SPINAL CORD INJURY: CORRELATES OF LESION ANATOMY TO THE DEVELOPMENT OF NEUROPATHIC PAIN AND SYSTEMATIC REVIEW OF CLINICAL TRIALS

A Dissertation

by

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ABSTRACT

Spinal cord injury (SCI) is a traumatic, life-altering injury that results in permanent neurological dysfunction, including chronic neuropathic pain. The mechanisms of SCI-associated pain are incompletely understood, although the numbers of SCI clinical trials testing therapeutic interventions are growing annually. In this dissertation, I will first explore the anatomical mechanisms of SCI pain, then describe a systematic analysis of the current state of SCI clinical trials.

To better understand the anatomical basis of SCI-associated neuropathic pain, specifically mechanical allodynia, we utilized a mouse cervical hemi-contusion model of SCI. We predicted that variability in lesion parameters might explain why some, but not all, experimental animals develop mechanical sensitivity after SCI. We found that 35% of animals exhibiting mechanical sensitivity had significantly increased dorsal horn neuronal sparing and that their tissue displacement at the time of impact was significantly lower. However, we observed no significant differences in dorsal horn nociceptive fiber density. Together, our data indicate that lesion size negatively correlates with the manifestation of at-level mechanical sensitivity and suggests that sparing of dorsal horn neurons may be required for the development of neuropathic pain.

In parallel to the large body of research characterizing pathophysiological mechanisms of SCI, candidate therapeutics continue to be evaluated in SCI clinical trials. Despite this, there is no comprehensive resource making SCI clinical trial information accessible to the lay public. Therefore, we performed a systematic analysis

of all the clinical trials registered in the U.S. National Library of Medicine (ClinicalTrials.gov) focused on improving outcomes after SCI. We annotated and categorized each trial according to the types of interventions tested and the outcome measures assessed. We observed that most trials have low enrollment and test single interventions. Commonly-used interventions include rehab/training/exercise and neuromodulation, and common outcomes focus on improving motor functions. Furthermore, we identify gaps in clinical trial reporting. Together, our work provides a comprehensive glimpse into the past, present, and future of SCI clinical trials, and suggests areas for improvement in clinical trial reporting.

DEDICATION

To my parents. My achievements are only possible because of your unconditional love, unwavering support, and consistent encouragement. Thank you for all the sacrifices you have made.

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CHAPTER I INTRODUCTION

1.1. Overview

Spinal cord injury (SCI) dramatically alters the ability of the brain to communicate with the body, resulting in widespread loss of neurological function. While significant progress has been made in understanding this complex neurological injury, there are no FDA-approved therapeutic interventions that can restore function following SCI. For this reason, there remains a great need to continue researching the pathophysiology of SCI and potential therapies.

Of particular interest to researchers, neuropathic pain is one of the most debilitating consequences of SCI that is estimated to affect over 60-80% of individuals living with SCI; for this reason, pain management has remained a top priority in the SCI community [1, 2]. Currently, there are limited treatment options for SCI-associated pain, and it is often resistant to conventional therapeutic strategies. While several pathophysiological alterations and neurochemical changes have been identified to contribute to chronic pain following SCI, the precise mechanisms driving SCI-associated neuropathic pain remain incompletely understood.

The focus of this dissertation is two-fold. First, I will explore the relation of anatomical characteristics of the lesioned spinal cord to the development of neuropathic pain-associated outcomes within a subset of animals. Neuropathic pain arises from damage to the somatosensory system and is categorized into at-level, below-level, and above-level pain in relation to the spinal level of injury. This project utilizes a clinically relevant cervical level 5 (C5) unilateral injury that has been shown to produce at-level

mechanical allodynia of the ipsilateral forepaw. Secondly, to evaluate clinical therapeutic options for SCI, I will present a systematic analysis of all the SCI clinical trials registered in the US National Library of Medicine (accessed through ClinicalTrials.gov). This work provides a comprehensive analysis of clinical trials focusing on improving outcomes after SCI and will be helpful for researchers, community members, and clinicians.

I will introduce SCI, etiology, stats, and lesion development in the introduction. I will then review and address known mechanisms contributing to neuropathic pain development after SCI and discuss the use of animal models in SCI research and for SCI-induced neuropathic pain. Finally, I will introduce the topic of clinical trials in SCI research.

1.2. Spinal Cord Injury

Spinal cord injury (SCI) is a traumatic, life-altering injury that frequently results in permanent loss of neurological function. The disruption of spinal cord neural circuitry leads to various symptoms, including paralysis, neuropathic pain, spasticity, autonomic dysreflexia, and the loss of bladder, bowel, respiratory, and sexual function [2-4]. Together, these neurological deficits can detract from quality of life and have devastating physical, social, financial, and occupational implications for individuals living with injury and their families [5].

Beyond the initial insult of tissue damage, several compounding processes further perpetuate tissue damage resulting in a complex injury paradigm, discussed below. This leads to a dynamic injury that changes over time. Historically, the injury's complex and

dynamic nature has complicated our understanding of SCI, leading us to believe that it was a fatal condition [6, 7]. Before the 1940s, the majority of SCI patients in the United States died within the first few weeks after injury [8]. As healthcare and our understanding of SCI began to improve, life expectancy after SCI steadily increased, as did expectations about enhancing the quality of life for those living with SCI [7, 9].

A more comprehensive understanding of the injury mechanisms is required to better treat SCI patients and improve outcome measures after injury. This launched decades of preclinical and clinical research initiatives. While significant progress has been made in deepening our understanding and testing therapeutic interventional strategies, there currently remains no FDA-approved treatment that can even partially restore lost neurological function after injury [10-12]. For this reason, we must continue research to elucidate the cellular and molecular mechanisms of SCI in hopes of developing novel effective treatments that improve quality of life.

1.2.1. SCI Etiology and Statistics

There are traumatic and non-traumatic causes of SCI. Traumatic SCI accounts for more than 90% [13]of all SCI and results from an external physical impact (such as a motor vehicle accident, sports injury, or a fall); non-traumatic SCI results from disease processes that generate the primary injury (such as a tumor, ischemic event, infection, or degenerative disk disease) [14]. According to the National Spinal Cord Injury Statistical Center (NSCISC), there are about 17,810 new SCI cases each year in the US, with approximately 294,000 individuals currently living with SCI [15, 16]. The leading cause of SCI is motor vehicle accidents which account for 38%, followed by falls accounting

for 32%, and acts of violence/sports injuries and recreational activities, accounting for 14% [17].

