frame-by-

frame analysis of video taken during training, we observe when an animal takes a weight-supported step, or a step where no other part of the animal except its limbs are on the treadmill. The wsBDNF group began with an average of 0 %WSS, consistent with its AOB score on the first day of training. By the end of training, we observed significant improvement ($p << 0.01$, paired t-test) with an average %WSS of 50.12 ± 15.37%. The ATX-R group did not show improvement in %WSS as a result of training. The NTX-R group, similar to the wsBDNF group, showed significant improvement from the start of training to the end.

We did not assess the normal, intact rats for their initial AOB scores, normalized zForce, or their %WSS.

C-3. Effect of Rehabilitation on Total Trunk Motor Area

We observed significant changes as a response to rehabilitation in the trunk motor cortex of the various SCI groups. To compare the cortical maps of the normal animals against the spinalized animals, we normalized the map data to the nonfacial motor cortex area (forelimb, neck, and trunk responses). We did this to eliminate error due to hindlimb responses observed in the intact animals that would not exist in the spinalized animal groups and bias the perception of data.

Consistent with previously published data, the ATX-R group had a significantly large total trunk area, as compared to the normal, intact group. Its total trunk area as a percentage of nonfacial motor area was 0.59 ± 0.06% (mean ± SEM). The normal group had a total trunk area of 0.35 ± 0.02% of the nonfacial motor cortex. The NTX-R group also had an increased total trunk area (0.48 ± 0.06%), though not significantly different

from normal or the ATX-R group. We observed that wsBDNF animals did not have a significant expansion of total trunk area as a result of robot rehabilitation with active stepping ($p < 0.05$, $F(3,26) = 8.47$, one-way ANOVA with Tukey Kramer post hoc corrections). Its total trunk area as a percentage of nonfacial motor area was $0.34 \pm 0.06\%$, significantly lower than normalized trunk area of the ATX-R group, but not from that of the NTX-R or the normal groups.

C-4. Changes in Total Trunk Motor Area by Segmental Level

To better understand the nuances of the change in total trunk area, we examined the distribution of total trunk motor area across trunk segments (external obliques, rectus abdominus, and longissimus) rostral and caudal to injury. Specifically, we observed mid-thoracic sites above the injury, and then low-thoracic and lumbar sites below the transection, as noted in the previous section (B. Materials and Methods). Again, to compare with data from normal rats, we normalized the data as a percentage of the nonfacial motor cortex. Sites along the map that corresponded to activation of muscles at different segmental levels were recorded as having responses in all of the segments.

At the mid-thoracic level, above injury, using one-way ANOVA with Tukey Kramer post hoc corrections, we observed that there was a significant increase in mid-thoracic trunk motor area in the ATX-R group, as compared to the normal group ($p = 0.0006$, $F(3,26) = 7.94$). The total trunk area as a percentage of nonfacial motor cortex in the ATX-R rehabilitation group was $0.54 \pm 0.05\%$. This was not significantly higher than any of the other SCI groups. In addition, there was no significant difference between the

intact group (0.26 ± 0.03%), the wsBDNF group (0.35 ± 0.06%), and the NTX-R group (0.39 ± 0.044%).

Below the site of injury, we observed similar total trunk area across all groups at the low-thoracic level. The normal group of intact animals had an average total trunk area of 0.28 ± 0.02% of nonfacial motor cortex. The ATX-R group had a slightly higher mean total trunk area of 0.34 ± 0.05%. The NTX-R group and the wsBDNF group had means of 0.35 ± 0.04% and 0.26 ± 0.12%, respectively. There was no significant differences between groups at this level ($p = 0.6371$, $F(3,26) = 0.57$, one-way ANOVA with Tukey Kramer post hoc corrections).

Further caudal to the transection site, we observed all transected animals had less trunk area corresponding to lumbar musculature than the normal animals (Normal: 0.27 ± 0.03%; ATX-R: 0%; NTX-R: 0.06 ± 0.05%; wsBDNF: 0.04 ± 0.02%). We observed that the normal animals had a significantly higher trunk area corresponding to the lumbar segmental level than the ATX-R animals ($p = 0.0034$, $F(3,26) = 5.87$, one-way ANOVA with Tukey Kramer post hoc corrections), but was not significantly different from the NTX-R and wsBDNF animals. In addition, there was a significant difference between the mean total trunk area of lumbar musculature in the ATX-R group and in the wsBDNF group. We found no significant difference between the NTX-R and the wsBDNF groups.

C-5. Changes in Total Trunk Area by Dorsal and Ventral Representation

In addition to observing changes at above and below the site of injury, we examined changes in representation of the dorsal and ventral aspects of the trunk in animals. Using the same musculature from the preceding section, we reorganized the data into ventral

(external obliques and rectus abdominus) and dorsal (longissimus) musculature. When recording observations of trunk musculature responses to stimulation in the cortex, we divided sites into three categories: dorsal and ventral, dorsal only, and ventral only. Again, to reduce bias in response as a result of hindlimb responses in the normal animals, we normalized all data to a percentage of nonfacial motor cortex.

For trunk area that corresponded to sites both on the dorsal and ventral trunk musculature of an animal, we observed many differences between the groups. In particular, the ATX-R group had the largest trunk area (0.29 ± 0.04%), which was significantly larger than those of the normal group (0.14 ± 0.03%) and the wsBDNF group (0.06 ± 0.02%) ($p = 0.0012$, $F(3,26) = 7.13$, one-way ANOVA with Tukey Kramer post hoc corrections). In addition, the wsBDNF group had the smallest trunk area for dorsal and ventral musculature activation, significantly smaller than those of the ATX-R group and the NTX-R group (0.25 ± 0.04%). There was no significant difference between the normal and the wsBDNF group.

As for total trunk area corresponding to dorsal musculature activation, we observed that the ATX-R group had the largest area (0.17 ± 0.04%). This was significantly larger than the dorsal trunk areas for the normal group and the wsBDNF group ($p = 0.003$, $F(3,26) = 6.02$, one-way ANOVA with Tukey Kramer post hoc corrections). There was no significant difference between the NTX-R group and the other groups.

We did not observe any significant differences across groups in trunk area corresponding to ventral trunk musculature ($p = 0.513$, $F(3,26) = 0.78$, one-way ANOVA with Tukey Kramer post hoc corrections).

C-6. Changes in Coactivation Density

Using nomenclature from our previous studies in ICMS cortical maps of the trunk motor cortex in rehabilitated SCI animals, we define "coactivation density" as the total number of muscles activated at a particular site in the trunk motor cortex. We used this to assess richness of muscle synergy representation per site in the cortex.

We observed that all transected animals had lower mean coactivation densities (ATX-R: 2.48 ± 0.20; NTX-R: 2.99 ± 0.27; wsBDNF: 1.91 ± 0.08) than the normal, intact animals, which had a mean coactivation density of 3.87 ± 0.20 muscles. This was significantly higher than in all of the other groups ($p << 0.01$, $F(3,26) = 21.75$, one-way ANOVA with Tukey Kramer post hoc corrections). In addition, the wsBDNF group had a significantly lower mean coactivation density than the other groups, in the same comparison.

C-7. Changes in Coactivation Density by Segmental Level

As in the total trunk area, we divided up trunk musculature by segmental levels above and below the site of injury to compare the coactivation densities and understand where the difference in richness of trunk motor synergy may be found.

Above the site of injury, at the mid-thoracic level on both the dorsal and ventral aspect of each animal, we found no significant differences between the groups ($p = 0.10$, $F(3,26) = 2.27$, one-way ANOVA with Tukey Kramer post hoc corrections). The ATX-R and the NTX-R groups had the highest mean coactivation densities (1.68 ± 0.17 and 1.70 ± 0.45 muscles, respectively). The wsBDNF group had a mean coactivation density of

1.35 ± 0.13 muscles. The normal, intact group had a mean coactivation density of 1.3 ± 0.10 muscles.

Below the site of injury at the low-thoracic level, we observed significant differences in synergy representation ($p = 0.012$, $F(3,26) = 4.44$, one-way ANOVA with Tukey Kramer post hoc corrections). The wsBDNF group had the lowest mean coactivation density of 1.00 ± 0.34 muscles, which was significantly lower than those of the normal group (1.88 ± 0.18 muscles) and the NTX-R group (2.05 ± 0.19 muscles). It was not significantly different from the ATX-R group (1.84 ± 0.13 muscles). In addition, there were no differences between the normal group, the ATX-R group, and the NTX-R group.

We also compared the coactivation densities at the lumbar levels of these groups of animals. All transected animals had lower mean coactivation densities compared to the normal, intact group (ATX-R: 0; NTX-R: 0.48 ±0.25; wsBDF: 0.50 ± 0.22 muscles). The normal group had the highest mean coactivation density, at 2.53 ± 0.28 muscles, which was significantly greater than all groups of transected animals ($p << 0.01$, $F(3,26) = 27.87$, one-way ANOVA with Tukey Kramer post hoc corrections).

C-8. Change in Coactivation Density by Dorsal and Ventral Representation

Finally, we compared the mean coactivation densities of the groups of animals based on the representation of the dorsal and ventral trunk musculature, similar to the evaluation of the total trunk area. We found no significant differences between the mean coactivation densities in both the dorsal trunk representation and in the ventral trunk

representation in the groups of animals ($p = 0.19$, $F(3,26) = 1.69$, one-way ANOVA with Tukey Kramer post hoc corrections).

C-9. Effect of Spinalization and Rehabilitation on Trunk Motor Cortex Migration

Next, we evaluated the location of the trunk motor cortex representation relative to the brain midline and the bregma landmark, across all of the groups of animals, as we had done in previous studies [147], [148]. In intact rats, the sites corresponding to trunk motor cortex are mostly caudal to the bregma line and within 2-3 mm of the midline of the brain. Using our maps, we calculated a "center of gravity" for the trunk motor representation in all of the animals, identifying the mean location of trunk sites relative to the midline (x-cog) and the bregma line (y-cog). We measured these locations in mm from midline and from bregma, with negative y-cog values indicating locations caudal to the bregma line. We compared the mean x-cog and y-cog between groups to identify any significant shifts in either direction.

In the x-direction, across all groups, there was no significant difference in the distance from the midline between groups ($p = 0.18$, $F(3,26) = 1.74$, one-way ANOVA with Tukey Kramer post hoc corrections). However, in the y-direction, we did find significant differences in y-cog between groups ($p = 0.0006$, $F(3,26) = 8.08$, one-way ANOVA with Tukey Kramer post hoc corrections). In particular, we observed that the normal group (-0.59 ± 0.32 mm) had a significantly lower y-cog than the ATX-R (1.11 ± 0.17 mm) and NTX-R groups (0.64 ± 0.22 mm). The y-cog of the wsBDNF group (-0.29 ± 0.42 mm) was also significantly different from that of the ATX-R group, though it was not significantly different from those of the NTX-R group or the normal group.

C-10. Overlap of Trunk and Forelimb Representation in Motor Cortex

In addition to the location of the trunk motor cortex representation relative to bregma, we observed accompanying changes to the coactivation between trunk and forelimb motor cortex. As this area rostral to the bregma line is the site of forelimb representation, we expected to observe changes in this overlap between these motor representation areas due to the migration of the trunk motor cortex further rostral, even though it was not a significant change.

To best describe this change, we reorganized our results into three categories of possible motor responses: trunk only, trunk and forelimb, and trunk and hindlimb. When comparing the number of sites that only corresponded to trunk motor responses across all three groups, we observed the normal group had significantly less sites than the NTX and the wsBDNF groups ($p = 0.0106$, $F(3,26) = 4.57$, one-way ANOVA with Tukey Kramer post hoc corrections). There were no significant differences between any of the other groups. In previous studies comparing various ATX rehabilitation paradigms with intact animals [148], there had been a significant increase in trunk only sites in the ATX model, but this significance disappeared in our ANOVA.

When comparing the changes in sites corresponding to coupling of trunk and forelimb motor responses, we observed that there was a significant difference between the intact group of animals and the ATX animals rehabilitated with robot ($p = 0.0358$, $F(3,26) = 3.31$, one-way ANOVA with Tukey Kramer post hoc corrections). There were no other significant differences among groups.

Finally, we compared the coupling of trunk and hindlimb motor responses across all groups. Unsurprisingly, as hindlimb responses were not observed in any of the

spinalized groups, there was a significant difference between the intact group and all of the other groups ($p << 0.01$, $F(3,26) = 174.66$, one-way ANOVA with Tukey Kramer post hoc corrections).

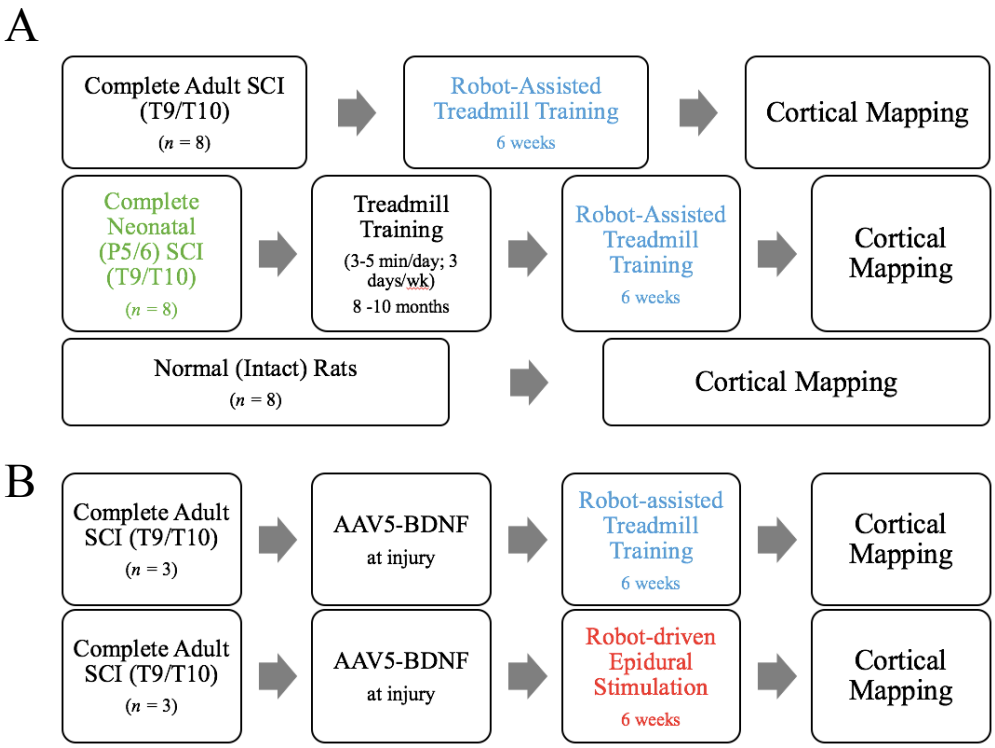


Figure 4-3. Overview of the rehabilitation scheme of the the six animals chosen for ICMS cortical mapping procedure. (A) Rehabilitation regimen for animals selected from previous studies in our lab. (B) Research design of the animals chosen for this study.

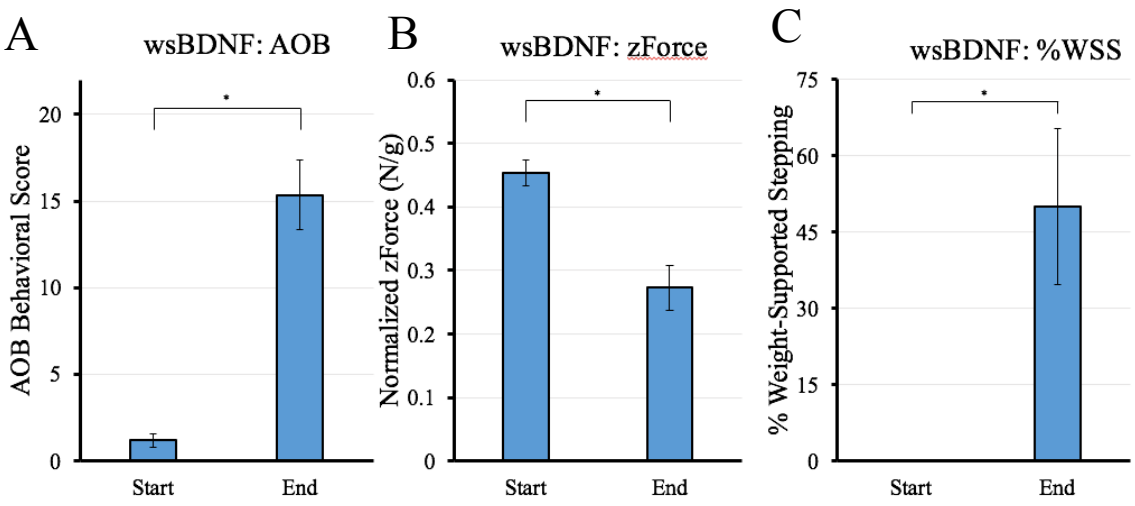


Figure 4-4. Locomotor recovery of the animals selected for this study. (A) Significant improvement in AOB behavior score, with a final mean AOB of 15.33 ± 2.01 (* $p << 0.001$, paired t-test). (B) wsBDNF animals relied significantly less on robot to maintain pelvic height at end of training (* $p = 0.0011$, paired t-test). (C) wsBDNF animals had a mean start %WSS of 0, but significantly improved to 50.18 ± 15.37% (* $p = 0.022$, paired t-test).

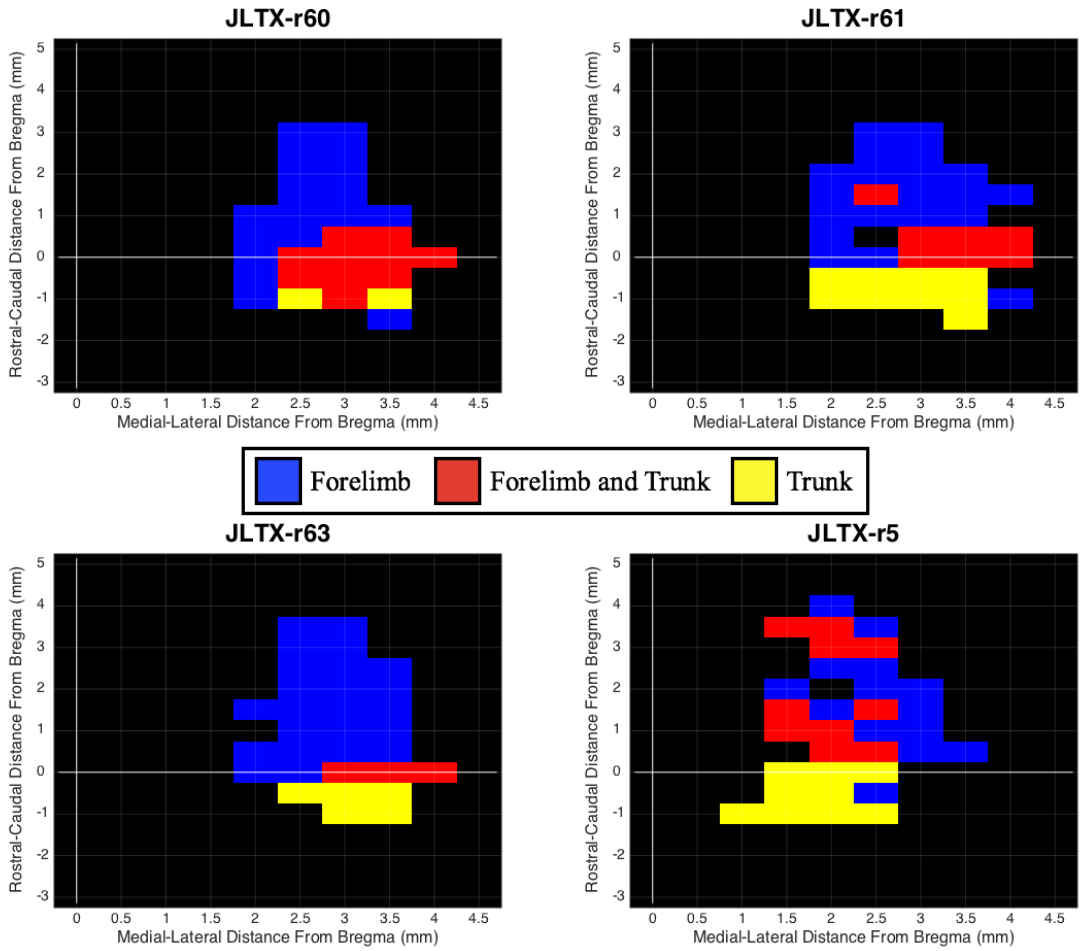


Figure 4-5. Representative cortical maps of select wsBDNF rats in this study, obtained via ICMS. Maps show only sites that activated forelimb and trunk musculature at 75 μA currents. In all of the maps, the bregma landmark is at (0,0). The rostral direction is in the positive y-direction, and caudal is in the negative y-direction. Lateral is in the positive x-direction, and medial is in the negative x-direction.

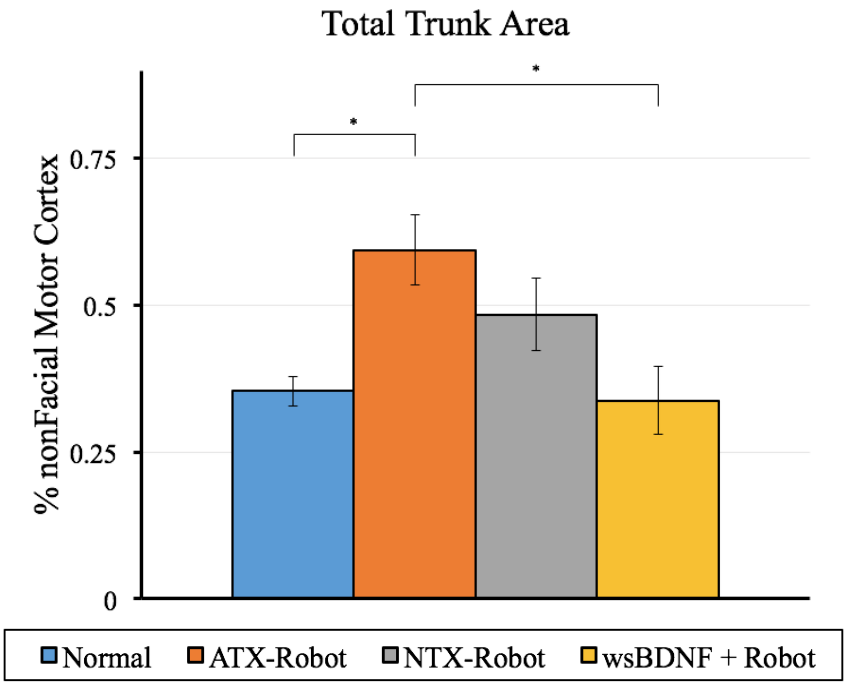


Figure 4-6. Total trunk motor area in the different groups of animals. Trunk motor area sites were determined by activation of trunk musculature as a response to ICMS. This was divided by the total number of sites corresponding to forelimb, neck, and trunk activation. To reduce error and bias in comparison with the motor cortex of normal, intact rats, trunk area was normalized to a percentage of the nonfacial motor cortex and hindlimb-exclusive responses were omitted. All data shown is mean ± SEM. There was a significant different between the total trunk area as a percentage of nonfacial motor cortex of normal rats and ATX rats rehabilitated with robot. This was also true between the ATX rats and the wsBDNF rats. (* $p < 0.05$, $F(3,26) = 8.47$, one-way ANOVA with Tukey Kramer post hoc corrections).

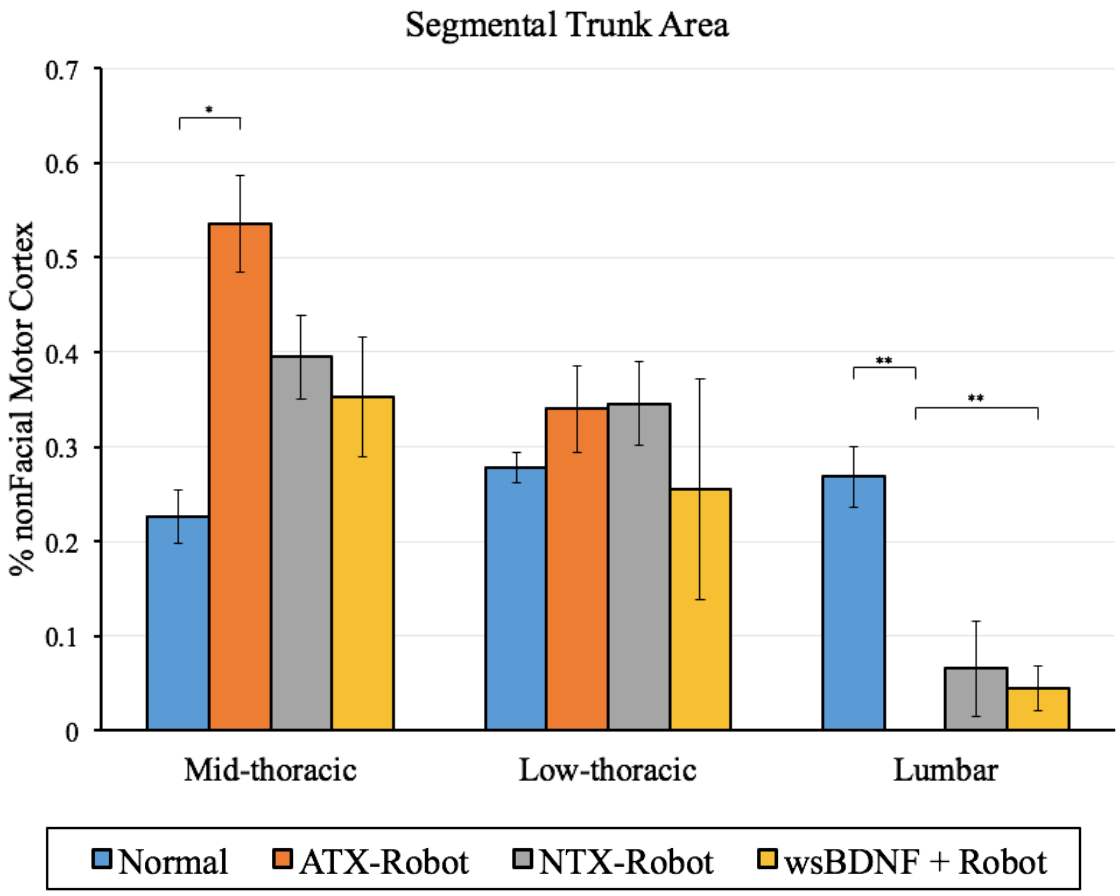


Figure 4-7. Segmental view of the changes to total trunk area as a percentage of nonfacial motor cortex, as a result of injury and rehabilitation. All data presented is in terms of mean ± SEM, and was normalized without hindlimb activity. The mid-thoracic level was above the site of injury, whereas the low-thoracic and lumbar levels were below the site of injury. Sites with activation of musculature at different levels were counted as contributing to those different levels. There was no significant difference between different groups in the low-thoracic region of trunk musculature. (*$p = 0.0006$, $F(3,26) = 7.94$), one-way ANOVA with Tukey Kramer post hoc corrections; ** $p = 0.0034$, $F(3,26) = 5.87$, one-way ANOVA with Tukey Kramer post hoc corrections).

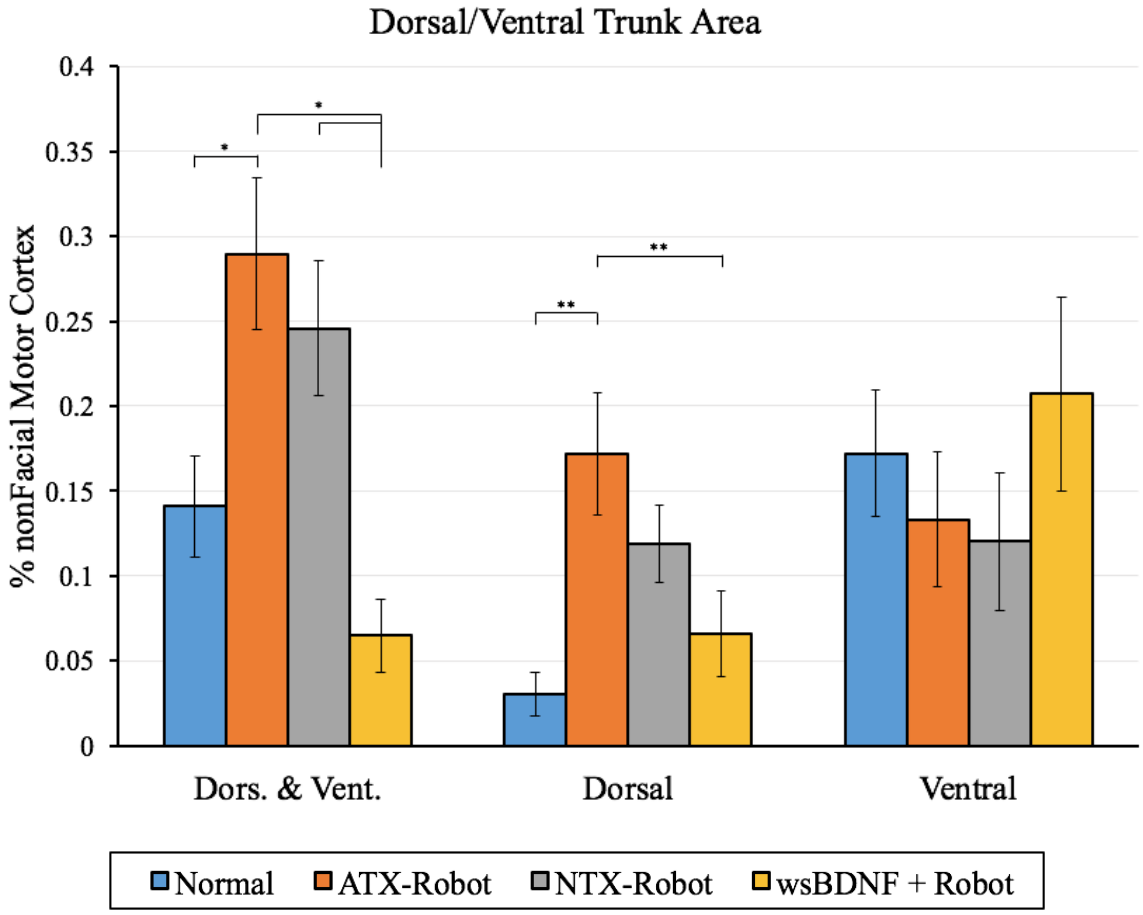


Figure 4-8. Changes in dorsal and ventral representation of the total trunk area as a percentage of nonfacial motor cortex, as a result of injury and rehabilitation. All data presented is in terms of mean ± SEM, and was normalized without hindlimb activity. All trunk responses were divided into three categories: dorsal and ventral responses, dorsal only, and ventral only. There was no significant difference in the ventral trunk musculature responses as a result of ICMS between the different groups of animals. (* $p = 0.0012$, $F(3,26) = 7.13$, one-way ANOVA with Tukey Kramer post hoc corrections ** $p = 0.003$, $F(3,26) = 6.02$, one-way ANOVA with Tukey Kramer post hoc corrections).

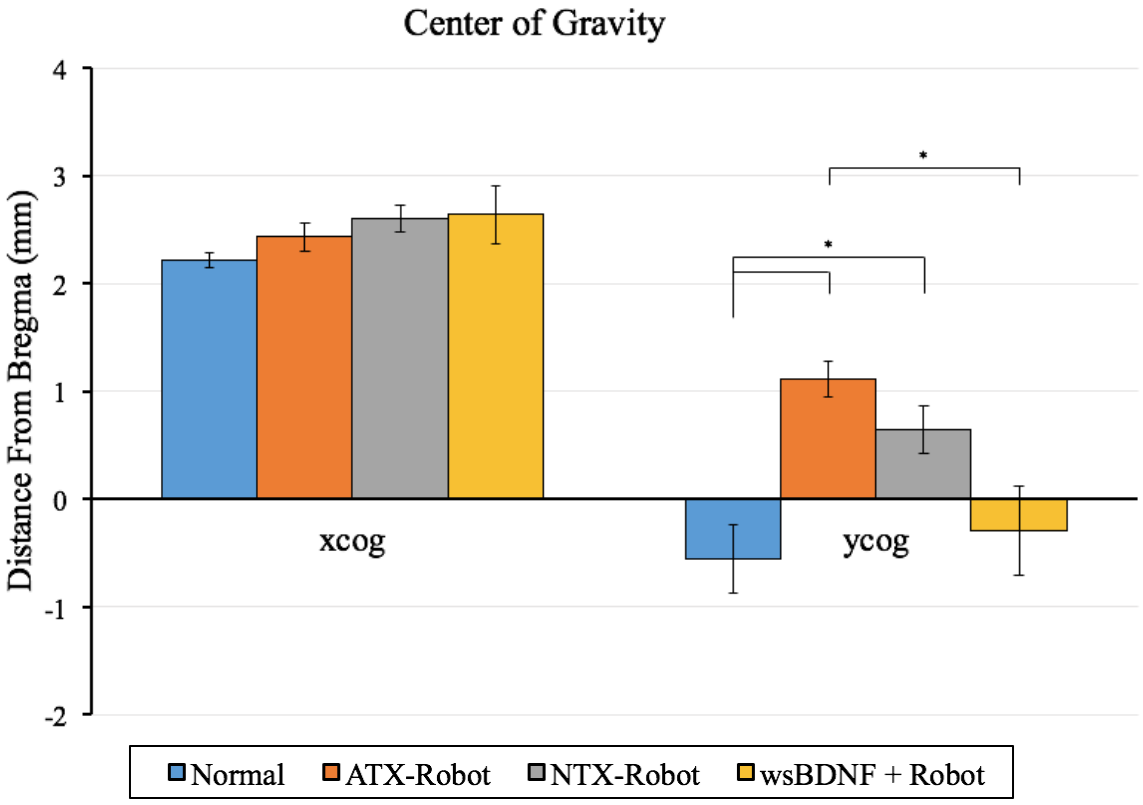


Figure 4-9. Relative location from bregma of trunk motor representation in the cortex following injury and rehabilitation. The bregma landmark was designated the point of origin (0, 0). Medial-lateral distance was calculated along the x-axis, with lateral movement in the positive x-direction. There was no significant difference in the x-center of gravity (cog) among the groups of animals. Rostral-caudal distance was calculated along the y-axis. Rostral movement was determed in the positive y-direction from bregma. Normal, intact animals have a significantly more caudal y-cog than the ATX and NTX animals rehabilitated with robot (* $p = 0.0006$, $F(3,26) = 8.08$, one-way ANOVA with Tukey Kramer post hoc corrections). The mean y-cog of the trunk motor cortex in wsBDNF rats was not significantly different from that of the normal, intact rats.