The cervical spinal cord is the most common area affected by SCI, accounting for approximately 50% of injuries, with cervical level 5 (C5) being the most common level affected [12]. The thoracic level makes up 35% of injuries, followed by the lumbar region at 11% [18].

Advancements in clinical care and medical procedures have improved, enabling individuals to live longer, up to decades, after the initial injury [9]. The life expectancy and associated symptoms highly depend on the injury's level and severity. For example, individuals needing indwelling catheterization have an increased mortality risk, whereas those not requiring catheterization have life expectancies of roughly 90% of normal life expectancy [19, 20].

Living with SCI often requires specialized care and frequent doctor appointments that can become an economic burden to individuals and their families. The mean number of all physician visits within the first year of injury is 31.7 among family physicians, emergency department physicians, and specialists [21]. Furthermore, the proportion of people living with SCI who are re-hospitalized in a given year has been reported to be between 27 and 57% [22-28]. It is estimated that the lifetime cost of SCI ranges from $1.2 to $5.4 million per person, with the average cost at $2.35 million [12].

1.2.2. Lesion Pathophysiology

Traumatic SCI consists of a primary injury followed by a secondary injury that further increases damage to the spinal cord over time [29]. Traumatic SCI is further

divided into an acute phase (less than 48 hours after injury), a subacute phase (48 hours – 14 days), an intermediate phase (14 days- 6 months), and a chronic phase (greater than 6 months). These phases are characterized by distinct changes in the pathophysiology [14]. The resulting damage and dynamic changes within the spinal cord from both phases of injury disrupt delicate and complex neural circuitry responsible for regulating how the brain and body communicate and interact with the outside world.

Primary injury consists of the initial mechanical force that acts on the spinal cord at the time of injury. During primary insult, bone fragments or disc materials generated from mechanical trauma can compress or even transect the spinal cord, with the most common form of primary injury classified as a contusion [29-32].

The secondary injury cascade is triggered within minutes, which expands the damage to the surrounding spinal cord tissue. Secondary injury is a complex cascade of biochemical and cellular changes within the spinal cord that work as a feed-forward loop in which the inflammatory response propagates further damage resulting in the recruitment of more inflammation in a vicious cycle. For this reason, the processes of secondary injury represent a target for the development of pharmacological strategies to limit the expansion of damaged spinal cord tissue [33].

In the acute and subacute phase of SCI, the disruption of vasculature and associated ischemia during the primary injury leads to a compromised blood-brain-barrier which can cause severe hemorrhage and expose the cord to an influx of inflammatory cells (neutrophils, macrophages, etc.), cytokines (tumor necrosis factor alpha (TNF-α),

interleukin 1 beta (IL-1β)) and vasoactive peptides within minutes of the injury [14, 34-36].

In the subacute injury phase, ongoing necrosis of neurons and glia occurs due to ischemia, inflammation, and excitotoxicity, which activate microglia by releasing ATP and potassium [33, 37]. Progressively, the spinal cord continues to swell, leading to further mechanical compression of the cord, which can expand into surrounding tissue multiple segments from the initial point of injury. This inflammation perpetuates necrotic cell death, which releases ATP and potassium ions, which trigger microglial activation and additional release of proinflammatory cytokines, thus recruiting more peripheral inflammatory cells [35]. As phagocytes work to clear out cellular debris, cytotoxic free radicals (nitrogen and oxygen) are produced, resulting in protein and lipid oxidation and oxidative damage to DNA [38-40]. As more and more neurons die, an accumulation of extracellular glutamate contributes to the excitotoxicity of neighboring neurons [41, 42]. Together, these biochemical changes and inflammation perpetuate the ongoing inflammation and ischemia and contribute to the cytotoxic microenvironment of the lesion in a feed-forward fashion [43, 44].

During the intermediate and chronic stages of SCI, the spinal cord undergoes dynamic alterations in the vasculature and extracellular matrix, accompanied by reorganization of both local and distal neural circuitry [33]. In response to atrophy of the spinal cord due to cell death, cystic cavitations form, surrounded by an astrocytic (or glial) scar. This type of cystic lesion core has been deemed a significant barrier to regeneration, cell migration, and axon outgrowth [14, 45-47]. Additionally, the glial scar

had been thought to inhibit axon regeneration further. However, recent evidence shows that it plays a critical role in corralling damaged tissue from uninjured spinal parenchyma and may benefit potential regeneration [48-50]. Within the perilesional region, astrocytes, pericytes, and ependymal cells produce chondroitin sulfate proteoglycans (CSPGs) as part of the fibrous glial scar. CSPGs bind leukocyte common antigen-related receptors, such as protein tyrosine phosphatase (PTPo), which activate GFPase RhoA and Rho-associated protein kinase (ROCK), leading to the collapse of axonal growth cones failing to regenerate [51-53].

1.2.3. Injury Complexity and Heterogeneity

In addition to the complex and dynamic changes that occur after injury, additional injury characteristics, such as cause, level, severity, and time since injury, further influence the wide variety of outcomes observed between individuals [54, 55]. SCI is not a static condition and evolves over time [56]. Understanding and appreciating the complexity of these injury factors is a crucial consideration in developing potential therapeutic interventions [57].

For example, in assessing the injury severity as a variable, the extent to which an individual living with SCI recovers function is highly dependent on the injury level and severity. In patients classified with incomplete paraplegia, impairment of sensory and motor function in the lower extremities often undergo some extent of recovery in locomotor ability 76% of the time within one year of injury [58, 59]. However, patients classified as complete paraplegia experience limited recovery of lower limb function [60].

Additionally, recent work has elucidated the importance of age as a biological variable in preclinical studies [61]. This is an essential consideration as the mean age of individuals at the time of injury has increased from 28 to 43 years old [17]. A recent meta-analysis of clinical reports has identified age as a significant variable associated with worsened neurological function and reduced recovery [62]. Preclinical reports have found that females often recover more locomotion abilities after SCI, which is believed to be based on the role of hormones in neural protection and immune modulation [63].

These biological variables even differentially impact neuropathic pain. Clinical reports have indicated that neuropathic pain is positively correlated to increasing age at the time of injury [64-68]. While it appears that biological sex does not correspond to the development of pain clinically between males and females [64], preclinical reports have indicated that biological sex does influence pharmacological inhibition [69-73].