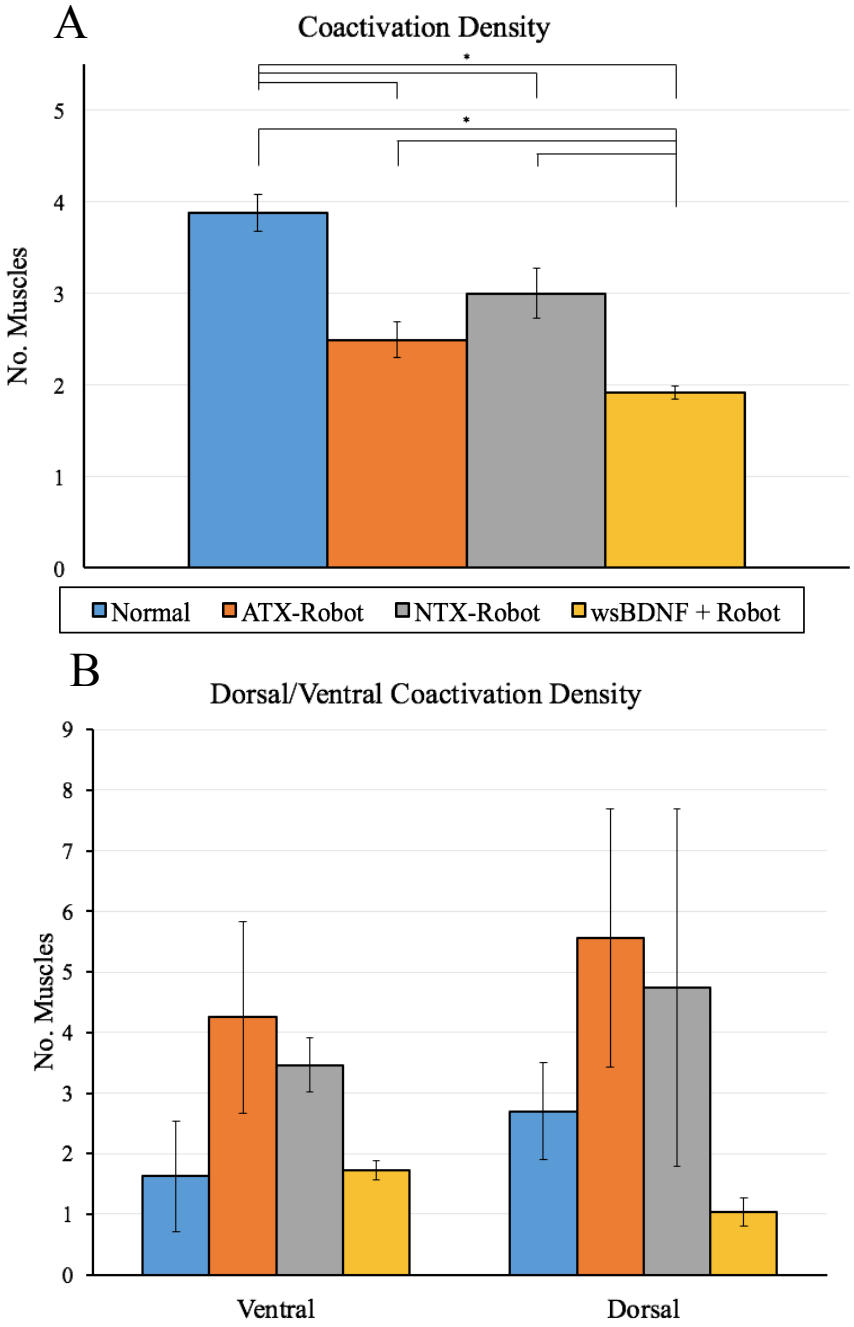


Figure 4-10. (A) Changes to the density of coactivation of trunk musculature as a result of injury and rehabilitation. Coactivation density is defined as the total number of muscles activated per site of trunk activation, determined by EMG analysis. There was no significant different between the NTX and ATX groups rehabilitated with robot. The normal group of intact rats had significantly more coactivation of trunk musculature per site than any of the other groups. The wsBDNF animals had significalty less coactivation density per site than the other groups. (*$p << 0.01$, $F(3,26) = 21.75$, one-way ANOVA with Tukey Kramer post hoc corrections). (B) Changes to the coactivation density of trunk musculature viewed by dorsal and ventral aspects of the animal. There were no significant different across groups. All data is presented as mean ± SEM.

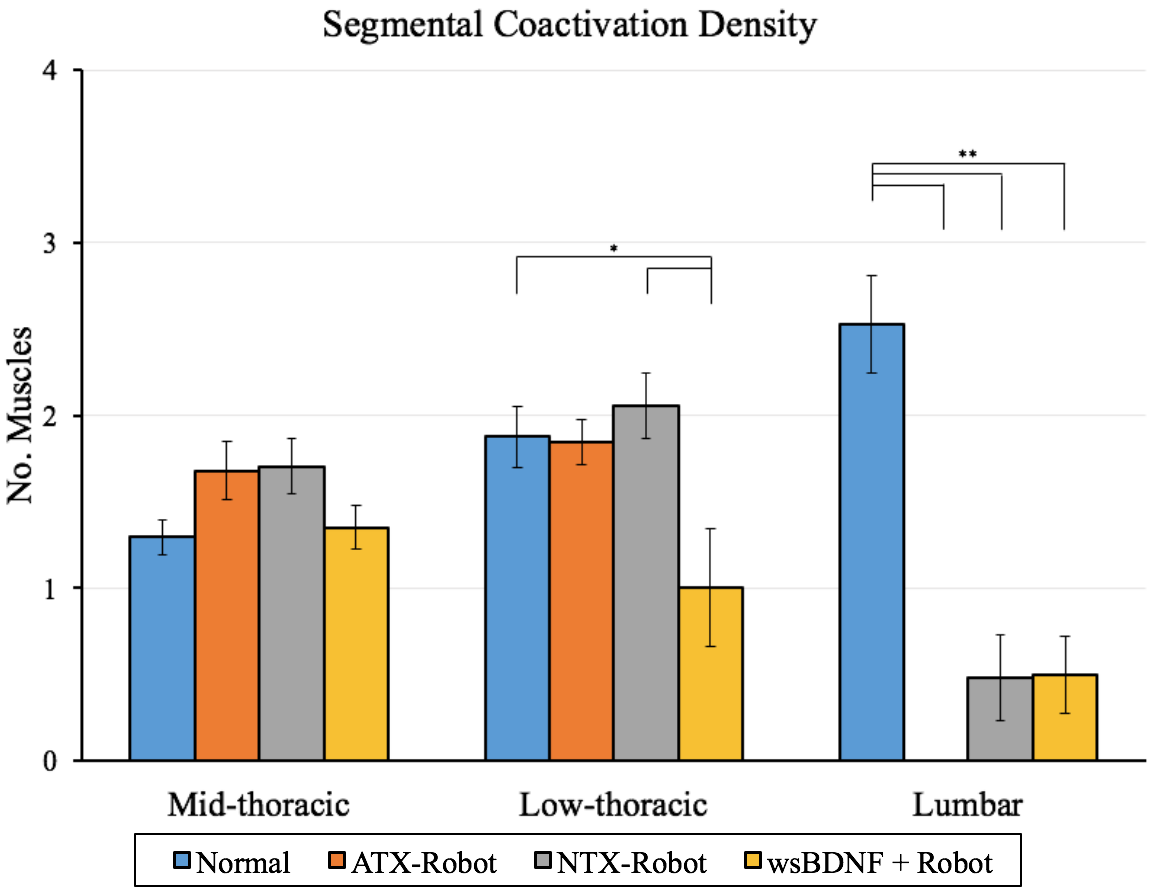


Figure 4-11. Changes in coactivation density per site of trunk activation, divided by levels above and below the site of transection. The mid-thoracic level is above the site of injury, and the other two levels are below. All data is presented as mean ± SEM. There was no significant different in coactivation density of sites in the mid-thoracic level. In the low-thoracic region, the wsBDNF rats had significantly less coactivation per site than both the intact animals and the NTX rats rehabilitated with robot. At the lumbar region, intact animals had significalty more coactivation per site than all of the other groups of animals. (*$p = 0.012$, $F(3,26) = 4.44$, one-way ANOVA with Tukey Kramer post hoc corrections; ** $p << 0.01$, $F(3,26) = 27.87$, one-way ANOVA with Tukey Kramer post hoc corrections).

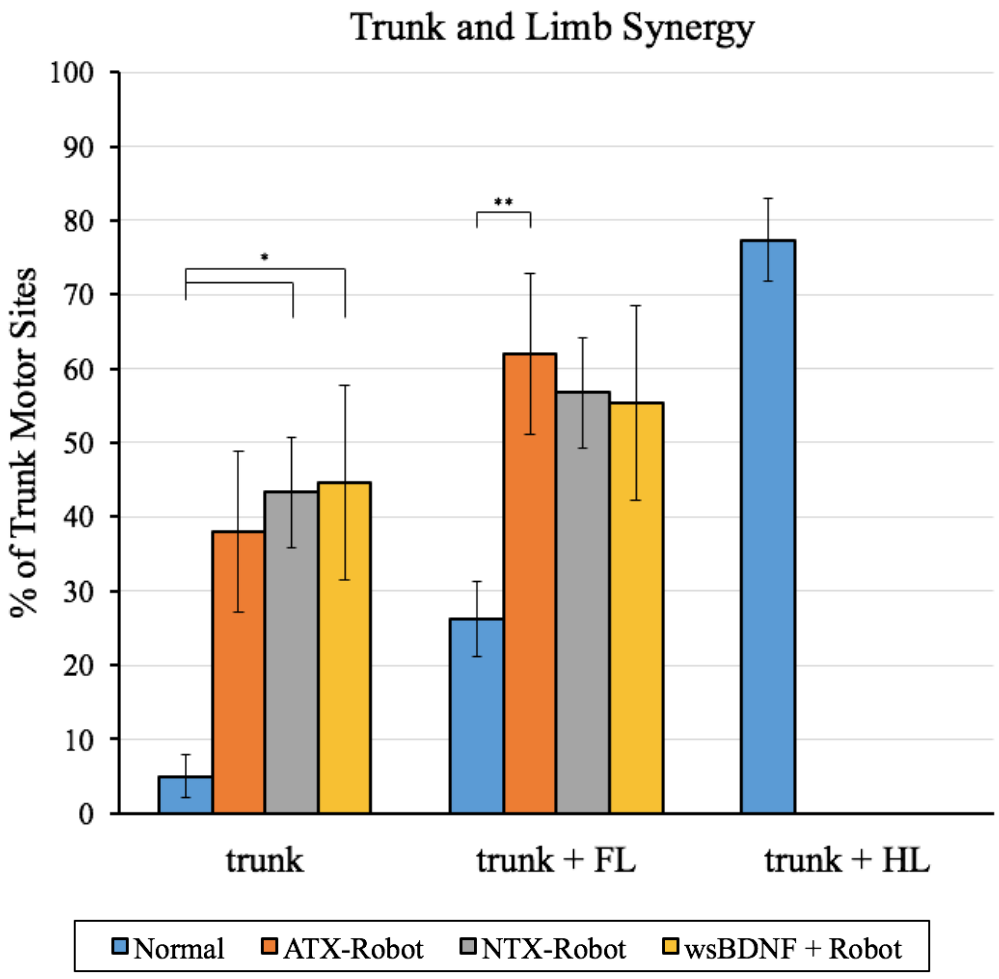


Figure 4-12. Changes to the coactivations of trunk musculature with the limbs, as a result of injury and rehabilitation. Responses in the musculature as a result of ICMS in the cortex was divided into three categories: trunk only, trunk and forelimb (FL) responses, and trunk and hindlimb (HL) responses. Predictably, there were no trunk and HL responses in any of the transected animals. There was no statistical test done in that case. Intact rats had significantly less trunk only responses than the NTX animals trained with robot and the wsBDNF animals. (* $p = 0.0106$, $F(3,26) = 4.57$, one-way ANOVA with Tukey Kramer post hoc corrections). In addition, intact rats had significantly less trunk and forelimb responses than the ATX animals trained with robot assistance (** $p = 0.0358$, $F(3,26) = 3.31$, one-way ANOVA with Tukey Kramer post hoc corrections). All data is presented as mean ± SEM).

D. Discussion

In this chapter, we examined the changes in the trunk motor cortex in response to SCI and active, stepping rehabilitation in the ATX model to understand the extent of reorganization that occurs with active stepping and robot assistance. There have been many studies investigating how complete SCI itself leads to somatotopic reorganization of the motor cortex [141], [142], a phenomenon that has also been observed and studied in the somatosensory cortex, as well [143], [144]. Recently, there has been interest in understanding how the process of locomotor rehabilitation affects cortical reorganization. Previous work in our lab was the first to show and characterize changes in trunk motor cortex representation as a result of SCI in the adult rat model (ATX) who were rehabilitated without active stepping [148]. We have also shown the effects of active stepping rehabilitation on the trunk motor cortex of adult rats transected as neonates (NTX) [147]. Each of these paradigms of SCI have characteristic changes and effects on the trunk motor cortex, which will be discussed further later. However, there exists in a gap in our understanding of the role of active stepping rehabilitation in the ATX model, to which we believe our current study will provide insight. In this study, we describe the extent to which active stepping patterns in the ATX model affect the total trunk motor area in the cortex, as well as change the synergies and coactivation found within this area.

Our interest in the trunk motor cortex lies in its central role in our rehabilitation modality of robot-assistance during treadmill training. Previous studies in humans and in the quadrupedal models for locomotion have emphasized the importance of the trunk in gait stability, as well as movement [12], [14], [200]. In a healthy human or intact model, the neural networks for locomotion are managed by supraspinal control, in the brain,

which coordinates with spinal networks to provide activation of necessary musculature for movement. However, in SCI, there is a disruption of the normal architecture of the central nervous system, and descending cortical control can be interrupted – completely, in some cases. There is still intact neuronal circuitry below the site of injury that can be manipulated by external interventions – pharmacological [56], [161], [201] and epidural stimulation [40], [89], [156], for example – to produce rhythmic locomotor activity, and in some cases, robust stepping.

Nonetheless, this does not exclude the importance of the cortex in locomotion and rehabilitation in the context of movement with complete SCI. Previous studies have shown that the cortex is essential for postural stability and propulsion of movement after SCI [165], [202]. Indeed, in our lab's previous investigations into the role of the trunk motor cortex in SCI rehabilitation, we discovered that, in the NTX model, animals whose trunk motor cortices are lesioned prior to recovery to adulthood do not develop weight-supported stepping [140]. Moreover, when previously stepping NTX animals are lesioned in the trunk motor cortex they lose their ability to weight support step (Oza – unpublished work). In the ATX model where animals were passively rehabilitated without stepping, we discovered that the trunk motor cortex expanded into deafferented areas and developed new synergies with the forelimbs, significantly changing from normal topography. As a result, we believe that there is a crucial interplay between spinal networks and trunk motor cortex in motor control to allow for successful rehabilitation with weight-supported stepping in SCI. Therefore, understanding the role and changes in trunk motor cortex is an essential component to developing effective rehabilitation regimens in SCI.

As a result, we employ a unique trunk-based robotic rehabilitation treatment that we have shown to be effective in increasing overall locomotion patterns, as well as increased weight-supported stepping in the NTX model [31] and in the ATX model induced to step with robot-driven epidural stimulation [160]. In Chapter 3, we have also shown how our method of robotic intervention at the pelvis can significantly improve the ability of ATX animals induced to step with viral delivery of BDNF caudal to the injury. Training is crucial for locomotor recovery in the SCI model [18], [19], and our robot provides a framework for effective and significant rehabilitation in the rat model.

Therefore, in our current study, we attempted to fill in the gaps between the known literature in trunk motor cortical reorganization – NTX with active stepping and ATX with passive rehabilitation – by studying ATX animals that were rehabilitated with active stepping induced by BDNF and/or robot-driven epidural stimulation (wsBDNF animals). We chose animals from our previous chapters based on their ability to provide weight-supported stepping. We treated these animals with Adeno-associated viral delivery of brain-derived neurotrophic factor (BDNF) to promote functional recovery, as had been observed previously in cats [56] and in rats [71]. Although BDNF changes the balance between excitatory and inhibitory neurotransmission in the affected neuronal networks [70], it can also result in a partial, but highly significant collapse in function, as observed by Ziemlínska *et al*., Blits *et al*., and in Chapter 2. However, we discovered that we could prevent this collapse, and mitigate loss of function, by combining AAV5-BDNF treatment with robot-driven rehabilitation techniques, such as robot-driven epidural stimulation.

We chose ATX animals based on their functional recovery after rehabilitation. The locomotor function of the animals was characterized by robust right and left hindlimb alternations of large amplitude, significantly improved weight support as a result of training, and regular plantar foot placement during stepping. We did not discriminate between animals that had been treated with robot-driven epidural stimulation or without. As our therapies (AAV5-BDNF, robot assistance at the pelvis, and robot-driven epidural stimulation) act below the site of injury, we do not believe that there are significant separate effects on the cortex, as there is no ascending sensory information that is relayed to the brain. We also only selected animals that did not have collapses in function, or had decreases in ability, regardless of pathological collapse. It was important for us to have ATX animals that were rehabilitated fully, as they have developed new methods of walking, learning to integrate a neuronal isolated, but activated trunk and hindlimbs into their conscious forelimb locomotor patterns. As novel skill acquisition is associated with changes in representation in the cortex [150] – [152], [203], and in the spinal cord [204], we hypothesize that these rehabilitated animals will have the most significant changes in their trunk motor cortex.