Together, these variables dramatically influence not only the outcomes the individual may experience but also the effectiveness of potential therapeutic strategies. There is growing evidence and interest in understanding how these variables can be used to understand and better predict the efficacy of therapeutic approaches.

1.3. SCI-Induced Neuropathic Pain

1.3.1. Neuropathic Pain

SCI results in a wide variety of neurological dysfunctions, including the development of neuropathic pain. Neuropathic pain arises from disease or injury to the somatosensory nervous system and encompasses burning sensations, electrical shock, dullness/aching, or pins and needles [74]. The development of neuropathic pain affects

up to 60-80% of individuals living with SCI, and the alleviation of chronic pain has been listed as a top priority in the SCI community [1, 75-77]. Several longitudinal studies have shown that many individuals living with SCI experience pain several years after initial injury [75, 78-80]. Neuropathic pain has even been suggested as a predictor of reduced quality of life, and there remains an unmet clinical need as this type of pain is often untreatable [81-84].

Neuropathic pain can manifest as at-level pain defined by its presence within a region spanning one dermatome rostral and three dermatomes caudal to the neurological level of injury, or as below-level pain, which occurs below three dermatomes caudal to the neurological level of injury [74]. Allodynia, which is pain due to a stimulus that does not usually provoke pain (such as a light touch), is more frequently experienced with at-level pain because that region is more likely to retain partial sensory function [80]. Additionally, the development of hyperalgesia is characterized as an exaggerated response to a previously painful stimulus [85]. After an injury, a patient may experience one or many combinations of pain sensations [86]. It has been reported that pain is persistent in 60-65% of the SCI population [87]. The mechanisms underlying the development of neuropathic pain are incompletely understood and directly limit the development of novel therapeutic interventions targeting chronic pain.

1.3.2. Pain Circuits

Sensing pain is vital to survival as it alerts us to potential dangers and hazards in our environment. In the intact nervous system, pain sensation is a complex combination of both physical sensation and an emotional experience linked to actual or potential

tissue damage [88]. The pain pathway is composed of elements in the peripheral and central nervous systems [89]. The peripheral nervous system contains nerves and ganglia that innervate the skin, muscles, organs, and viscera, connecting distal regions of the body to the central nervous system [88]. The central nervous system consists of the brain and spinal cord, which are primarily responsible for integrating and interpreting the signals from the peripheral nervous system [88].

There are three general stages in the perception of pain: 1) the reception of pain signals from a stimulus by peripheral sensory neurons; 2) the transmission of signals from the periphery to the dorsal horn via the dorsal roots; 3) the transmission of these signals to the brain via the central nervous system where it is perceived as painful [88].

The perception of pain is usually initiated by external stimuli (such as a noxious chemical, mechanical or thermal stimuli) in the periphery by a specialized subset of sensory neurons called nociceptors. These nociceptors include medium-sized myelinated Aδ fibers and small-diameter, unmyelinated C-fibers that project onto the spinal cord's dorsal horn [93, 95]. The dorsal horn of the spinal cord is highly organized into Rexed laminae. It consists of complex excitatory and inhibitory networks that regulate the signaling of dorsal horn projection neurons [90-92]. The specific termination for these primary afferents in the dorsal horn depends on their sensory modality and the region of the body that they innervate [90]. After processing sensory information by the dorsal horn, somatosensory information is passed to supraspinal centers. For example, the thalamus is a supraspinal structure that receives projections from multiple ascending pathways [93].

1.3.3. Mechanisms of Neuropathic Pain After SCI

Several distinct changes occur in pain circuitry after SCI, which are attributed to the development of chronic pain, ranging from changes in the peripheral nervous system, the spinal cord itself, and the brain.

1.3.3.1. Peripheral Mechanisms

While SCI primarily results in damaged tissue of the spinal cord, characteristic changes in peripheral nociceptor function and spontaneous activity at all levels of the central nervous system have been shown to contribute to neuropathic pain [94-97].

Increased excitability of at-level DRG has been linked to reduced expression of the Kv3.4 potassium channel, while below-level nociceptor spontaneous activity has been linked to increased expression of Nav1.8 sodium channels [94, 97, 98]. The dysregulation of these voltage-gated channels results in repetitive spiking and elongated action potentials [97, 99]. Increasing activation of DRG soma leads to ATP release that activates neuronal interactions with surrounding satellite glial cells, which can further influence the excitability of DRG neurons [100, 101]. Satellite cells become reactive in DRG from sensory regions innervating dermatomes of at-level SCI pain and have been shown to express connexin-43, a gap junction protein known to contribute to the development of neuropathic pain in animal models [102, 103]. Additionally, it has been shown that chronic depolarization of resting membrane potential is maintained by cAMP signaling, which further contributes to chronic pain [104].

Elevated spontaneous activity of DRGs has been linked to increased vocalization of at-level stimulation in animal models [96]. Furthermore, SCI leads to an enhanced growth state of nociceptive neurons at and below-level DRGs [96]. This increased nociceptor sprouting has been observed in the dorsal horns at and below-level of SCI [105-108].

1.3.3.2. Spinal Mechanisms

As described earlier, SCI causes severe pathological changes within the spinal cord through primary and secondary injury mechanisms. Some of these alterations and inflammation-based injury mechanisms have been associated with pain, including reactive gliosis, spinal disinhibition, and spinal hyperexcitability [4].

SCI induces changes to microglia and astrocytes proximal and distal to the lesion epicenter [95, 109, 110]. A mix of resident microglia and peripheral monocyte-derived macrophages infiltrate injured tissue in a pro-inflammatory state which is vital for wound healing processes that have been shown to modulate neuronal excitability [110-112] followed by a switch to anti-inflammatory phenotypes [113]. After CNS damage, microglia and macrophages fail to switch to anti-inflammatory phenotypes and continue releasing pro-inflammatory cytokines, which have been linked to contributing to the development of neuropathic pain [114-116].

Reactive gliosis is an innate reaction to injury by up-regulation of the glial-fibrillary acidic protein (GFAP) and p-p38 MAPK in astrocytes. Several studies have modulated levels of GFAP to evaluate its connection to pain behaviors through astrocyte-specific deletion [117, 118] and pharmacological inhibition [119, 120]. Spinal

disinhibition occurs due to the reduction of local GABAergic inhibition. GABAergic inhibition is vital in gaiting sensory stimuli, and the loss of such inhibition can result in dysregulation, leading to hypersensitivity to innocuous stimuli [121]. The loss of GABAergic inhibitory interneurons or GABA-synthesizing enzymes has been shown to contribute to neuropathic pain [122-124].

Spinal hyperexcitability can result from partial loss of descending fiber tracts responsible for inhibitory control of sensory circuitry [125]. However, alterations in descending fiber tracts have been shown to differentially affect neuropathic pain and sensory response at or below- the level of injury. For example, serotonin fibers extensively sprout above injury yet are reduced below the level of injury [126, 127].

1.3.3.3. Supraspinal Mechanisms

After SCI, dynamic reorganization of neural circuitry has been observed primarily due to the deafferentation [128]. Neuropathic pain has been correlated to the level of reorganization within the primary somatosensory cortex [129, 130]. Specifically, the thalamus, essential for relaying sensory information to the somatosensory cortex, undergoes specific changes that correlate to neuropathic pain. Reduced levels of thalamic GABA, increased burst firing patterns, and hyperresponsive firing of thalamic neurons have been reported [131-134]. Additionally, fMRI has been utilized to show changes in thalamic anatomy [135, 136] and decreased thalamic perfusion [136-138] in relation to the development of neuropathic pain.

1.4. Animal Models

The utilization of animal models has contributed significantly to understanding the pathophysiology of SCI and has been instrumental in testing preclinical therapeutic strategies. Very little of our understanding of the complex pathophysiological processes of SCI comes from human studies; most of it comes from animal models of SCI, which utilize a variety of animal species and injury paradigms [33, 139].

In 1911, the first SCI animal model was established using a weight drop to study the effect of SCI experimentally [140]. Since then, several animal models have been used to further our understanding of SCI and its potential therapeutic interventions. While no animal model of SCI will genuinely be able to recapitulate the nuances of human SCI, animal models have proven valuable for understanding the complex events following SCI and testing potential therapeutic strategies and interventions.

The inclusion of animals in research is continually questioned from an ethical and financial standpoint, especially in neuroscience (SCI specifically), as the translation of preclinical success has only sometimes resulted in clinical success in humans [141-144]. In response, it is of the utmost importance that researchers carefully consider which animal model is optimal for their research question and what outcome measures will be assessed.

1.4.1. Types of SCI Injury Models

Animal models of SCI include contusion, compression, and transection injuries. These model injuries can be performed at any level of the cord and with various intensities based on the research question being analyzed. According to Akhtar et al.,

ideal SCI models should meet the following conditions: 1) produce damage similar to clinical SCI; 2) be controlled and reproducible; 3) involve a simple technique that is easy to study and 4) utilize equipment that is easy to make and straightforward to produce [145].

As most clinical SCI presents as a contusion (or compression) injury, a contusion is one of the most commonly utilized injury models [146]. Allen established the first contusion model paradigm in 1911, where a weight was dropped dorsally onto canine dura, and most current models of contusion injuries are based on this same principle [147]. Current contusion models utilize weight-drop, electromagnetic, or pneumatic impactor devices to deliver blunt forces to the spinal cord and can be administered at graded severities [148-154]. A recent review published in 2017 shows the most common method for administering contusion was using the Weight Drop method (37.5%), followed by New York University (NYU) impactor (27.4%), and Infinite Horizon impactor (20.6%) [155]. Furthermore, most cervical contusions utilize either a unilateral contusion or a hemi contusion due to life-threatening adverse effects that could occur after a complete cervical lesion [155, 156].

Compression SCI models are helpful in studying spinal canal occlusion and are valuable in the study of the timing of decompression. It is currently estimated that more than half of the compression models utilize an aneurism clip as the source of ischemia [155, 157, 158]. Other compression models, such as the balloon-induction method or calibrated forceps and spacers, can be used to establish compression of the spinal cord [155, 159, 160].

While transection of the spinal cord does not often occur clinically, transection injury models help study the effects of scaffolds, biomaterials, neural regeneration, and tissue engineering strategies [155, 156, 161]. Transection models are specifically useful because they allow for the assessment of axonal regeneration and subsequent functional recovery mediated through the therapeutic intervention being tested. Transection models include complete transection of the spinal cord or unilateral (partial) transections and are accomplished using fine surgical scissors to induce transection [155].

1.4.2. Outcome Assessments

An important consideration in using animal models of SCI is the outcome measures that can be assessed. The ultimate indicator of a successful experiment in SCI research is often linked to improvement in behavior without a worsening of function or condition [141]. This position reflects the urgent demand to improve the quality of life for individuals living with SCI. For this reason, behavioral testing and understanding the mechanisms that lead to functional improvement have become essential elements of experimental design.

In terms of behavior, animals cannot directly indicate what they are feeling or experiencing to experimenters. Hence, scientists must depend on reliable and quantifiable behaviors to assess outcomes such as motor function, strength, and sensory function.

One of the most visible symptoms after SCI is the loss of motor function (paralysis), and the recovery of motor functions can be assessed through various outcome measures focusing on the locomotion of the forelimb or hindlimbs of an animal

model. Recovery of walking or stepping is often measured in animal models using the Basso, Beattie, Bresnahan (BBB) scale in rats [162], the Basso Mouse Scale (BMS) in mice [163], and through the objective gait analysis systems, such as the CatWalk Gait Analysis [164]. Evaluation of grooming behaviors can also indicate locomotor recovery through grooming motion analysis [165, 166]. To better differentiate between the restoration of descending function compared to pure spinal function, outcomes that challenge the motor system by requiring brain and brainstem processing can be implemented through the use of the horizontal ladder [167], grid walk [168, 169] or narrow beam test [170]. Locomotor testing has also evaluated walking in shallow water or swimming tasks [171-173]. Fine motor function of the forelimb can be assessed using tasks dependent on reaching and grasping, such as the pellet reaching task [174], rotating knob/lever manipulation [175, 176], or through manipulation of small objects (such as pasta or a cereal treats) [177, 178].

Assessments of limb strength are also an important metric that can be measured in animal models of SCI. Forelimb grip strength measures the force an animal can hold on to through a force sensor [179], and the inclined plane test indirectly measures trunk stability, proprioception or sensation, and unilateral limb strength [180].

Assessments of sensory function outcomes are important in evaluating sensory dysfunction and in testing therapeutic interventions to mediate pain. There are several different sensations that can be tested in animal models. In response to temperature, the Hargraves assay measures allodynia through an infrared thermal heat stimulus [181], and the acetone test evaluates allodynia stimulated by a cold temperature [182]. In response

to mechanical stimulation, similar to a pinprick, the von Frey assay measures mechanical allodynia in response to mechanical stimulation [183, 184].