D-1. Total Trunk Motor Cortex Area

To compare the trunk motor cortex amongst groups, we normalized the trunk data to a percentage of the nonfacial motor cortex. We selected sites that corresponded to forelimb, trunk, and neck movements in response to stimulus, excluding the hindlimb in our selection of nonfacial responses. This eliminated any bias as a result of hindlimb responses in the intact animals, which would artificially decrease the representation of the

trunk motor cortex as a percentage of the total motor cortex, as transected animals do not have hindlimb representation.

In the context of loss of motor output as a result of limb amputation, spinal cord injury, or peripheral nerve injuries, the motor cortex begins to reorganize by a predictable loss of motor representation corresponding to specific injury. Furthermore, adjacent motor areas enlarge and begin to encroach into these deafferented areas [145], which can have pathological sequelae, such as phantom limb sensation [124] or allodynia [205]. In the ATX model with passive rehabilitation, we demonstrated this phenomenon in the trunk motor cortex, where the overall total area of the trunk motor cortex significantly increased, as a percentage of the nonfacial motor cortex [148]. Though some expansion did occur in the NTX model with active rehabilitation, the difference was not significant. We discovered that similar to the NTX model, the total trunk motor cortices in our ATX animals rehabilitated with active stepping did not have significant expansion. It is of particular interest to us, considering that both the NTX and the wsBDNF animals had high locomotor function while trained on robot, characterized by weight-supported stepping.

To elucidate further the differences between the total trunk area in the groups of animals, we compared the total trunk motor cortex area based on the responses in the different segmental levels of the trunk musculature – above injury at the mid-thoracic level, and below injury at the low-thoracic and lumbar levels. Unlike the non-stepping ATX animals, wsBDNF animals had significantly more lumbar representation in the cortex, similar to the NTX model, which did not have a significant difference compared to the ATX and the intact animals, but still had lumbar responses. The presence of lumbar

representation in the ATX model allows us to speculate that the wsBDNF animals were able to integrate the induced activity of the trunk muscles and hindlimbs by BDNF into their natural locomotion, thereby enriching trunk representation in the cortex and recruiting trunk caudal to injury. Observing muscular responses below the site of injury in response to cortical stimulus is not uncommon – it has been observed in humans [206] and in rats [140], [147]. Both ventral and dorsal trunk musculature are mechanically coupled above and below the site of injury as a result of the anatomy of these muscles. In addition, there may be multi-segmental distribution of motor nerve pools, as they exit the spinal cord, allowing for control above the site of injury to muscles below (Udoekwere – unpublished). We also verified the completeness of transection at the time of sacrifice of the animal, as well as with histology, to exclude the possibility of an incomplete injury resulting in motor responses from stimulus. The presence of lumbar representation in both the NTX and wsBDNF groups further advances our belief that the cortex is an essential component to weight-supported stepping locomotion after SCI, although the extent to which this is the case is still unclear.

In observations of the wsBDNF animals, we find that that these animals have strong muscle tone in their trunk musculature, presumably as a result of activation by BDNF in the spinal networks. We believe this spinal, autonomous activation of the musculature provides a sturdy base and stability for locomotion in the wsBDNF model. When we examine the differences in total trunk motor area in the cortex according to the dorsal, ventral, and both dorsal and ventral musculature, we observe that the trunk motor cortex of the wsBDNF animals looks like that of the intact animals, and is significantly lower from both the passive ATX and the NTX model. The mean total trunk motor area

is also lower than that of the intact animals, though it is not significantly lower. This suggests to us that the interplay between the spinal networks and cortex has changed in these wsBDNF rats, due to the effects of BDNF. While the presence of cortical responses still suggests a role for cortical control in rehabilitation after SCI, we suspect the increased activation of spinal networks by BDNF has significantly changed the dynamics in the interplay of control of trunk, allowing for a larger role for spinal networks.

D-2. Changes to Coactivation Densities

The change in the dynamics of control is further supported by analyzing the change in coactivation densities among the different groups. We defined coactivation density in our previous studies of the trunk motor cortex [147], [148] as the total number of trunk sites activated per site in the trunk motor cortex as a result of stimulus. In the context of a normal, intact rat, coactivation density gives us a glimpse into how the trunk motor cortex coordinates trunk activation for locomotion. In the case of the intact animal, the coactivation density is significantly higher than those of all spinalized groups, suggesting a high level of organization of muscle groups in the coordination of movement, gait, and postural stability. This is lost in the spinalized animals, but particularly so in the wsBDNF group, which has a significantly lower mean coactivation density than all other groups. Earlier, we suggested that BDNF activates trunk musculature in the spinal networks, emphasizing spinal control over cortical control in rehabilitation post-SCI. In the successfully rehabilitated wsBDNF animals, these animals have adapted to the BDNF-associated trunk activation, integrating trunk and hindlimb movements into their movement patterns. The corresponding changes in the cortex suggests that these animals

have adapted successfully, which is consistent with previous studies in cortical reorganization following novel skill acquisition [150] – [152], [203]. When we examine the coactivation density differences further, by segmental levels above and below the site of injury, we discover that the main differences lie at the low-thoracic and lumbar musculature. This further bolsters our belief that the activation of trunk musculature due to BDNF is a key component to changing the nature of the relationship between spinal cord and cortex in locomotion in these animals.

D-3. Muscle Synergy

An interpretation of our findings that we must consider is what the relationship between the spinal networks and cortical control suggests in the context of muscle synergy and modularity. There are many different theories of how the cortex controls and integrates different muscle activations to provide seamless movement or accomplish a task [104], [207]. Some studies hypothesize that muscles create synergies with other complementary muscles to for movement. This allows for a "hierarchical control strategy" [105], wherein multiple simple synergies can be combined to perform a task. Based on previous work in our lab in the frog model [107], [108], we believe that muscle synergies are a relatively primitive means to coordinate movement, which have become integrated from evolutionarily conserved neural systems into more complex movements for more complex vertebrates [106]. In intact animals, we believe the motor cortex is responsible for activating and coordinating these synergies downstream in the spinal cord [208]. In the case of injury, however, these "primitive" synergies become isolated from supraspinal control, and thus, movements can be elicited separately [114], which is what we may be

observing in the wsBDNF animals where BDNF is activating these rudimentary synergies.

In the case of complete SCI, we observe there is an overall decrease in cortical control of these trunk muscle synergies, or an increase in fractionation of cortical control. In the case of the wsBDNF animals, this fractionation is even more significant. This is consistent with studies that have demonstrated that there are changes to and reorganization of modularity as a result of pathology, such as stroke [115]. Indeed, neural injury can change the ways that synergies interact with one another – by adding, merging, or fractionating synergy – or even stay the same [118]. Yet, our wsBDNF animals are able to produce weight-supported steps and have high function, compared to the passively rehabilitation ATX animals and the NTX animals. Thus, our present study provides valuable insight into how muscle synergies coded in the spinal network can be exploited for rehabilitation in the absence of direct cortical control. The fact that we still observe trunk representation – especially low-thoracic and lumbar muscles – suggest that the cortex is still essential to locomotor rehabilitation, but it may play a smaller role than before to modulate or fine-tune trunk musculature as the rat adapts and integrates an activated trunk and hindlimb movement into its newly acquired gait.

D-4. Displacement of Trunk Motor Cortex

We also observed that the different rehabilitation paradigms had significantly different effects on the displacement of the trunk motor cortex relative to the bregma line. In our previous study exploring passive rehabilitation in the ATX model, we observed that there was a significant rostral migration of the trunk motor cortex away from the bregma

landmark [148]. This was accompanied by increased coupling of forelimb and trunk muscles, as a result of the loss of hindlimb activity. This is consistent with previous studies that have observed and documented this phenomenon in humans with SCI [209]. This rostral shift was observed, but not as significant in the NTX model. Indeed, Oza *et al*. demonstrated that the trunk motor cortex in the NTX model exhibited more "normal topography" [147], with trunk and forelimb coupling not significantly different from the intact animals.

In the case of the wsBDNF animals, we find that the mean center of gravity in the y-direction is not significantly different from the intact animals, as well, but is significantly closer to bregma than the passively rehabilitated ATX model. Furthermore, though it is not significantly different from that of the NTX model, its mean is negative, which is closer to normal than the positive y- cog value for the NTX model. Giszter *et al*. [140] and Oza *et al*. (Oza – unpublished) have both shown the significance of this area of the trunk motor cortex in weight-supported stepping in the NTX model by lesioning this area and observing failure to obtain or the loss of WSS, respectively. This result in our wsBDNF animals further emphasizes not only the cortical control necessary for WSS after SCI, but also bolsters the importance of this specific area of the trunk motor cortex.

In addition, we also observed there were not significant differences to trunk and forelimb coupling in the wsBDNF animals from the intact groups of animals and from the passively rehabilitated ATX animals. Given that these animals behave and move differently from both the intact and ATX animals, this suggests to us that these wsBDNF animals have developed new ways of moving, similar to our earlier assertions that this is

a newly acquired skill. We now provide evidence at the cortical level that this may be the case.

The mechanisms of cortical reorganization and plasticity have been investigated in many different studies. The concept of neuroplasticity in the adult nervous system has been widely accepted. Though not as malleable as the neonatal nervous system, adult brains retain some plasticity that can be shaped and molded by training [210]. However, there is a time-dependent component related to training that is necessary to take advantage of this plasticity [211]. In the short-term, unmasking of connecting intracortical connections can take affect almost immediately as a result of injury [212]. Other processes, such as long-term potentiation [213] and cortical synaptogenesis [211] may take longer to affect change in the adult brain. Unfortunately, our current study cannot address this time component to the reorganization of the trunk motor cortex, as it does not discriminate between the short-term and long-term processes involved in the changes we observed. Further study into the timeframes of reorganization is an avenue of investigation that would be invaluable to understanding the differences between the NTX model and the wsBDNF model. It would also allow us to compare timeframes of recovery and timeframes of reorganization, which could provide insight into optimal rehabilitation regimens in adult SCI.

E. Conclusion

Our present work has shown how active rehabilitation with BDNF in the ATX model may change the topography and organization of the trunk motor cortex. In doing so, it also reveals the intricacies of the relationship between spinal networks and cortex in

control of the trunk for locomotion and gait stability. Further studies involving active rehabilitation using methods other than BDNF may shed more light into whether this change in the dynamics of that relationship is due to the mechanism of action of BDNF or is a result of adaptations in the ATX model. Also, we believe this study further bolsters our belief that muscle synergies and modules of simple, primitive movements are hardwired into the spinal cord, and can be accessed and manipulated at the cortical level in intact animals. From a clinical perspective, the results of this study may help us to understand better how rehabilitation can be used as an effective tool at the cortical level, to mitigate any pathology from cortical reorganization resulting from nerve injuries.

CHAPTER 5: SUMMARY

The work presented in this dissertation is the first investigation into how robotic technology may be used in combination with viral delivery of BDNF, in a rat transected completely as an adult. It uses novel techniques and rehabilitation paradigms unique to our laboratory to devise effective locomotor recovery in the complete SCI model. The overall goal of this work was to understand how biological therapies, such as exogenous neurotrophin delivery, and robotic therapies might interact – synergistically or antagonistically – in a rehabilitating animal, so as to employ or translate these methods into clinical applications. As a result of investigating these techniques, we also developed greater understanding and appreciation for the cortical and spinal cord neural mechanisms that underlie quadrupedal (and bipedal) locomotion.

In Specific Aim I, we successfully attempted to adapt our unique robot-assisted treadmill training technique to the ATX model of SCI to increase overall locomotor behavior, body weight support, and weight-supported stepping. Previous work in our lab has shown this to be an effective means of rehabilitation in the NTX model, where robot assistance can help an animal to discover the neural control to integrate already existing hindlimb activity – regardless of how robust – into natural quadrupedal locomotion. Outside of the neonatal model, this has been shown to work in only when ATX animals are induced to step with robot-driven epidural stimulation. The work in Aim I showed that exogenous BDNF delivery can be used to induce stepping in the ATX model, and those stepping patterns can be enhanced by the use of robot interaction at the pelvis. The work in this aim also corroborated the existence of a partial, but highly significant, collapse in function observed previously in studies using BDNF to induce stepping in

ATX rats. Collapse not only hinders locomotor recovery, but has serious co-morbid health effects, such as recurrent bladder infections, skin lesions, and muscle wasting. Although collapse did occur in our animals trained with robot assistance, our work showed that robotic interventions can significantly improve functionality before loss of function, and may indeed help to maintain a high level of function, after collapse. We were also able to characterize specifically how collapse in function affects locomotor behavior, body weight support, and weight-supported stepping patterns.

To further understand the nature of exogenous BDNF's interactions with a rehabilitating spinal cord, we attempted to combine viral delivery of BDNF with robot-driven epidural stimulation, in Specific Aim IIA. Statistical analysis of the experimental data showed that ATX animals treated with robot-driven epidural stimulation are able to significantly improve hindlimb function. Their recovery is characterized by robust alternations of the hindlimbs, body weight support, and plantar foot placement during stepping. Based on our battery of outcome measures, however, the improvement observed in these animals is not significantly different from that observed in the animals of Aim I, who received exogenous BDNF and robot-assistance only. However, of particular interest to us is the role epidural stimulation played in preventing the collapse of function observed with exogenous BDNF use in the locomotor rehabilitation. This is the first occurrence of this collapse being prevented, in the literature, and we believe this to be a highly significant discovery. This has meaningful clinical applications, as we apply epidural stimulation to reduce the co-morbid effects of exogenous BDNF use to provide better general health in our treated animals.

Furthermore, in Specific Aim IIB, we discovered that exogenous BDNF has a significant effect on the parameters of epidural stimulation, particularly stimulus intensity. ATX animals treated with AAV5-BDNF had significantly lower thresholds to elicit hindlimb motor activity, as compared to control animals that received a sham virus, regardless of whether the BDNF-treated animals received robot-driven or conventional epidural stimulation. This was observed on the first day of training (ten days after injury and injection) and persisted throughout training. Indeed, the significance in the differences between groups increased as a function of time. This finding is not entirely surprising, as BDNF induces significant structural changes to the excitability of the spinal cord and motor neural networks.

Finally, in Aim III, we used intracortical microstimulation to further understand the effects of active rehabilitation on the trunk motor cortex in the ATX rat. Based on the literature and previous studies in our lab, our weight-supporting, stepping ATX animals filled a gap in our knowledge of how rehabilitation can affect cortical representation. We discovered that active stepping can significantly reduce trunk expansion observed in passively rehabilitated ATX animals. This occurs mostly at the level of the mid-thoracic trunk region, where there is less expansion of this musculature into adjacent areas. This was coupled with a reduced rostral migration of the trunk motor cortex overall.

Furthermore, our results from Aim III also give us further insight into the relationship between the motor cortex and spinal cord networks in control of trunk musculature in the context of locomotion. By delivering exogenous BDNF to the spinal cord after injury to induce stepping, we observed increased fractionation of trunk control in the cortex, as seen by significantly reduced coactivation density of sites of trunk

muscle activation in the weight-supporting ATX animals. However, the existence of trunk activation sites in the cortices of these animals strongly support the role of cortex in locomotion, though BDNF administration has changed the fundamental relationship between the spinal neural networks and supraspinal control.

In this way, our study also further elucidates our understanding of muscle synergies and motor primitives in locomotion. Loss of direct supraspinal control in this case creates an isolated system wherein low-level, "primitive" synergies may be expressed, and indeed, required to elicit trunk and hindlimb locomotor behavior. Exogenous BDNF may serve to "amplify" these synergies, and observe the basic units of trunk and hindlimb activation required for locomotion. Further investigations using EMG and spinal neural recordings may deepen our understanding of the results in this aim.

By developing a new and innovative rehabilitation scheme in the treatment of complete SCI in the adult animal, we have expanded our knowledge of the relationship between the cortical and spinal systems involved in locomotion, though we are very far from a complete understanding. From a clinical perspective, we have also investigated novel means to make current therapies more efficient and effective. In addition, we have shown that combining therapies can not only improve recovery, but serve to mitigate pathological sequelae derived from individual treatments. We believe this work provides a foundation for much further investigation into combination therapies for locomotor rehabilitation of SCI.

BIBLIOGRAPHY

[1] J. J. E. Adriaansen, F. W. A. van Asbeck, E. Lindeman, L. H. V. van der Woude, S. de Groot, and M. W. M. Post, "Secondary health conditions in persons with a spinal cord injury for at least 10 years: design of a comprehensive long-term cross-sectional study," *Disabil. Rehabil.*, vol. 35, no. 13, pp. 1104–1110, Jun. 2013.

[2] J. Adriaansen, M. Post, S. Groot, F. Asbeck, J. Stolwijk-Swüste, M. Tepper, and E. Lindeman, "Secondary health conditions in persons with spinal cord injury: A longitudinal study from one to five years post-discharge," *J. Rehabil. Med.*, vol. 45, no. 10, pp. 1016–1022, 2013.