While it is important to measure behavioral changes, it is equally important to evaluate the mechanisms driving behavioral outcomes. Scientists often measure pathophysiological metrics of SCI such as lesion size, lesion length, white/gray matter sparing, or axon regeneration [185]. It has been shown that increased lesion severity of the spinal cord does not linearly increase functional deficits; therefore, the inclusion of several injury measurements is necessary [141, 149, 168, 186-188]. These metrics are important in evaluating pathology and can be used to validate injury severity and elucidate the pathological basis of behavior. For example, the evaluation of tissue sparing after the injury is important because even small amounts of spared tissue can result in significant recovery of function over time regardless of the amount of spinal cord damage [141, 188, 189].

1.4.3. Modeling SCI Neuropathic Pain

It is essential to evaluate pain mechanisms as there are differences in the incidence and phenotype of pain across different injury levels. A review by Kramer et al. in 2016 highlights that models of SCI neuropathic pain primarily use contusion injuries, focus on thoracic injury level (over 90% of studies), evaluate above-level pain, and analyze the modality of mechanical and thermal pain [190]. The most common form of SCI is a cervical contusion, and the most common level affected is cervical level 5 [12, 191]. However, it is estimated that 80-90% of animal models studying SCI utilize thoracic injury paradigms [155, 190]. Cervical, thoracic, and lumbar spinal levels are

fundamentally different, and findings in one region are not necessarily translatable to other areas of injury [153]. These region-specific differences have justified the development of cervical SCI models in recent years to better model the clinical condition [149].

The development of a clinically relevant cervical 5 spinal level unilateral contusion has recently been established to study the development of neuropathic pain in mice [192-194]. While this injury is clinically relevant, it has also been shown to drive the development of neuropathic pain through multiple mechanisms. These mechanisms include gliosis of microglia and astrocytes, downregulation of glutamate transporter GLT1, and activation of dorsal horn neurons innervated by forepaw sensory neurons [192, 193, 195]. Furthermore, this injury model shows increased activation of dorsal horn projection neurons, including protein kinase C (PKC) and calretinin excitatory dorsal horn interneuron populations, with a marked decrease in activation of neuronal nitric oxide synthase (nNos) inhibitory interneurons [195].

Overall, the development and further use of clinically relevant injury models will allow for further characterization of both pathophysiological and mechanistic changes that contribute to the development and maintenance of neuropathic pain. Ultimately, gaining a more detailed and precise understanding of these circuits can lead to the development of novel, targeted therapeutic interventions.

1.5. Clinical Translation for SCI

Decades of preclinical and clinical research have improved our understanding of SCI, yet few treatment options are available [10, 11, 35, 196, 197]. Since 2016, the

National Institute of Neurological Disorders and Stroke (NINDS) alone has spent over $70 million each year to fund applied research on spinal cord structure, function, mechanisms of injury, and secondary consequences of SCI (report from report.nih.gov/funding/categorical-spending#/). This funding, among other sources, has allowed for the establishment of various animal models, which, combined with advances in cellular and molecular techniques, have further improved our understanding of SCI [141, 151, 152, 155, 156, 198-203]. A steady growth in knowledge of underlying injury mechanisms has led to an increase in the number of clinical trials, encouraging scientists, clinicians, and individuals/families living with SCI [204].

Over 1,400 clinical trials have been registered to ClinicalTrials.gov, with 1,149 testing of them testing interventional therapeutic strategies to improve the lives of individuals living with SCI. However, despite all of the gained knowledge and the sheer number of clinical trials, there remains no FDA-approved therapeutic that can even partially restore neurological function after injury, nor is there a clear consensus on the standard of care [144, 205]. While many factors may contribute to this lack of translation of preclinical success into the clinic, the SCI community initiated collaborative efforts to close the gaps among clinicians, researchers, funding agencies, industry partnerships, and individuals living with SCI. The development of the North American Spinal Cord Injury Consortium (NASCIC) is dedicated to promoting a unified voice among the SCI community on behalf of those living with SCI. Regarding establishing the standard of care guidelines, the International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) released a series of papers reviewing clinical trials for SCI. It made

recommendations regarding inclusion/exclusion criteria, ethics, clinical trial design, clinical trial outcome measures, and the required statistical power considering spontaneous recovery after SCI [206-209].

Similarly, the Spinal Cord Outcomes Partnership Endeavor (SCOPE) was established to promote academic-scientific-industry partnerships to streamline and promote treatment options for SCI [210]. And most recently, the NIH hosted a conference titled "SCI 2020: Launching a Decade of Disruption in Spinal Cord Injury Research," where gaps in scientific knowledge were identified/discussed, and priorities for SCI research were established [211]. Furthermore, these efforts are not limited to the US; international efforts, such as The International Spinal Cord Society (ISCoS), have been formed [212]. Organizations and actions like these are essential in breaking down barriers, facilitating the exchange of knowledge, experience/expertise, and encouraging collaborative efforts to streamline the path to finding and producing effective treatments.

In addition to such collaborative efforts, it is imperative to understand what has been done in the field of clinical trials for SCI. Several excellent reviews have been published focusing on advances in SCI therapeutics [213-217]. However, these reviews focus on only a select few trials and ultimately do not make general conclusions about the outcomes or speak to the evolution of clinical trials for SCI. To address this knowledge gap, we performed a systematic analysis of over 1,000 interventional clinical trials focusing on SCI using publicly available data extracted from ClinicalTrials.gov. We organically defined 14 classes of intervention and 37 classes of outcome measures which were used to annotate all clinical trials. Ultimately, we provide a comprehensive

glimpse into the past, present, and future of SCI clinical trials and suggest areas of improvement in clinical trial reporting. Chapter 3 will discuss the findings of this systematic analysis.

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CHAPTER II DORSAL HORN NEURONAL SPARING PREDICTS THE DEVELOPMENT OF AT-LEVEL MECHANICAL ALLODYNIA FOLLOWING CERVICAL SPINAL CORD INJURY IN MICE*

2.1. Abstract

Spinal cord injury (SCI) frequently results in immediate and sustained neurological dysfunction, including intractable neuropathic pain in approximately 60–80% of individuals. SCI induces immediate mechanical damage to spinal cord tissue followed by a period of secondary injury in which tissue damage is further propagated, contributing to the development of anatomically unique lesions. Variability in lesion size and location influences the degree of motor and sensory dysfunction incurred by an individual. We predicted that variability in lesion parameters may also explain why some, but not all, experimental animals develop mechanical sensitivity after SCI. To characterize the relationship of lesion anatomy to mechanical allodynia, we utilized a mouse cervical hemicontusion model of SCI that has been shown to lead to the development and persistence of mechanical allodynia in the ipsilateral forelimb after injury. At four weeks post-SCI, the numbers and locations of surviving neurons were quantified along with total lesion volume and nociceptive fiber sprouting. We found that the subset of animals exhibiting mechanical allodynia had significantly increased

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neuronal sparing in the ipsilateral dorsal horn around the lesion epicenter compared to animals that did not exhibit mechanical allodynia. Additionally, we failed to observe significant differences between groups in nociceptive fiber density in the dorsal horn around the lesion epicenter. Notably, we found that impactor probe displacement upon administration of the SCI surgery was significantly lower in sensitive animals compared with not-sensitive animals. Together, our data indicate that lesion severity negatively correlates with the manifestation of at-level mechanical hypersensitivity and suggests that sparing of dorsal horn neurons may be required for the development of neuropathic pain.

2.2. Introduction

Spinal cord injury (SCI) typically results in lifelong neurological deficits including the development and persistence of neuropathic pain, defined as "pain caused by a lesion or disease of the somatosensory system" [1]. Neuropathic pain resulting from SCI is typically categorized into two types: hyperalgesia, which is characterized by exaggerated pain responses to noxious stimulation; or allodynia, which is characterized by pain in response to normally innocuous stimulation. Neuropathic pain typically manifests as sharp, shooting or burning pain sensations, and severely detracts from quality of life [1, 2]. Clinical studies have found that 60-80% of individuals with SCI experience neuropathic pain, which may persist throughout life [3-5]. There are limited treatment options available to manage neuropathic pain, and SCI patients with chronic neuropathic pain typically only experience a 20-30% reduction in pain intensity following treatment [6]. Thus, there remains a great unmet need to develop more

effective therapies to mitigate SCI-associated pain in order to improve quality of life for those living with SCI.

A major roadblock to the development of effective treatments for chronic SCI-associated neuropathic pain is our incomplete understanding of how central nervous system injury alters the neural substrates underlying pain signaling. The spinal cord dorsal horn is the main site of input and processing of sensory information. Following SCI, multiple pathophysiological changes have been shown to contribute to the development of chronic neuropathic pain [7]. These include hyperexcitability of spinal cord dorsal horn neurons [7-9], hyperexcitability and heightened activity of primary dorsal root ganglion neurons [8, 10-14], sprouting of primary afferent fibers into the dorsal horn [15-17], and glial activation [10, 18-22]. The most immediate effect of SCI is loss of neurons at the lesion epicenter due to mechanical tissue damage; however, it is not clear how lesion size or location affects the development of pain. Experimental studies have shown that the extent of tissue loss at the lesion epicenter in rats does not predict the development of pain-associated outcomes [23]; however, it is still unclear whether the preservation of specific neuronal populations is correlated with such outcomes. This information will be critical for understanding how neuropathic pain develops, and for identifying more targeted therapeutic treatments that can successfully combat neuropathic pain.

Notably, neuropathic pain is only experienced by a subset of individuals with SCI, with some individuals experiencing pain more frequently than others [3, 5]. In parallel, some experimental animal studies have shown that pain-associated behaviors

such as allodynia and hyperalgesia only develop in a subset of animals following SCI [23]. While it is unclear why pain behaviors develop in some animals but not others, this observation presents a useful opportunity to study whether the anatomical characteristics of the lesioned spinal cord are associated with the development of neuropathic pain-associated outcomes in a subset of animals within a single experimental cohort. To address this, we utilized a rodent model of cervical level 5 (C5) unilateral hemi-contusion SCI that has been previously reported to produce mechanical allodynia of the ipsilateral forepaw [18, 23-25]. We found that in the first 28 days post-SCI, approximately 35% of mice exhibited mechanical allodynia (a neuropathic pain-associated outcome) of the ipsilateral forelimb, whereas 65% of mice did not. Interestingly, we found that in the subset of animals that developed sensitivity, motor impairment was less severe and neuronal density in the ipsilateral dorsal horn was significantly greater than that of the non-sensitive counterparts. These findings suggest that variability in the force-defined contusion, which in turn affects dorsal horn neuronal sparing, plays a significant role in determining whether animals will develop neuropathic pain-associated outcomes after SCI.

2.3. Materials and Methods

2.3.1. Ethics Statement

All animal studies were performed in stringent compliance with *NIH Guidelines for Animal Care and Use of Laboratory Animals*. All experiments utilizing animals were approved by the Texas A&M University Institutional Animal Care and Use Committee. All efforts were made to minimize pain and distress.

2.3.2. Animals

Thirty female C57BL/6 mice (6 weeks, approximately 20g; The Jackson Laboratory) were housed 5 per cage, in a 12-hour (6:00AM-6:00PM) light cycle, with food and water accessible at all times. Mice were randomized to receive SCI (N=20) or laminectomy only (N=10). During the study, 2 animals in the SCI group died immediately after contusion and 1 laminectomy animal died 2 weeks post-surgery. One subject from the SCI group was excluded from the post-hoc analysis, because it exhibited paw withdrawal thresholds more than 4 standard deviations above its normal range of the ipsilateral forepaw. This produced final group sizes of N=17 for SCI, and N=9 for laminectomy.