[3] W. T. Phillips, B. J. Kiratli, M. Sarkarati, G. Weraarchakul, J. Myers, B. A. Franklin, I. Parkash, and V. Froelicher, "Effect of spinal cord injury on the heart and cardiovascular fitness," *Curr. Probl. Cardiol.*, vol. 23, no. 11, pp. 641–716, 1998.

[4] K. J. Suda, U. C. Patel, R. Sabzwari, L. Cao, S. Ramanathan, J. N. Hill, and C. T. Evans, "Bacterial susceptibility patterns in patients with spinal cord injury and disorder (SCI/D): an opportunity for customized stewardship tools," *Spinal Cord*, Mar. 2016.

[5] S. Grillner, "Locomotion in vertebrates: central mechanisms and reflex interaction," *Physiol Rev*, vol. 55, no. 2, pp. 247–304, Apr. 1975.

[6] H. Forssberg, S. Grillner, and S. Rossignol, "Phase dependent reflex reversal during walking in chronic spinal cats," 1975.

[7] H. Forssberg, S. Grillner, and S. Rossignol, "Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion," *Brain Res.*, vol. 132, no. 1, pp. 121–139, 1977.

[8] H. Forssberg, "Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion," *J. Neurophysiol.*, vol. 42, no. 4, 1979.

[9] K. TAKAHASHI, H. NISHIMURA, N. WADA, and M. TOKURIKI, "Rhythmical Discharges Recorded from Cervical and Trunk Muscle Nerves Produced by Stimulation of Mesencephalic Locomotor Region (MLR) in the Decerebrated Cat.," *J. Vet. Med. Sci.*, vol. 59, no. 10, pp. 951–952, 1997.

[10] M. J. Comerford and S. L. Mottram, "Movement and stability dysfunction – contemporary developments," *Man. Ther.*, vol. 6, no. 1, pp. 15–26, 2001.

[11] G. G. Gregersen and D. B. Lucas, "An in vivo study of the axial rotation of the human thoracolumbar spine.," *J. Bone Joint Surg. Am.*, vol. 49, no. 2, pp. 247–62, Mar. 1967.

[12] C. Anders, H. Wagner, C. Puta, R. Grassme, A. Petrovitch, and H.-C. Scholle, "Trunk muscle activation patterns during walking at different speeds.," *J. Electromyogr. Kinesiol.*, vol. 17, no. 2, pp. 245–52, Apr. 2007.

[13] A. Gramsbergen, H. . Geisler, H. Taekema, and L. . van Eykern, "The activation of back muscles during locomotion in the developing rat," *Dev. Brain Res.*, vol. 112, no. 2, pp. 217–228, 1999.

[14] P. S. Sung, K.-J. Lee, and W.-H. Park, "Coordination of trunk and pelvis in young and elderly individuals during axial trunk rotation.," *Gait Posture*, vol. 36, no. 2, pp. 330–1, Jun. 2012.

[15] M. Falgairolle, M. de Seze, L. Juvin, D. Morin, and J.-R. Cazalets, "Coordinated network functioning in the spinal cord: An evolutionary perspective," *J. Physiol.*, vol. 100, no. 5, pp. 304–316, 2006.

[16] A. L. Behrman, P. M. Nair, M. G. Bowden, R. C. Dauser, B. R. Herget, J. B. Martin, C. P. Phadke, P. J. Reier, C. R. Senesac, F. J. Thompson, and D. R. Howland, "Locomotor Training Restores Walking in a Nonambulatory Child With Chronic, Severe, Incomplete Cervical Spinal Cord Injury," *Phys. Ther.*, vol. 88, no. 5, pp. 580–590, May 2008.

[17] E. J. Fox, N. J. Tester, S. A. Kautz, D. R. Howland, D. J. Clark, C. Garvan, A. L. Behrman, A. S. I. Association, A. Behrman, S. Harkema, A. Behrman, S. Harkema, A. Behrman, P. Nair, M. Bowden, R. Dauser, B. Herget, J. Martin, C. Phadke, P. Reier, C. Senesac, F. Thompson, D. Howland, J. Beres-Jones, S. Harkema, A. Berkowitz, E. Bizzi, V. Cheung, A. d'Avella, P. Saltiel, M. Tresch, E. Bizzi, F. Mussa-Ivaldi, S. Giszter, E. Bizzi, M. Tresch, P. Saltiel, A. d'Avella, M. Bowden, D. Clark, S. Kautz, B. Bregman, M. Goldberger, B. Calancie, B. Needham-Shropshire, P. Jacobs, K. Willer, G. Zych, B. Green, V. Cheung, A. Turolla, M. Agostini, S. Silvoni, C. Bennis, P. Kasi, S. Paganoni, P. Bonato, E. Bizzi, V. Cheung, A. d'Avella, E. Bizzi, V. Cheung, A. d'Avella, M. Tresch, E. Bizzi, D. Clark, L. Ting, F. Zajac, R. Neptune, S. Kautz, J. Cohen, D. Damiano, S. DeJong, A. d'Avella, P. Saltiel, E. Bizzi, V. Dietz, V. Dietz, S. Harkema, V. Dietz, R. Muller, G. Colombo, M. Dimitrijevic, Y. Gerasimenko, M. Pinter, B. Dobkin, S. Harkema, P. Requejo, V. Edgerton, A. Domingo, G. Sawicki, D. Ferris, N. Dominici, Y. Ivanenko, G. Cappellini, A. d'Avella, V. Mondi, M. Cicchese, A. Fabiano, T. Silei, A. Di Paolo, C. Giannini, R. Poppele, F. Lacquaniti, G. Earhart, P. Stein, V. Edgerton, N. Tillakaratne, A. Bigbee, R. de Leon, R. Roy, D. Ferris, K. Gordon, J. Beres-Jones, S. Harkema, H. Forssberg, S. Grillner, J. Halbertsma, S. Rossignol, E. Fox, S. Kautz, K. Day, S. Suter, D. Clark, D. Howland, A. Behrman, E. Fox, N. Tester, C. Phadke, P. Nair, C. Senesac, D. Howland, A. Behrman, R. Gentner, J. Classen, L. Gizzi, J. Nielsen, F. Felici, Y. Ivanenko, D. Farina, L. Gizzi, J. Nielsen, F. Felici, J. Moreno, J. Pons, D. Farina, S. Grillner, S. Grillner, P. Wallen, K. Saitoh, A. Kozlov, B. Robertson, S. Grillner, P. Zangger, S. Harkema, S. Harkema, S. Hurley, U. Patel, P. Requejo, B. Dobkin, V. Edgerton, C. Hart, S. Giszter, C. Hart, S. Giszter, H. Hayes, L. VanHiel, S. Chvatal, L. Ting, K.

Tansey, R. Trumbower, R. Holdefer, L. Miller, Y. Ivanenko, G. Cappellini, N. Dominici, R. Poppele, F. Lacquaniti, Y. Ivanenko, R. Grasso, M. Zago, M. Molinari, G. Scivoletto, V. Castellano, V. Macellari, F. Lacquaniti, Y. Ivanenko, R. Poppele, F. Lacquaniti, M. Kadaba, H. Ramakrishnan, M. Wootten, J. Gainey, G. Gorton, G. Cochran, W. Kargo, S. Giszter, W. Kargo, S. Giszter, A. Kozlov, M. Huss, A. Lansner, J. Kotaleski, S. Grillner, T. Lamb, J. Yang, D. Lee, H. Seung, M. Maegele, S. Muller, A. Wernig, V. Edgerton, S. Harkema, E. Marder, D. Bucher, C. McGowan, R. Neptune, D. Clark, S. Kautz, L. Mortin, P. Stein, F. Mussa-Ivaldi, S. Giszter, E. Bizzi, S. Nadeau, G. Jacquemin, C. Fournier, Y. Lamarre, S. Rossignol, R. Neptune, D. Clark, S. Kautz, S. Patrick, J. Noah, J. Yang, T. Petersen, M. Kliim-Due, S. Farmer, J. Nielsen, C. Phadke, S. Wu, F. Thompson, A. Behrman, J. Roh, V. Cheung, E. Bizzi, S. Rossignol, R. Dubuc, J. Gossard, S. Safavynia, G. Torres-Oviedo, L. Ting, R. Stoloff, E. Zehr, D. Ferris, D. Sutherland, R. Olshen, L. Cooper, S. Woo, L. Ting, L. Ting, J. Macpherson, L. Ting, . McKay, G. Torres-Oviedo, L. Ting, G. Torres-Oviedo, J. Macpherson, L. Ting, M. Tresch, P. Saltiel, E. Bizzi, M. Visintin, H. Barbeau, M. Vitale, J. Goss, H. Matsumoto, D. Roye, L. Vogel, J. Lubicky, T. Wannier, C. Bastiaanse, G. Colombo, V. Dietz, D. Winter, A. Fuglevand, S. Archer, E. Zehr, E. Zehr, J. Balter, D. Ferris, S. Hundza, P. Loadman, and R. Stoloff, “Modular control of varied locomotor tasks in children with incomplete spinal cord injuries.,” *J. Neurophysiol.*, vol. 110, no. 6, pp. 1415–25, Sep. 2013.

[18] J. A. Hogson, R. R. Roy, R. D. de Leon, B. H. Dobkin, and V. R. Edgerton, “Can the mammalian lumbar spinal cord learn a motor task?,” *Med Sci Sport. Exc.*, vol. 12, pp. 1491–7, 1994.

[19] R. M. Ichiyama, G. Courtine, Y. P. Gerasimenko, G. J. Yang, R. van den Brand, I. A. Lavrov, H. Zhong, R. R. Roy, and V. R. Edgerton, “Step training reinforces specific spinal locomotor circuitry in adult spinal rats.,” *J. Neurosci.*, vol. 28, no. 29, pp. 7370–5, Jul. 2008.

[20] V. R. Edgerton, N. J. K. Tillakaratne, A. J. Bigbee, R. D. de Leon, and R. R. Roy, “Plasticity of the spinal neural circuitry after injury.,” *Annu. Rev. Neurosci.*, vol. 27, pp. 145–167, 2004.

[21] V. R. Edgerton, R. D. de Leon, S. J. Harkema, J. A. Hodgson, N. London, D. J. Reinkensmeyer, R. R. Roy, R. J. Talmadge, N. J. Tillakaratne, W. Timoszyk, and A. Tobin, “Retraining the injured spinal cord,” *J. Physiol.*, vol. 533, no. 1, pp. 15–22, May 2001.

[22] M.-P. Cote and J.-P. Gossard, “Step Training-Dependent Plasticity in Spinal Cutaneous Pathways,” *J. Neurosci.*, vol. 24, no. 50, pp. 11317–11327, Dec. 2004.

[23] J. C. Petruska, R. M. Ichiyama, D. L. Jindrich, E. D. Crown, K. E. Tansey, R. R. Roy, V. R. Edgerton, and L. M. Mendell, “Changes in Motoneuron Properties and Synaptic Inputs Related to Step Training after Spinal Cord Transection in Rats,” *J. Neurosci.*, vol. 27, no. 16, pp. 4460–4471, Apr. 2007.

[24] R. D. De Leon, J. A. Hodgson, R. R. Roy, and V. R. Edgerton, “Full weight-bearing hindlimb standing following stand training in the adult spinal cat.,” *J. Neurophysiol.*, vol. 80, no. 1, pp. 83–91, Jul. 1998.

[25] V. R. Edgerton and R. R. Roy, “Robotic training and spinal cord plasticity,” *Brain Res. Bull.*, vol. 78, no. 1, pp. 4–12, 2009.

[26] V. Dietz and R. Müller, “Degradation of neuronal function following a spinal cord injury: mechanisms and countermeasures.,” *Brain*, vol. 127, no. Pt 10, pp. 2221–31, Oct. 2004.

[27] L. L. Cai, A. J. Fong, C. K. Otoshi, Y. Liang, J. W. Burdick, R. R. Roy, and V. R. Edgerton, “Implications of Assist-As-Needed Robotic Step Training after a Complete Spinal Cord Injury on Intrinsic Strategies of Motor Learning,” *J. Neurosci.*, vol. 26, no. 41, pp. 10564–10568, Oct. 2006.

[28] D. Aoyagi, W. E. Ichinose, S. J. Harkema, D. J. Reinkensmeyer, and J. E. Bobrow, “A Robot and Control Algorithm That Can Synchronously Assist in Naturalistic Motion During Body-Weight-Supported Gait Training Following Neurologic Injury,” *IEEE Trans. Neural Syst. Rehabil. Eng.*, vol. 15, no. 3, pp. 387–400, Sep. 2007.

[29] H. I. Krebs and N. Hogan, “Robotic Therapy,” *Am. J. Phys. Med. Rehabil.*, vol. 91, no. 11 Suppl 3, pp. S290–S297, 2012.

[30] U. I. Udoekwere, A. Ramakrishnan, L. Mbi, and S. F. Giszter, “Robot application of elastic fields to the Pelvis of the spinal transected rat: A tool for detailed assessment and rehabilitation,” *Annu. Int. Conf. IEEE Eng. Med. Biol. - Proc.*, pp. 3684–3687, 2006.

[31] U. I. Udoekwere, C. S. Oza, and S. F. Giszter, “A pelvic implant orthosis in rodents, for spinal cord injury rehabilitation, and for brain machine interface research: Construction, surgical implantation and validation,” *J. Neurosci. Methods*, vol. 222, pp. 199–206, 2014.

[32] A. J. Fong, L. L. Cai, C. K. Otoshi, D. J. Reinkensmeyer, J. W. Burdick, R. R. Roy, and V. R. Edgerton, “Spinal Cord-Transected Mice Learn to Step in Response to Quipazine Treatment and Robotic Training,” *J. Neurosci.*, vol. 25, no. 50, pp. 11738–11747, Dec. 2005.

[33] D. J. Stelzner, W. B. Ershler, and E. D. Weber, “Effects of spinal transection in neonatal and weanling rats: Survival of function,” *Exp. Neurol.*, vol. 46, no. 1, pp. 156–177, Jan. 1975.

[34] S. F. Giszter, M. R. Davies, and V. Graziani, “Coordination strategies for limb forces during weight-bearing locomotion in normal rats, and in rats spinalized as neonates.,” *Exp. brain Res.*, vol. 190, no. 1, pp. 53–69, Sep. 2008.

[35] F. H. Hsieh and S. F. Giszter, “Robot-driven spinal epidural stimulation compared with conventional stimulation in adult spinalized rats,” *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS*, no. October, pp. 5807–5810, 2011.

[36] P. a. Guertin and I. Steuer, “Key central pattern generators of the spinal cord,” *J. Neurosci. Res.*, vol. 87, no. 11, pp. 2399–2405, 2009.

[37] K. G. Pearson and S. Rossignol, “Fictive motor patterns in chronic spinal cats.,” *J. Neurophysiol.*, vol. 66, no. 6, pp. 1874–87, Dec. 1991.

[38] D. A. McCrea and I. A. Rybak, “Organization of mammalian locomotor rhythm and pattern generation,” *Brain Res. Rev.*, vol. 57, no. 1, pp. 134–146, 2008.

[39] T. Hoellinger, M. Petieau, M. Duvinage, T. Castermans, K. Seetharaman, A.-M. Cebolla, A. Bengoetxea, Y. Ivanenko, B. Dan, and G. Cheron, “Biological oscillations for learning walking coordination: dynamic recurrent neural network functionally models physiological central pattern generator,” *Front. Comput. Neurosci.*, vol. 7, 2013.

[40] Y. DIMITRIJEVIC, MILAN R.GERASIMENKO and M. M. PINTER, “Evidence for a Spinal Central Pattern Generator in Humans,” *Ann. New York Acad. Sci.*, vol. 860, no. 1, pp. 360–376, 1998.

[41] H. Nishimaru, H. Takizawa, and N. Kudo, “5-Hydroxytryptamine-induced locomotor rhythm in the neonatal mouse spinal cord in vitro,” 2000.

[42] H. Nishimaru and N. Kudo, “Formation of the central pattern generator for locomotion in the rat and mouse,” *Brain Res. Bull.*, vol. 53, no. 5, pp. 661–669, 2000.

[43] N. Dominici, Y. P. Ivanenko, G. Cappellini, a. d’Avella, V. Mondi, M. Cicchese, a. Fabiano, T. Silei, a. Di Paolo, C. Giannini, R. E. Poppele, and F. Lacquaniti, “Locomotor Primitives in Newborn Babies and Their Development,” *Science (80-.).*, vol. 334, no. 6058, pp. 997–999, 2011.

[44] M. Stanojevic, A. Kurjak, A. Salihagić-Kadić, O. Vasilj, B. Miskovic, A. N. Shaddad, B. Ahmed, and S. Tomasović, “Neurobehavioral continuity from fetus to neonate,” *J. Perinat. Med.*, vol. 39, no. 2, Jan. 2011.

[45] F. Delcomyn, “Neural basis of rhythmic behavior in animals.,” *Science*, vol. 210, no. 4469, pp. 492–8, Oct. 1980.

[46] F. Bretzner and T. Drew, “Contribution of the motor cortex to the structure and the timing of hindlimb locomotion in the cat: a microstimulation study.,” *J. Neurophysiol.*, vol. 94, no. 1, pp. 657–72, Jul. 2005.

[47] E. Jankowska, M. G. M. Jukes, S. Lund, and A. Lundberg, “The Effect of DOPA on the Spinal Cord 6. Half-centre organization of interneurones transmitting

effects from the flexor reflex afferents," *Acta Physiol. Scand.*, vol. 70, no. 3–4, pp. 389–402, Jul. 1967.