2.3.3. Surgery

Spinal cord hemi-contusion at spinal cord cervical level 5 (C5) was performed as previously described [18, 25]. Animals were anesthetized with anesthetic cocktail consisting of ketamine (25 mg/kg), xylazine (5.8 mg/kg) and acepromazine (0.25 mg/kg). Anesthesia was maintained with 1.0-1.5% inhaled isoflurane for the duration of the surgery, and heating pads were used to maintain body temperature. Animals were shaved from the base of the skull to approximately halfway down their backs, and betadine solution was used to clean the skin before incision. A bead sterilizer was used to sterilize surgical instruments; surgical consumables were autoclaved. A small incision was made in the skin overlying approximately cervical level 2 (C2) to thoracic vertebral level 2 (T2). After exposure of the spinal column, C2 was used as a landmark to identify

the C5 vertebral segment. A partial laminectomy was performed to expose the spinal cord at the C5 level on the right side of the spinal column. For animals in the SCI group, the spinal column was stabilized using micro Adson forceps attached to the surgical platform at the process of T2. An Infinite Horizon Impactor device (IH-0400, Precision Systems and Instrumentation; Lexington, KY) was used to administer a force-defined contusion. Force of 40 kilodynes, a 2 second dwell time, and an impactor tip of 0.7mm diameter were used. Stabilizing forceps were removed immediately after contusion, and 4-0 prolene sutures were used to close muscle incisions, followed by wound clip closure of the skin. Antibiotic powder (Neo-Predef, Zoetis Inc, Kalamazoo, MI) was applied to the sutured muscles prior to skin closure. Post-operative care consisted of subcutaneous injection of banamine (0.05 mg/kg) and ampicillin (0.05 mg/kg) in lactated Ringer's (0.5 mL) once daily for 3 days, and animal cages remained half on / half off heating pads for 72 hours post-surgery. Daily health checks were performed for the duration of the study in addition to monitoring animal grooming behaviors and weight.

2.3.4. Assessment of Mechanical Allodynia

To assess mechanical allodynia, we used the von Frey assay [26, 27]. Behavior testing was conducted in a blinded fashion once weekly beginning 2 weeks prior to injury (baseline) and continuing through 28 days post-surgery. Mice were habituated to the testing environment and testing chambers 1 hour daily for 5 days prior to baseline testing. Behavior testing was performed in a dedicated quiet behavioral suite at the same time every day during the animals' light cycle. Before each testing session, animals were acclimated to the testing room conditions for 30 minutes and were then acclimated in the

testing chambers for an additional 45 minutes. Mice were unrestrained in custom-built clear acrylic testing chambers (11cm x 8cm x 5cm) placed on a metal mesh platform. Compared to larger testing chambers, the small area and low ceiling reduced the frequency of exploratory and rearing behaviors, thus encouraging full contact and plantar placement of each paw on the platform (unpublished observations). The Electronic von Frey (EVF) and accompanying BIO-CIS Software were used to monitor mechanical allodynia (BIO-CIS; Pinellas Park, USA). To obtain a paw withdrawal, the spring-tip filament was continuously applied to the plantar surface of the animal's paw to obtain a continuous reading of force (grams) to the point where the animal rapidly withdrew its paw. Paw withdrawal was defined as a fast movement of the paw away from the stimulus [28]. Spontaneous movements were not considered withdrawal, nor were instances when the animal was exhibiting aggressive or avoidant behavior toward the stimulus (such as grabbing the spring tip sensor or attempting to bite the sensor). Each mouse was repeatedly tested for a total of five trials on each paw. The highest and lowest values were removed, and the middle 3 values were averaged. A minimum of 120 seconds interval time between trials of the same paw was used to reduce avoidant behavior and sensitization of the paw. Fruit loop cereal pieces were administered as treats between testing periods [27]. A total of 5 baseline scores were obtained for each paw of each individual animal, to generate a 'normal range' equaling the mean paw withdrawal threshold ± 2 standard deviations. Mice were determined post hoc to exhibit allodynia if they scored below their normal range for at least three out of the four post-surgical time points assessed (Fig. 2-5). Mice were excluded from the study if they

scored more than 2 standard deviations above their normal range for at least three out of the four post-surgical time points, demonstrating loss of sensory function.

2.3.5. Forelimb Grooming Score

Monitoring of animal grooming behavior has been established as an assay for determining the range of motion of mouse forelimbs [29]. To assess grooming behavior, animals were placed in a clear bin with a smooth bottom and observed for a 5-minute period. To encourage grooming behavior, a small water dropper was used to place a drop of water on the top of the animal's head. A score of 0-5 was assigned depending on the ability of the animal's forepaw to reach different regions of its face during grooming behavior, as previously described [29]. A score of 0 indicates that the forepaw did not make contact with the animal's head at all, a 1 indicates that the forepaw came into contact with the bottom jaw, a 2 indicates touch of the top of snout, a 3 indicates contact with area below the eyes, a 4 indicates above eyes and front of ears and a 5 indicates full range of motion all the way to the back of ears. The highest score achieved during the 5-minute period was recorded for each animal. All animals in this study exhibited a score of 5 prior to surgery. Grooming scores were assessed daily for 14 days post-surgery.

2.3.6. Tissue Collection

Mice were deeply anesthetized after behavioral testing at 28 days post-surgery using an overdose (three times the normal anesthetic dose) of ketamine/xylazine/acepromazine cocktail injected intraperitoneally. Once deeply anesthetized, animals were then perfused transcardially with approximately 50 mL each

of chilled 0.1 M phosphate-buffered saline followed by chilled 4% paraformaldehyde. Spinal columns were removed and post-fixed in 4% paraformaldehyde at 4°C overnight, and then moved to 30% sucrose for cryoprotection and storage at 4°C. Approximately 7 mm of cervical spinal cord spanning from spinal segments C2-C7 was collected, using dorsal root ganglia as landmarks.

2.3.7. Sectioning and Selection of Representative C4, C5 and C6 Spinal Segments

Serial transverse spinal cord cryosections (20 μm section thickness collected in a series of 12 slides) were used for immunostaining. The tissue was embedded in Optimal Cutting Temperature medium and frozen on dry ice. Cryosections were directly mounted onto gelatin coated slides and stored at -20°C. By sectioning the same anatomical regions of tissue from each subject and directly mounting tissue sections to slides, tissue sections could be accurately mapped to specific spinal segments. This method allowed us to select equivalent tissue sections from each animal, taken rostral to the injury at C4, at the lesion epicenter at C5, and caudal to the injury at C6. We verified accuracy of this method using a mouse spinal cord atlas to visually inspect and verify each tissue section during analysis [30].

2.3.8. Histology

All tissue sections were stored at -20°C and thawed at room temperature before staining. Tissue was first washed 3 times for 10 min each with tris-buffered saline (TBS) in coplin jars to remove residual tissue embedding medium. Slides were incubated in 5% donkey serum in TBS + 0.1% Triton-X-100 (TBS-T), and then incubated with primary

antibodies in 5% donkey serum in TBS-T for 24 hours in a humidifying chamber at 4°C. Tissue sections were stained with the following primary antibodies: neuronal marker NeuN (1:800, Millipore, ABN90), astrocyte marker glial fibrillary acidic protein (GFAP, 1:1000, Millipore, AB5541), and nociceptive fiber marker calcitonin gene-related peptide (CGRP, 1:1000, Abcam, ab36001). Tissue was then washed in TBS before incubation with AlexaFluor-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) in 5% donkey serum in TBS-T for 2 hours in the humidifying chamber at room temperature. Slides were rinsed in TBS containing the nuclear label DAPI (5 μg/mL, Sigma Aldrich, D9542), rinsed in deionized water, allowed to dry, and coverslipped with Mowiol mounting medium.