[48] E. Jankowska, M. G. M. Jukes, S. Lund, and A. Lundberg, "The Effect of DOPA on the Spinal Cord 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors," *Acta Physiol. Scand.*, vol. 70, no. 3–4, pp. 369–388, Jul. 1967.

[49] D. Feraboli-Lohnherr, J. Y. Barthe, and D. Orsal, "Serotonin-induced activation of the network for locomotion in adult spinal rats," *J. Neurosci. Res.*, vol. 55, no. 1, pp. 87–98, 1999.

[50] S. Hjorth and S. B. Auerbach, "Autoreceptors remain functional after prolonged treatment with a serotonin reuptake inhibitor," *Brain Res.*, vol. 835, no. 2, pp. 224–228, 1999.

[51] M. Antri, J.-Y. Barthe, C. Mouffle, and D. Orsal, "Long-lasting recovery of locomotor function in chronic spinal rat following chronic combined pharmacological stimulation of serotonergic receptors with 8-OHDPAT and quipazine.," *Neurosci. Lett.*, vol. 384, no. 1–2, pp. 162–7, Jan. 2005.

[52] P. J. Harvey, X. Li, Y. Li, and D. J. Bennett, "5-HT2 receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury.," *J. Neurophysiol.*, vol. 96, no. 3, pp. 1158–70, Sep. 2006.

[53] A. R. Harvey, S. J. Lovett, B. T. Majda, J. H. Yoon, L. P. G. Wheeler, and S. I. Hodgetts, "Neurotrophic factors for spinal cord repair: Which, where, how and when to apply, and for what period of time?," *Brain Res.*, vol. 1619, pp. 36–71, Sep. 2015.

[54] D. R. Howland, B. S. Bregman, A. Tessler, and M. E. Goldberger, "Transplants enhance locomotion in neonatal kittens whose spinal cords are transected: a behavioral and anatomical study.," *Exp. Neurol.*, vol. 135, no. 2, pp. 123–45, Oct. 1995.

[55] T. Mitsui, I. Fischer, J. S. Shumsky, and M. Murray, "Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats.," *Exp. Neurol.*, vol. 194, no. 2, pp. 410–31, Aug. 2005.

[56] V. S. Boyce, M. Tumolo, I. Fischer, M. Murray, and M. a Lemay, "Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats.," *J. Neurophysiol.*, vol. 98, no. 4, pp. 1988–1996, 2007.

[57] L. Schnell, R. Schneider, R. Kolbeck, Y. A. Barde, and M. E. Schwab, "Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion.," *Nature*, vol. 367, no. 6459, pp. 170–3, Jan. 1994.

[58] L. N. Novikova, L. N. Novikov, and J.-O. Kellerth, “Differential effects of neurotrophins on neuronal survival and axonal regeneration after spinal cord injury in adult rats.,” *J. Comp. Neurol.*, vol. 452, no. 3, pp. 255–63, Oct. 2002.

[59] B. S. Seebach, V. Arvanov, and L. M. Mendell, “Effects of BDNF and NT-3 on Development of Ia/Motoneuron Functional Connectivity in Neonatal Rats,” *J Neurophysiol*, vol. 81, no. 5, pp. 2398–2405, May 1999.

[60] S. Cohen-Cory, A. H. Kidane, N. J. Shirkey, and S. Marshak, “Brain-derived neurotrophic factor and the development of structural neuronal connectivity.,” *Dev. Neurobiol.*, vol. 70, no. 5, pp. 271–88, Apr. 2010.

[61] J. M. Conner, J. C. Lauterborn, Q. Yan, C. M. Gall, and S. Varon, “Distribution of Brain-Derived Neurotrophic Factor (BDNF) Protein and mRNA in the Normal Adult Rat CNS: Evidence for Anterograde Axonal Transport,” *J. Neurosci.*, vol. 17, no. 7, pp. 2295–2313, Apr. 1997.

[62] F. Gomez-Pinilla, Z. Ying, P. Opazo, R. R. Roy, and V. R. Edgerton, “Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle,” *Eur. J. Neurosci.*, vol. 13, no. 6, pp. 1078–1084, Mar. 2001.

[63] Z. Ying, R. R. Roy, V. R. Edgerton, and F. Gómez-Pinilla, “Voluntary exercise increases neurotrophin-3 and its receptor TrkC in the spinal cord,” *Brain Res.*, vol. 987, no. 1, pp. 93–99, Oct. 2003.

[64] O. Ikeda, M. Murakami, and H. Ino, “Acute up-regulation of brain-derived neurotrophic factor expression resulting from experimentally induced injury in the rat spinal cord,” *Acta …*, vol. 102, no. 3, pp. 239–245, 2001.

[65] R. Gulino, S. A. Lombardo, A. Casabona, G. Leanza, and V. Perciavalle, “Levels of brain-derived neurotrophic factor and neurotrophin-4 in lumbar motoneurons after low-thoracic spinal cord hemisection,” *Brain Res.*, vol. 1013, no. 2, pp. 174–181, 2004.

[66] D. J. Donnelly and P. G. Popovich, “Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury,” *Exp. Neurol.*, vol. 209, no. 2, pp. 378–388, 2008.

[67] D. Soppet, E. Escandon, J. Maragos, D. S. Middlemas, S. W. Reid, J. Blair, L. E. Burton, B. R. Stanton, D. R. Kaplan, T. Hunter, K. Nikolics, and L. F. Parada, “The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor.,” *Cell*, vol. 65, no. 5, pp. 895–903, 1991.

[68] W. D. Snider, “Functions of the neurotrophins during nervous system development: What the knockouts are teaching us,” *Cell*, vol. 77, no. 5, pp. 627–638, 1994.

[69] D. J. Liebl, W. Huang, W. Young, and L. F. Parada, “Regulation of Trk Receptors Following Contusion of the Rat Spinal Cord,” *Exp. Neurol.*, vol. 167, no. 1, pp. 15–26, 2001.

[70] E. Ziemlińska, S. Kügler, M. Schachner, I. Wewiór, J. Czarkowska-Bauch, and M. Skup, “Overexpression of BDNF increases excitability of the lumbar spinal network and leads to robust early locomotor recovery in completely spinalized rats,” *PLoS One*, vol. 9, no. 2, 2014.

[71] V. S. Boyce, J. Park, F. H. Gage, and L. M. Mendell, “Differential effects of brain-derived neurotrophic factor and neurotrophin-3 on hindlimb function in paraplegic rats,” *Eur. J. Neurosci.*, vol. 35, no. 2, pp. 221–232, 2012.

[72] B. Blits, M. Oudega, G. . Boer, M. Bartlett Bunge, and J. Verhaagen, “Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function,” *Neuroscience*, vol. 118, no. 1, pp. 271–281, Apr. 2003.

[73] P. Lu, A. Blesch, and M. H. Tuszynski, “Neurotrophism without neurotropism: BDNF promotes survival but not growth of lesioned corticospinal neurons,” *J. Comp. Neurol.*, vol. 436, no. 4, pp. 456–470, Aug. 2001.

[74] L. N. Novikova, L. N. Novikov, and J.-O. Kellerth, “Survival effects of BDNF and NT-3 on axotomized rubrospinal neurons depend on the temporal pattern of neurotrophin administration,” *Eur. J. Neurosci.*, vol. 12, no. 2, pp. 776–780, Feb. 2000.

[75] K. Ollivier-Lanvin, I. Fischer, V. Tom, J. D. Houlé, and M. A. Lemay, “Either brain-derived neurotrophic factor or neurotrophin-3 only neurotrophin-producing grafts promote locomotor recovery in untrained spinalized cats.,” *Neurorehabil. Neural Repair*, vol. 29, no. 1, pp. 90–100, Jan. 2015.

[76] M. Koda, M. Hashimoto, M. Murakami, K. Yoshinaga, O. Ikeda, M. Yamazaki, S. Koshizuka, T. Kamada, H. Moriya, H. Shirasawa, S. Sakao, and H. Ino, “Adenovirus vector-mediated in vivo gene transfer of brain-derived neurotrophic factor (BDNF) promotes rubrospinal axonal regeneration and functional recovery after complete transection of the adult rat spinal cord.,” *J. Neurotrauma*, vol. 21, no. 3, pp. 329–37, Mar. 2004.

[77] V. S. Boyce and L. M. Mendell, “Neurotrophins and spinal circuit function,” *Front. Neural Circuits*, vol. 8, no. June, p. 59, 2014.

[78] K. Fouad, D. J. Bennett, R. Vavrek, and A. Blesch, “Long-term viral brain-derived neurotrophic factor delivery promotes spasticity in rats with a cervical spinal cord hemisection,” *Front. Neurol.*, vol. 4 NOV, no. November, pp. 1–9, 2013.

[79] C. Chau, H. Barbeau, and S. Rossignol, “Early locomotor training with clonidine in spinal cats.,” *J. Neurophysiol.*, vol. 79, no. 1, pp. 392–409, Jan. 1998.

[80] Y. P. Gerasimenko, R. M. Ichiyama, I. A. Lavrov, G. Courtine, L. Cai, H. Zhong, R. R. Roy, and V. R. Edgerton, "Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats.," *J. Neurophysiol.*, vol. 98, no. 5, pp. 2525–36, Nov. 2007.

[81] R. L. Meisel and B. Rakerd, "Induction of hindlimb stepping movements in rats spinally transected as adults or as neonates," *Brain Res.*, vol. 240, no. 2, pp. 353–356, May 1982.

[82] S. Grillner and P. Zangger, "On the central generation of locomotion in the low spinal cat," *Exp. Brain Res.*, vol. 34, no. 2, pp. 241–261, 1979.

[83] J. Duysens and H. W. A. . Van de Crommert, "Neural control of locomotion; Part 1: The central pattern generator from cats to humans," *Gait Posture*, vol. 7, no. 2, pp. 131–141, Mar. 1998.

[84] I. Lavrov, C. J. Dy, A. J. Fong, Y. Gerasimenko, G. Courtine, H. Zhong, R. R. Roy, and V. R. Edgerton, "Epidural stimulation induced modulation of spinal locomotor networks in adult spinal rats.," *J. Neurosci.*, vol. 28, no. 23, pp. 6022–9, Jun. 2008.

[85] R. M. Ichiyama, Y. P. Gerasimenko, H. Zhong, R. R. Roy, and V. R. Edgerton, "Hindlimb stepping movements in complete spinal rats induced by epidural spinal cord stimulation," *Neurosci. Lett.*, vol. 383, no. 3, pp. 339–344, 2005.

[86] G. Courtine, Y. Gerasimenko, R. van den Brand, A. Yew, P. Musienko, H. Zhong, B. Song, Y. Ao, R. M. Ichiyama, I. Lavrov, R. R. Roy, M. V Sofroniew, and V. R. Edgerton, "Transformation of nonfunctional spinal circuits into functional states after the loss of brain input," *Nat. Neurosci.*, vol. 12, no. 10, pp. 1333–1342, Sep. 2009.

[87] Y. P. Gerasimenko, V. D. Avelev, O. A. Nikitin, and I. A. Lavrov, "Initiation of locomotor activity in spinal cats by epidural stimulation of the spinal cord," *Neurosci. Behav. Physiol.*, vol. 33, no. 3, pp. 247–254, 2003.

[88] C. N. Shealy, J. T. Mortimer, and J. B. Reswick, "Electrical inhibition of pain by stimulation of the dorsal columns: preliminary clinical report.," *Anesth. Analg.*, vol. 46, no. 4, pp. 489–491, 1967.

[89] S. Harkema, Y. Gerasimenko, J. Hodes, J. Burdick, C. Angeli, Y. Chen, C. Ferreira, A. Willhite, E. Rejc, R. G. Grossman, and V. R. Edgerton, "Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study," *Lancet*, vol. 377, no. 9781, pp. 1938–1947, 2011.

[90] Y. Gerasimenko, R. R. Roy, and V. R. Edgerton, "Epidural stimulation: Comparison of the spinal circuits that generate and control locomotion in rats, cats and humans," *Exp. Neurol.*, vol. 209, pp. 417–425, 2008.

[91] S. M. Danner, U. S. Hofstoetter, B. Freundl, H. Binder, W. Mayr, F. Rattay, and K. Minassian, “Human spinal locomotor control is based on flexibly organized burst generators.,” *Brain*, vol. 138, no. Pt 3, pp. 577–88, Mar. 2015.

[92] Y. P. Gerasimenko, I. a. Lavrov, G. Courtine, R. M. Ichiyama, C. J. Dy, H. Zhong, R. R. Roy, and V. R. Edgerton, “Spinal cord reflexes induced by epidural spinal cord stimulation in normal awake rats,” *J. Neurosci. Methods*, vol. 157, pp. 253–263, 2006.

[93] T. Iwahara, Y. Atsuta, E. Garcia-Rill, and R. D. Skinner, “Spinal cord stimulation-induced locomotion in the adult cat,” *Brain Res. Bull.*, vol. 28, no. 1, pp. 99–105, 1992.

[94] B. Jilge, K. Minassian, F. Rattay, M. M. Pinter, F. Gerstenbrand, H. Binder, and M. R. Dimitrijevic, “Initiating extension of the lower limbs in subjects with complete spinal cord injury by epidural lumbar cord stimulation.,” *Exp. brain Res.*, vol. 154, no. 3, pp. 308–26, Feb. 2004.

[95] K. Minassian and U. S. Hofstoetter, “Spinal Cord Stimulation and Augmentative Control Strategies for Leg Movement after Spinal Paralysis in Humans,” *CNS Neurosci. Ther.*, vol. 22, p. n/a–n/a, 2016.

[96] P. Musienko, J. Heutschi, L. Friedli, R. van den Brand, and G. Courtine, “Multi-system neurorehabilitative strategies to restore motor functions following severe spinal cord injury,” *Exp. Neurol.*, vol. 235, no. 1, pp. 100–109, 2012.

[97] N. Wenger, E. M. Moraud, S. Raspopovic, M. Bonizzato, J. DiGiovanna, P. Musienko, M. Morari, S. Micera, and G. Courtine, “Closed-loop neuromodulation of spinal sensorimotor circuits controls refined locomotion after complete spinal cord injury,” *Sci. Transl. Med.*, vol. 6, no. 255, pp. 255ra133–255ra133, 2014.

[98] V. R. Edgerton, S. J. Kim, R. M. Ichiyama, Y. P. Gerasimenko, and R. R. Roy, “Rehabilitative therapies after spinal cord injury.,” *J. Neurotrauma*, vol. 23, no. 3–4, pp. 560–570, 2006.

[99] M. D. Klein Breteler, K. J. Simura, and M. Flanders, “Timing of muscle activation in a hand movement sequence.,” *Cereb. Cortex*, vol. 17, no. 4, pp. 803–15, Apr. 2007.

[100] G. Torres-Oviedo, J. M. Macpherson, and L. H. Ting, “Muscle synergy organization is robust across a variety of postural perturbations.,” *J. Neurophysiol.*, vol. 96, no. 3, pp. 1530–46, Sep. 2006.

[101] G. Torres-Oviedo and L. H. Ting, “Muscle synergies characterizing human postural responses.,” *J. Neurophysiol.*, vol. 98, no. 4, pp. 2144–56, Oct. 2007.

[102] A. d’Avella, A. Portone, L. Fernandez, and F. Lacquaniti, “Control of Fast-Reaching Movements by Muscle Synergy Combinations,” *J. Neurosci.*, vol. 26,

no. 30, pp. 7791–7810, Jul. 2006.

[103] L. H. Ting, “Dimensional reduction in sensorimotor systems: a framework for understanding muscle coordination of posture,” in *Progress in brain research*, vol. 165, 2007, pp. 299–321.

[104] L. H. Ting and J. L. McKay, “Neuromechanics of muscle synergies for posture and movement,” *Curr. Opin. Neurobiol.*, vol. 17, no. 6, pp. 622–628, 2007.

[105] G. E. Loeb, I. E. Brown, and E. J. Cheng, “A hierarchical foundation for models of sensorimotor control,” *Exp. Brain Res.*, vol. 126, no. 1, pp. 1–18, 1999.

[106] F. a Mussa-Ivaldi, S. F. Giszter, and E. Bizzi, “Linear combinations of primitives in vertebrate motor control.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 91, no. 16, pp. 7534–7538, 1994.

[107] C. B. Hart, “Modular Premotor Drives and Unit Bursts as Primitives for Frog Motor Behaviors,” *J. Neurosci.*, vol. 24, no. 22, pp. 5269–5282, 2004.

[108] C. B. Hart and S. F. Giszter, “A neural basis for motor primitives in the spinal cord.,” *J. Neurosci.*, vol. 30, no. 4, pp. 1322–1336, 2010.

[109] T. Drew, J. Kalaska, and N. Krouchev, “Muscle synergies during locomotion in the cat: a model for motor cortex control,” *J. Physiol.*, vol. 586, no. 5, pp. 1239–1245, Mar. 2008.

[110] J. Massion, “Postural Control Systems in Developmental Perspective,” *Neurosci. Biobehav. Rev.*, vol. 22, no. 4, pp. 465–472, 1998.

[111] V. J. Santos and F. J. Valero-Cuevas, “Reported Anatomical Variability Naturally Leads to Multimodal Distributions of Denavit-Hartenberg Parameters for the Human Thumb,” *IEEE Trans. Biomed. Eng.*, vol. 53, no. 2, pp. 155–163, Feb. 2006.

[112] K. A. Thoroughman, M. S. Fine, and J. A. Taylor, “Trial-by-trial motor adaptation: a window into elemental neural computation,” *Prog. Brain Res.*, vol. 165, pp. 373–382, 2007.

[113] W. J. Kargo and D. A. Nitz, “Early skill learning is expressed through selection and tuning of cortically represented muscle synergies.,” *J. Neurosci.*, vol. 23, no. 35, pp. 11255–69, Dec. 2003.

[114] S. F. Giszter and C. B. Hart, “Motor primitives and synergies in spinal cord and after injury – the current state of play,” *Ann N Y Acad Sci*, vol. 1279, pp. 114–126, 2014.

[115] B. Rohrer, S. Fasoli, H. I. Krebs, R. Hughes, B. Volpe, W. R. Frontera, J. Stein, and N. Hogan, “Movement smoothness changes during stroke recovery,” *J.*

Neurosci. Off. J. Soc. Neurosci., vol. 22, no. 18, pp. 8297–8304, 2002.

[116] L. Dipietro, H. I. Krebs, S. E. Fasoli, B. T. Volpe, J. Stein, C. Bever, and N. Hogan, "Changing motor synergies in chronic stroke.," *J. Neurophysiol.*, vol. 98, no. 2, pp. 757–68, Aug. 2007.

[117] T. Hayes Cruz and Y. Y. Dhaher, "Evidence of Abnormal Lower-Limb Torque Coupling After Stroke: An Isometric Study * Supplemental Materials and Methods," *Stroke*, vol. 39, no. 1, pp. 139–147, Jan. 2008.

[118] V. C. K. Cheung, A. Turolla, M. Agostini, S. Silvoni, C. Bennis, P. Kasi, S. Paganoni, P. Bonato, and E. Bizzi, "Muscle synergy patterns as physiological markers of motor cortical damageCheung, V. C. K., Turolla, A., Agostini, M., Silvoni, S., Bennis, C., Kasi, P., … Bizzi, E. (2012). Muscle synergy patterns as physiological markers of motor cortical damage. Proceed," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109, no. 36, pp. 14652–6, 2012.

[119] J. O. Ojemann and D. L. Silbergeld, "Cortical stimulation mapping of phantom limb rolandic cortex," *J. Neurosurg.*, vol. 82, no. 4, pp. 641–644, Apr. 1995.

[120] L. G. COHEN, S. BANDINELLI, T. W. FINDLEY, and M. HALLETT, "MOTOR REORGANIZATION AFTER UPPER LIMB AMPUTATION IN MAN," *Brain*, vol. 114, no. 1, 1991.

[121] J. P. Donoghue and J. N. Sanes, "Peripheral nerve injury in developing rats reorganizes representation pattern in motor cortex.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 84, no. 4, pp. 1123–6, Feb. 1987.

[122] J. P. Donoghue, S. Suner, and J. N. Sanes, "Dynamic organization of primary motor cortex output to target muscles in adult rats II. Rapid reorganization following motor nerve lesions," *Exp. Brain Res.*, vol. 79, no. 3, pp. 492–503, 1990.

[123] A. Karl, N. Birbaumer, W. Lutzenberger, L. G. Cohen, and H. Flor, "Reorganization of motor and somatosensory cortex in upper extremity amputees with phantom limb pain.," *J. Neurosci.*, vol. 21, no. 10, pp. 3609–18, May 2001.

[124] E. Raffin, N. Richard, P. Giraux, and K. T. Reilly, "Primary motor cortex changes after amputation correlate with phantom limb pain and the ability to move the phantom limb," *Neuroimage*, vol. 130, pp. 134–144, 2016.

[125] P. Giraux, a Sirigu, F. Schneider, and J. M. Dubernard, "Cortical reorganization in motor cortex after graft of both hands.," *Nat. Neurosci.*, vol. 4, no. 7, pp. 691–692, 2001.

[126] S. Röricht, J. Machetanz, K. Irlbacher, L. Niehaus, E. Biemer, and B. U. Meyer, "Reorganization of human motor cortex after hand replantation," *Ann. Neurol.*, vol. 50, no. 2, pp. 240–249, 2001.

[127] J. H. Kaas, “Topographic Maps are Fundamental to Sensory Processing,” *Brain Res. Bull.*, vol. 44, no. 2, pp. 107–112, 1997.

[128] J. H. Kaas, “Topographic Maps in the Brain,” in *International Encyclopedia of the Social & Behavioral Sciences*, 2001, pp. 15771–15775.

[129] A. Nieoullon and L. Rispal-Padel, “Somatotopic localization in cat motor cortex,” *Brain Res.*, vol. 105, no. 3, pp. 405–422, 1976.

[130] H. C. Kwan, W. A. MacKay, J. T. Murphy, and Y. C. Wong, “Spatial organization of precentral cortex in awake primates. II. Motor outputs.,” *J. Neurophysiol.*, vol. 41, no. 5, pp. 1120–31, Sep. 1978.

[131] C. G. Phillips, “CORTICAL MOTOR THRESHOLD AND THE THRESHOLDS AND DISTRIBUTION OF EXCITED BETZ CELLS IN THE CAT,” *Q. J. Exp. Physiol. Cogn. Med. Sci.*, vol. 41, no. 1, pp. 70–84, Jan. 1956.

[132] H. Devanne, F. Cassim, C. Ethier, L. Brizzi, A. Thevenon, and C. Capaday, “The comparable size and overlapping nature of upper limb distal and proximal muscle representations in the human motor cortex,” *Eur. J. Neurosci.*, vol. 23, no. 9, pp. 2467–2476, May 2006.

[133] P. D. Cheney and E. E. Fetz, “Functional classes of primate corticomotoneuronal cells and their relation to active force.,” *J. Neurophysiol.*, vol. 44, no. 4, pp. 773–91, Oct. 1980.

[134] E. E. Fetz and P. D. Cheney, “Postspike facilitation of forelimb muscle activity by primate corticomotoneuronal cells.,” *J. Neurophysiol.*, vol. 44, no. 4, pp. 751–72, Oct. 1980.

[135] E. J. Buys, R. N. Lemon, G. W. Mantel, and R. B. Muir, “Selective facilitation of different hand muscles by single corticospinal neurones in the conscious monkey.,” *J. Physiol.*, vol. 381, pp. 529–49, Dec. 1986.

[136] R. N. Lemon, “Functional properties of monkey motor cortex neurones receiving afferent input from the hand and fingers.,” *J. Physiol.*, vol. 311, pp. 497–519, Feb. 1981.

[137] B. J. McKiernan, J. K. Marcario, J. H. Karrer, P. D. Cheney, H. Asanuma, I. Rosen, H. Asanuma, A. Arnold, P. Zarzecki, H. Asanuma, P. Zarzecki, E. Jankowska, T. Hongo, S. Marcus, A. Belhaj-Saïf, J. H. Karrer, P. D. Cheney, K. M. B. Bennett, R. N. Lemon, E. J. Buys, R. N. Lemon, G. W. H. Mantel, R. B. Muir, P. D. Cheney, E. E. Fetz, P. D. Cheney, E. E. Fetz, K. Mewes, P. D. Cheney, J. W. Kenton, R. W. Thompson, B. J. McKiernan, R. Lininger, J. W. A. Trank, P. D. Cheney, K. Mewes, G. Widener, J. F. M. Clough, D. Kernell, C. G. Phillips, J. G. Colebatch, J. C. Rothwell, B. L. Day, P. D. Thompson, C. D. Marsden, J. P. Donoghue, S. Leibovic, J. N. Sanes, E. E. Fetz, P. D. Cheney, E. E. Fetz, P. D. Cheney, D. C. German, E. E. Fetz, P. D. Cheney, K. Mewes, S. Palmer, D.

Flament, P. A. Fortier, E. E. Fetz, A. Fourment, A. Belhaj-Saïf, B. Maton, A. Fourment, B. Maton, A. Belhaj-Saïf, J. Gibbs, L. M. Harrison, J. A. Stephens, A. R. Gibson, J. C. Houk, N. J. Kohlerman, P. Glees, S. Q. He, R. P. Dum, P. L. Strick, S. Q. He, R. P. Dum, P. L. Strick, D. R. Humphrey, G. W. Huntley, E. G. Jones, E. Jankowska, Y. Padel, R. Tanaka, J. H. Karrer, B. J. McKiernan, J. K. Marcario, R. Belhaj-Saïf, P. D. Cheney, R. J. Kasser, P. D. Cheney, H. C. Kwan, W. A. MacKay, J. T. Murphy, Y. C. Wong, H. C. Kwan, W. A. MacKay, J. T. Murphy, Y. C. Wong, R. N. Lemon, R. S. Johansson, G. Westling, R. N. Lemon, G. W. Mantel, R. N. Lemon, G. W. H. Mantel, R. B. Muir, R. N. Lemon, R. B. Muir, G. W. H. Mantel, G. E. Loeb, C. Gans, B. J. McKiernan, J. K. Marcario, J. H. Karrer, P. D. Cheney, K. Mewes, P. D. Cheney, K. Mewes, P. D. Cheney, L. E. Miller, J. C. Houk, E. Palmer, P. Ashby, R. Porter, R. N. Lemon, J. B. Preston, M. C. Shende, K. Uemura, S. M. Rao, J. R. Binder, T. A. Hammeke, P. A. Bandettini, J. A. Bobholz, J. A. Frost, B. M. Myklebust, J. N. Sanes, J. P. Donoghue, V. Thangaraj, R. R. Edelman, S. Warach, M. Schiepatti, C. Trompetto, G. Abbruzzese, Y. Shinoda, J. Yokota, T. Futami, Y. Shinoda, P. Zarzecki, H. Asanuma, T. Sinkjaer, L. Miller, T. Andersen, J. C. Houk, W. N. Smith, and E. E. Fetz, “Corticomotoneuronal postspike effects in shoulder, elbow, wrist, digit, and intrinsic hand muscles during a reach and prehension task.,” *J. Neurophysiol.*, vol. 80, no. 4, pp. 1961–80, Oct. 1998.

[138] A. D. Miller, “Localization of motoneurons innervating individual abdominal muscles of the cat,” *J. Comp. Neurol.*, vol. 256, no. 4, pp. 600–606, Feb. 1987.

[139] S. F. Giszter, W. J. Kargo, M. Davies, M. Shibayama, H. Barbeau, S. Rossignol, D. M. Basso, M. S. Beattie, J. C. A. Bresnehan, C. A. Bates, D. J. Stelzner, M. Belanger, T. Drew, J. Provencher, S. A. Rossignol, D. R. Bernstein, D. J. Stelzner, B. S. Bregman, B. S. Bregman, P. J. Reier, H. Cheng, Y. Cao, L. Olsen, H. Cheng, L. A. Olsen, J. W. Commisiong, Y. Sauve, M. Cox, D. R. Humphrey, J. M. Donatelle, J. P. Donoghue, K. L. Kerman, F. F. Ebner, J. P. Donoghue, J. N. Sanes, J. P. Donoghue, J. N. Sanes, J. P. Donoghue, S. P. Wise, V. R. Edgerton, R. R. Roy, J. A. Hodgson, R. J. Prober, C. P. De Guzman, R. de Leon, H. Flor, T. Elbert, S. Knecht, C. Wienbruch, C. Pantev, N. Birnbaumer, W. Larbig, E. Taub, G. W. Hiebert, M. A. Gorassini, W. Jiang, A. Prochazka, K. G. Pearson, B. T. Himes, M. E. Goldberger, A. Tessler, D. R. Howland, B. S. Bregman, A. Tessler, M. E. Goldberger, D. H. Hubel, T. N. Weisel, H. Hummelsheim, M. Wiesendanger, Y. Itoh, R. F. Waldeck, A. Tessler, M. J. Pinter, Y. Iwashiti, S. Kawaguchi, M. Murata, T. E. Jones, T. Schallert, J. H. Kaas, M. M. Merzenich, H. P. Killackey, D. Miya, S. F. Giszter, F. Mori, V. Adipudi, A. Tessler, M. Murray, F. Mori, B. T. Himes, M. Kuwada, M. Murray, A. Tessler, J. B. Ranck, D. J. Schreyer, E. G. Jones, D. J. Schreyer, E. G. Jones, P. H. Settlage, W. G. Bingham, H. M. Suckle, A. F. Borge, C. N. Woolsey, J. S. Soblosky, M. A. Matthews, J. F. Davidson, S. L. Tabor, M. E. Carey, D. J. Stelzner, W. B. Ershler, E. D. Weber, D. J. Stelzner, E. D. Weber, J. A. Prendergast, E. J. Tehovnik, P. M. E. Waite, J. T. Wall, C. G. Cusick, E. D. Weber, D. J. Stelzner, and J. S. Yeomans, “Fetal transplants rescue axial muscle representations in M1 cortex of neonatally transected rats that develop weight support.,” *J. Neurophysiol.*, vol. 80, no. 6, pp.

3021–30, Dec. 1998.

[140] S. Giszter, M. R. Davies, A. Ramakrishnan, U. I. Udoekwere, and W. J. Kargo, “Trunk sensorimotor cortex is essential for autonomous weight-supported locomotion in adult rats spinalized as P1/P2 neonates.,” *J. Neurophysiol.*, vol. 100, no. 2, pp. 839–851, 2008.

[141] N. Jain, K. C. Catania, and J. H. Kaas, “Deactivation and reactivation of somatosensory cortex after dorsal spinal cord injury.,” *Nature*, vol. 386, no. 6624, pp. 495–8, Apr. 1997.

[142] E. Sydekum, A. Ghosh, M. Gullo, C. Baltes, M. Schwab, and M. Rudin, “Rapid functional reorganization of the forelimb cortical representation after thoracic spinal cord injury in adult rats.,” *Neuroimage*, vol. 87, pp. 72–9, Feb. 2014.

[143] T. Endo, C. Spenger, T. Tominaga, S. Brené, and L. Olson, “Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling.,” *Brain*, vol. 130, no. Pt 11, pp. 2951–61, Nov. 2007.

[144] J. Aguilar, D. Humanes-Valera, E. Alonso-Calviño, J. G. Yague, K. A. Moxon, A. Oliviero, and G. Foffani, “Spinal cord injury immediately changes the state of the brain.,” *J. Neurosci.*, vol. 30, no. 22, pp. 7528–37, Jun. 2010.

[145] C. W. Wu and J. H. Kaas, “Reorganization in primary motor cortex of primates with long-standing therapeutic amputations.,” *J. Neurosci.*, vol. 19, no. 17, pp. 7679–7697, 1999.

[146] A. Ghosh, E. Sydekum, F. Haiss, S. Peduzzi, B. Zorner, R. Schneider, C. Baltes, M. Rudin, B. Weber, and M. E. Schwab, “Functional and Anatomical Reorganization of the Sensory-Motor Cortex after Incomplete Spinal Cord Injury in Adult Rats,” *J. Neurosci.*, vol. 29, no. 39, pp. 12210–12219, Sep. 2009.

[147] C. S. Oza and S. F. Giszter, “Trunk Robot Rehabilitation Training with Active Stepping Reorganizes and Enriches Trunk Motor Cortex Representations in Spinal Transected Rats,” *J. Neurosci.*, vol. 35, no. 18, pp. 7174–7189, 2015.

[148] C. S. Oza and S. F. Giszter, “Plasticity and alterations of trunk motor cortex following spinal cord injury and non-stepping robot and treadmill training,” *Exp. Neurol.*, vol. 256, pp. 57–69, 2014.

[149] R. J. Nudo, “Mechanisms for recovery of motor function following cortical damage,” *Curr. Opin. Neurobiol.*, vol. 16, no. 6, pp. 638–644, 2006.

[150] J. A. Kleim, S. Barbay, and R. J. Nudo, “Functional Reorganization of the Rat Motor Cortex Following Motor Skill Learning,” *J Neurophysiol*, vol. 80, no. 6, pp. 3321–3325, Dec. 1998.

[151] J. A. Kleim, S. Barbay, N. R. Cooper, T. M. Hogg, C. N. Reidel, M. S. Remple,

and R. J. Nudo, “Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex.,” *Neurobiol. Learn. Mem.*, vol. 77, no. 1, pp. 63–77, Jan. 2002.

[152] M. A. Perez, B. K. S. Lungholt, K. Nyborg, and J. B. Nielsen, “Motor skill training induces changes in the excitability of the leg cortical area in healthy humans.,” *Exp. brain Res.*, vol. 159, no. 2, pp. 197–205, Nov. 2004.

[153] S. F. Giszter, G. Hockensmith, A. Ramakrishnan, and U. I. Udoekwere, “How spinalized rats can walk: Biomechanics, cortex, and hindlimb muscle scaling - Implications for rehabilitation,” *Ann. N. Y. Acad. Sci.*, vol. 1198, pp. 279–293, 2010.

[154] N. Dancause and R. J. Nudo, “Shaping plasticity to enhance recovery after injury.,” *Prog. Brain Res.*, vol. 192, pp. 273–95, Jan. 2011.

[155] S. F. Giszter, M. R. Davies, and V. Graziani, “Motor strategies used by rats spinalized at birth to maintain stance in response to imposed perturbations.,” *J. Neurophysiol.*, vol. 97, no. 4, pp. 2663–75, Apr. 2007.

[156] I. Lavrov, C. J. Dy, A. J. Fong, Y. Gerasimenko, G. Courtine, H. Zhong, R. R. Roy, and V. R. Edgerton, “Epidural stimulation induced modulation of spinal locomotor networks in adult spinal rats.,” *J. Neurosci.*, vol. 28, no. 23, pp. 6022–6029, 2008.

[157] S. Tashiro, M. Shinozaki, M. Mukaino, F. Renault-Mihara, Y. Toyama, M. Liu, M. Nakamura, and H. Okano, “BDNF Induced by Treadmill Training Contributes to the Suppression of Spasticity and Allodynia After Spinal Cord Injury via Upregulation of KCC2,” *Neurorehabil. Neural Repair*, 2014.

[158] V. J. Tom and J. D. Houlé, “Intraspinal microinjection of chondroitinase ABC following injury promotes axonal regeneration out of a peripheral nerve graft bridge,” *Exp. Neurol.*, vol. 211, no. 1, pp. 315–319, 2008.

[159] J. C. Petruska, B. Kitay, V. S. Boyce, B. K. Kaspar, D. D. Pearse, F. H. Gage, and L. M. Mendell, “Intramuscular AAV delivery of NT-3 alters synaptic transmission to motoneurons in adult rats,” *Eur. J. Neurosci.*, vol. 32, no. 6, pp. 997–1005, 2010.

[160] F. H. Hsieh and S. F. Giszter, “Robot-driven spinal epidural stimulation compared with conventional stimulation in adult spinalized rats,” *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS*, pp. 5807–5810, 2011.

[161] M. Antri, D. Orsal, and J. Y. Barthe, “Locomotor recovery in the chronic spinal rat: Effects of long-term treatment with a 5-HT2 agonist,” *Eur. J. Neurosci.*, vol. 16, no. 3, pp. 467–476, 2002.

[162] B. Blits, M. Oudega, G. J. Boer, M. B. Bunge, and J. Verhaagen, “Adeno-

associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function," *Neuroscience*, vol. 118, no. 1, pp. 271–281, 2003.

[163] J. V. Coumans, T. T.-S. Lin, H. N. Dai, L. MacArthur, M. McAtee, C. Nash, and B. S. Bregman, "Axonal Regeneration and Functional Recovery after Complete Spinal Cord Transection in Rats by Delayed Treatment with Transplants and Neurotrophins," *J. Neurosci.*, vol. 21, no. 23, pp. 9334–9344, Dec. 2001.

[164] F. Gómez-Pinilla, Z. Ying, R. R. Roy, R. Molteni, and V. R. Edgerton, "Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity.," *J. Neurophysiol.*, vol. 88, no. 5, pp. 2187–95, Nov. 2002.

[165] G. Desroches, D. Gagnon, S. Nadeau, and M. R. Popovic, "Effects of sensorimotor trunk impairments on trunk and upper limb joint kinematics and kinetics during sitting pivot transfers in individuals with a spinal cord injury.," *Clin. Biomech. (Bristol, Avon)*, vol. 28, no. 1, pp. 1–9, Jan. 2013.

[166] N. Dominici, U. Keller, H. Vallery, L. Friedli, R. van den Brand, M. L. Starkey, P. Musienko, R. Riener, and G. Courtine, "Versatile robotic interface to evaluate, enable and train locomotion and balance after neuromotor disorders.," *Nat. Med.*, vol. 18, no. 7, pp. 1142–7, Jul. 2012.

[167] B. H. Dobkin, D. Apple, H. Barbeau, M. Basso, A. Behrman, D. Deforge, J. Ditunno, G. Dudley, R. Elashoff, L. Fugate, S. Harkema, M. Saulino, and M. Scott, "Methods for a randomized trial of weight-supported treadmill training versus conventional training for walking during inpatient rehabilitation after incomplete traumatic spinal cord injury.," *Neurorehabil. Neural Repair*, vol. 17, no. 3, pp. 153–67, Sep. 2003.

[168] S. Hussain, S. Q. Xie, and G. Liu, "Robot assisted treadmill training: mechanisms and training strategies.," *Med. Eng. Phys.*, vol. 33, no. 5, pp. 527–33, Jun. 2011.

[169] X. M. Xu, V. Guénard, N. Kleitman, P. Aebischer, and M. B. Bunge, "A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord.," *Exp. Neurol.*, vol. 134, no. 2, pp. 261–72, Aug. 1995.

[170] G. W. Hiebert, K. Khodarahmi, J. McGraw, J. D. Steeves, and W. Tetzlaff, "Brain-derived neurotrophic factor applied to the motor cortex promotes sprouting of corticospinal fibers but not regeneration into a peripheral nerve transplant," *J. Neurosci. Res.*, vol. 69, no. 2, pp. 160–168, 2002.

[171] B.-L. He, Y. Ba, X. Wang, S. Liu, G. Liu, S. Ou, Y. Gu, X. Pan, and T.-H. Wang, "BDNF expression with functional improvement in transected spinal cord treated with neural stem cells in adult rats," *Neuropeptides*, vol. 47, no. 1, pp. 1–7, Feb. 2013.

[172] M.-P. Côté, G. A. Azzam, M. A. Lemay, V. Zhukareva, and J. D. Houlé, "Activity-Dependent Increase in Neurotrophic Factors Is Associated with an Enhanced Modulation of Spinal Reflexes after Spinal Cord Injury," Feb. 2011.

[173] H. Wang, N.-K. Liu, Y. P. Zhang, L. Deng, Q.-B. Lu, C. B. Shields, M. J. Walker, J. Li, and X.-M. Xu, "Treadmill training induced lumbar motoneuron dendritic plasticity and behavior recovery in adult rats after a thoracic contusive spinal cord injury.," *Exp. Neurol.*, vol. 271, pp. 368–78, Sep. 2015.

[174] L. Frank, S. J. Wiegand, J. A. Siuciak, R. M. Lindsay, and J. S. Rudge, "Effects of BDNF infusion on the regulation of TrkB protein and message in adult rat brain.," *Exp. Neurol.*, vol. 145, no. 1, pp. 62–70, May 1997.

[175] A. Haapasalo, I. Sipola, K. Larsson, K. E. O. Akerman, P. Stoilov, S. Stamm, G. Wong, and E. Castren, "Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms.," *J. Biol. Chem.*, vol. 277, no. 45, pp. 43160–7, Nov. 2002.

[176] X. F. Zhou, L. F. Parada, D. Soppet, and R. a Rush, "Distribution of trkB tyrosine kinase immunoreactivity in the rat central nervous system," *Brain Res*, vol. 622, no. 1–2, pp. 63–70, 1993.

[177] M. Antri, C. Mouffle, D. Orsal, and J. Y. Barthe, "5-HT1A receptors are involved in short- and long-term processes responsible for 5-HT-induced locomotor function recovery in chronic spinal rat," *Eur. J. Neurosci.*, vol. 18, no. 7, pp. 1963–1972, 2003.

[178] E. S. Landry, N. P. Lapointe, C. Rouillard, D. Levesque, P. B. Hedlund, and P. a. Guertin, "Contribution of spinal 5-HT1A and 5-HT7 receptors to locomotor-like movement induced by 8-OH-DPAT in spinal cord-transected mice," *Eur. J. Neurosci.*, vol. 24, no. 2, pp. 535–546, 2006.

[179] C. A. Angeli, V. R. Edgerton, Y. P. Gerasimenko, and S. J. Harkema, "Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans," *Brain*, vol. 137, no. 5, pp. 1394–1409, 2014.

[180] S. Grillner, "Neuroscience. Human locomotor circuits conform.," *Science*, vol. 334, no. 6058, pp. 912–3, Nov. 2011.

[181] M. Lafreniere-Roula and D. A. McCrea, "Deletions of rhythmic motoneuron activity during fictive locomotion and scratch provide clues to the organization of the mammalian central pattern generator.," *J. Neurophysiol.*, vol. 94, no. 2, pp. 1120–32, Aug. 2005.

[182] S. Harkema, Y. Gerasimenko, J. Hodes, J. Burdick, C. Angeli, Y. Chen, C. Ferreira, A. Willhite, E. Rejc, R. G. Grossman, and V. R. Edgerton, "Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study.,"

Lancet (London, England), vol. 377, no. 9781, pp. 1938–47, Jun. 2011.

[183] V. R. Edgerton and S. Harkema, "Epidural stimulation of the spinal cord in spinal cord injury: current status and future challenges.," *Expert Rev. Neurother.*, vol. 11, no. 10, pp. 1351–3, Oct. 2011.

[184] I. Lavrov, "Plasticity of Spinal Cord Reflexes After a Complete Transection in Adult Rats: Relationship to Stepping Ability," *J. Neurophysiol.*, vol. 96, no. 4, pp. 1699–1710, 2006.

[185] V. J. Tom, H. R. Sandrow-Feinberg, K. Miller, C. Domitrovich, J. Bouyer, V. Zhukareva, M. C. Klaw, M. a. Lemay, and J. D. Houlé, "Exogenous BDNF enhances the integration of chronically injured axons that regenerate through a peripheral nerve grafted into a chondroitinase-treated spinal cord injury site," *Exp. Neurol.*, vol. 239, no. 1, pp. 91–100, 2013.

[186] R. van den Brand, J. Heutschi, Q. Barraud, J. DiGiovanna, K. Bartholdi, M. Huerlimann, L. Friedli, I. Vollenweider, E. M. Moraud, S. Duis, N. Dominici, S. Micera, P. Musienko, and G. Courtine, "Restoring voluntary control of locomotion after paralyzing spinal cord injury.," *Science*, vol. 336, no. 6085, pp. 1182–5, Jun. 2012.

[187] J. G. McPherson, R. R. Miller, and S. I. Perlmutter, "Targeted, activity-dependent spinal stimulation produces long-lasting motor recovery in chronic cervical spinal cord injury.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 39, pp. 12193–8, Sep. 2015.

[188] S. J. Harkema, "Plasticity of interneuronal networks of the functionally isolated human spinal cord.," *Brain Res. Rev.*, vol. 57, no. 1, pp. 255–64, Jan. 2008.

[189] M. Knikou, "Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury.," *Exp. brain Res.*, vol. 228, no. 3, pp. 279–96, Jul. 2013.

[190] L. J. Volz, A. K. Rehme, J. Michely, C. Nettekoven, S. B. Eickhoff, G. R. Fink, and C. Grefkes, "Shaping Early Reorganization of Neural Networks Promotes Motor Function after Stroke.," *Cereb. Cortex*, Mar. 2016.

[191] E. L. Simões, I. Bramati, E. Rodrigues, A. Franzoi, J. Moll, R. Lent, and F. Tovar-Moll, "Functional expansion of sensorimotor representation and structural reorganization of callosal connections in lower limb amputees.," *J. Neurosci.*, vol. 32, no. 9, pp. 3211–20, Feb. 2012.

[192] J. J. Kew, M. C. Ridding, J. C. Rothwell, R. E. Passingham, P. N. Leigh, S. Sooriakumaran, R. S. Frackowiak, and D. J. Brooks, "Reorganization of cortical blood flow and transcranial magnetic stimulation maps in human subjects after upper limb amputation.," *J. Neurophysiol.*, vol. 72, no. 5, pp. 2517–24, Nov. 1994.

[193] C. R. Jutzeler, A. Curt, and J. L. K. Kramer, “Relationship between chronic pain and brain reorganization after deafferentation: A systematic review of functional MRI findings.,” *NeuroImage. Clin.*, vol. 9, pp. 599–606, Jan. 2015.

[194] J. B. Nielsen, C. Crone, and H. Hultborn, “The spinal pathophysiology of spasticity--from a basic science point of view.,” *Acta Physiol. (Oxf).*, vol. 189, no. 2, pp. 171–80, Feb. 2007.

[195] P. Langhorne, J. Bernhardt, and G. Kwakkel, “Stroke rehabilitation,” *Lancet*, vol. 377, no. 9778, pp. 1693–1702, 2011.

[196] J. Toldi, R. Laskawi, M. Landgrebe, and J. R. Wolff, “Biphasic reorganization of somatotopy in the primary motor cortex follows facial nerve lesions in adult rats.,” *Neurosci. Lett.*, vol. 203, no. 3, pp. 179–82, Jan. 1996.

[197] E. M. de Carvalho Abreu, L. P. S. Dias, F. P. S. Lima, A. R. de Paula Júnior, and M. O. Lima, “Cardiovascular autonomic control in paraplegic and quadriplegic.,” *Clin. Auton. Res.*, vol. 26, no. 2, pp. 117–26, Mar. 2016.

[198] S. Geyh, S. Kunz, R. Müller, and C. Peter, “Describing functioning and health after spinal cord injury in the light of psychological-personal factors.,” *J. Rehabil. Med.*, vol. 48, no. 2, pp. 219–34, Mar. 2016.

[199] Y. Liu, D. Kim, B. T. Himes, S. Y. Chow, T. Schallert, M. Murray, A. Tessler, and I. Fischer, “Transplants of Fibroblasts Genetically Modified to Express BDNF Promote Regeneration of Adult Rat Rubrospinal Axons and Recovery of Forelimb Function,” *J. Neurosci.*, vol. 19, no. 11, pp. 4370–4387, Jun. 1999.

[200] C. J. C. Lamoth, A. Daffertshofer, O. G. Meijer, and P. J. Beek, “How do persons with chronic low back pain speed up and slow down? Trunk-pelvis coordination and lumbar erector spinae activity during gait.,” *Gait Posture*, vol. 23, no. 2, pp. 230–9, Feb. 2006.

[201] E. A. Dugan and J. S. Shumsky, “A combination therapy of neural and glial restricted precursor cells and chronic quipazine treatment paired with passive cycling promotes quipazine-induced stepping in adult spinalized rats.,” *J. Spinal Cord Med.*, vol. 38, no. 6, pp. 792–804, Nov. 2015.

[202] G. Desroches, D. Gagnon, S. Nadeau, and M. Popovic, “Magnitude of forward trunk flexion influences upper limb muscular efforts and dynamic postural stability requirements during sitting pivot transfers in individuals with spinal cord injury.,” *J. Electromyogr. Kinesiol.*, vol. 23, no. 6, pp. 1325–33, Dec. 2013.

[203] R. J. Nudo, G. W. Milliken, W. M. Jenkins, and M. M. Merzenich, “Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys.,” *J. Neurosci.*, vol. 16, no. 2, pp. 785–807, 1996.

[204] D. L. Adkins, J. Boychuk, M. S. Remple, and J. A. Kleim, “Motor training induces

experience-specific patterns of plasticity across motor cortex and spinal cord.," *J. Appl. Physiol.*, vol. 101, no. 6, pp. 1776–82, Dec. 2006.

[205] D. De Ridder, S. Vanneste, K. Van Laere, and T. Menovsky, "Chasing map plasticity in neuropathic pain," *World Neurosurg.*, vol. 80, no. 6, pp. 901.e1–901.e5, 2013.

[206] P. Cariga, M. Catley, A. V Nowicky, G. Savic, P. H. Ellaway, and N. J. Davey, "Segmental recording of cortical motor evoked potentials from thoracic paravertebral myotomes in complete spinal cord injury.," *Spine (Phila. Pa. 1976).*, vol. 27, no. 13, pp. 1438–1443, 2002.

[207] M. C. Tresch and A. Jarc, "The case for and against muscle synergies," *Curr. Opin. Neurobiol.*, vol. 19, no. 6, pp. 601–607, 2009.

[208] R. N. Holdefer and L. E. Miller, "Primary motor cortical neurons encode functional muscle synergies," *Exp. Brain Res.*, vol. 146, no. 2, pp. 233–243, 2002.

[209] H. A. Seelen, Y. J. Potten, J. Drukker, J. P. Reulen, and C. Pons, "Development of new muscle synergies in postural control in spinal cord injured subjects," *J. Electromyogr. Kinesiol.*, vol. 8, no. 1, pp. 23–34, 1998.

[210] C. M. Bütefisch, "Plasticity in the human cerebral cortex: lessons from the normal brain and from stroke.," *Neuroscientist*, vol. 10, no. 2, pp. 163–173, 2004.

[211] J. A. Kleim, T. M. Hogg, P. M. VandenBerg, N. R. Cooper, R. Bruneau, and M. Remple, "Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning.," *J. Neurosci.*, vol. 24, no. 3, pp. 628–33, Jan. 2004.

[212] G. W. Huntley, "Correlation between patterns of horizontal connectivity and the extent of short-term representational plasticity in rat motor cortex," *Cereb. Cortex*, vol. 7, no. 2, pp. 143–156, 1997.

[213] G. Hess and J. P. Donoghue, "Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps," *J Neurophysiol*, vol. 71, no. 6, pp. 2543–2547, Jun. 1994.

Vita

John Kim Lee was born in El Paso, Texas on June 24, 1986. He grew up in Germany and Belgium before attending high school at the International School of Yangon in Myanmar and Cairo American College in Egypt. He attended Columbia University, where he graduated with a degree in Astrophysics in 2008. He was an undergraduate researcher for two summers in the lab of Axel Scherer at the California Institute of Technology, investigating nanofluidics and micromanipulators. He also was a National Science Foundation fellow in the summer of 2007, when he conducted research in protoplanetary nebulae and stellar evolution at the Arecibo Observatory in Puerto Rico. After two years of research at the Tisch Multiple Sclerosis Research Center of New York, John enrolled in Drexel University's MD/PhD program. He finished the first two years of medical school, and joined the laboratory of Simon F. Giszter as a PhD candidate in the summer of 2012.

While in the Giszter lab, John had the privilege to attend the Lindau Nobel Laureate's Meeting in 2014 as an Alcoa Foundation fellow. He was also awarded the Drexel University Dean's Fellowship for Excellence in Collaborative or Themed Research in 2015.