2.3.9. Fluorescence Microscopy and Image Analysis

Image acquisition: A Nikon Eclipse fully motorized upright fluorescent microscope equipped with monochrome camera and Nikon NIS-Elements software was used for image acquisition. Slides stained with fluorescent dyes were stored protected from light and imaged in a dark room. Images were acquired using the same acquisition settings across all samples for each immunohistochemical label. Images were exported as TIFF files for analysis. All image analysis was performed in a blinded fashion using ImageJ software. Sample sizes: Any tissue section showing poor immunolabeling or poor tissue quality were excluded from image analysis. This led to the exclusion of tissue from 1 SCI-S animal (poor tissue quality affected all immunolabeling), 5 SCI-NS animals (affected CGRP labeling), and 4 laminectomy animals (affected CGRP labeling). 1 laminectomy animal also exhibited poor NeuN labeling.

2.3.10. Neuronal Quantification

Images of NeuN immunoreactivity were saved as TIFF files and imported into ImageJ software. The grey matter of the spinal cord was divided into 4 quadrants using the line tool in ImageJ: left dorsal (LD), right dorsal (RD), left ventral (RV) and left ventral (LV) (Fig. 2-3). A vertical line was drawn through the dorsal median sulcus, central canal and ventral median fissure landmarks to divide the ipsilateral and contralateral halves. A horizontal line was drawn through the lateral column and central canal to create dorsal and ventral sides. To count individual cells, the counter tool was used to keep track of the cell count and total neuron counts were saved as a mask. Neuronal cells were identified by positive staining of NeuN co-localized to DAPI. Total cell counts within each quadrant were averaged across 7 total tissue sections for each individual animal (two sections at C4, three at C5, and two at C6).

2.3.11. Lesion Volume Quantification

Lesion volume was quantified using two independent methods, 1) volume bounded by GFAP immunoreactivity and 2) volume of spared gray matter. Images of GFAP immunoreactivity were saved as TIFF files and imported into ImageJ software. For each image that contained an obvious $GFAP^+$ astroglial border, ROIs were drawn along the $GFAP^+$ border to obtain the lesion area bounded by GFAP. Total lesion volume was calculated with the following equation:

Total lesion volume = [average area of lesion] x [section thickness] x [number of sections containing lesion] x 12

To express lesion volume as a percentage of spared gray matter, images of NeuN immunoreactivity were saved as TIFF files and imported into ImageJ software. ROIs were drawn around the boundaries of the gray matter where surviving neurons were visible. The contralateral (left) side of the tissue always appeared fully intact, exhibiting normal NeuN immunoreactivity; therefore, it served as an internal control for each sample. The ipsilateral (right) side of the tissue was outlined in the same fashion, and percent neuronal sparing was calculated using the following equation:

Percent gray matter sparing = 100 – 100 x [(left area – right area)/left area]

2.3.12. Quantification of CGRP$^+$ Axon Density

Images of CGRP immunoreactivity were saved as TIFF files and imported into ImageJ software. ROIs were drawn around left and right dorsal horns (laminae I-IV). Images were thresholded using the auto local threshold function with the Phansalkar method [31]. The total number of above-threshold pixels was calculated for each ROI.

2.3.13. Statistical Analysis

GraphPad Prism 8 (GraphPad Software, Inc.; La Jolla, CA) was used to perform statistical analysis. All data are presented as mean ± SEM. Statistical significance was defined as $p<0.05$.

2.4. Results

2.4.1. Evidence Of Mechanical Allodynia Of Ipsilateral Forepaw In A Subset Of Animals

We first determined whether the C5 hemi-contusion SCI model used in this study would lead to the development of neuropathic pain-associated behaviors, as previously described [18, 23-25]. To evaluate the incidence of mechanical allodynia, mechanical withdrawal thresholds were evaluated for all four paws prior to surgery and weekly following surgery for 28 days. Before surgery, baselines were obtained for each animal and used to generate a "normal range" of individual paw withdrawal by taking the average of 5 trials ±2 standard deviations of those trials. Scores for each post-surgical time point were normalized to individual baseline scores. For a given paw, if an animal scored below its normal range on 3 out of the 4 post-surgical time points assessed, the paw was categorized as "sensitive", indicating the presence of mechanical allodynia (Fig. 2-5). Individual animals' scores for each paw are shown in Fig. 2-6.

We found that none (0 out of 9) of the sham-operated animals met the criteria for "sensitivity" for any of the four paws, and post-surgical scores did not vary significantly from baseline (pre-surgical) scores in most cases (Fig. 2-1A-D). One exception was that sham animals exhibited contralateral forepaw withdrawal scores that were significantly higher than baseline at 21 DPI (Fig. 2-1A), due to two animals exhibiting desensitization (greater than 2 standard deviations above baseline) at that time point. In contrast, in 35.3% (6 out of 17) of animals with SCI, we observed the development of mechanical allodynia of the ipsilateral forepaw, with scores greater than two standard deviations below baseline at least 3 of the 4 time points post-injury. Group averages for SCI-S

animals were significantly lower than baseline at 7, 14 and 28 DPI, and approached significance at 21 DPI ($p = 0.0504$) (Fig. 2-1B). Of these 6 animals, 3 of the animals scored in the "sensitive" range for all four post-surgical time points. We did not observe any significant changes in paw withdrawal thresholds of the contralateral forepaws or hindpaws after SCI (Fig. 2-1A, C), although the ipsilateral hindpaws of 2 out of 17 animals (11.7%) were categorized as sensitive (Fig. 2-1D). Only one of the two animals that exhibited hindpaw sensitivity also exhibited forepaw sensitivity. Overall, of the SCI animals that did not meet our criteria for forepaw sensitivity, only 2 of these animals scored in the sensitive range only once, either on 14- or 21 DPI. In contrast, all 6 of the animals that did meet the criteria for forepaw sensitivity exhibited forepaw sensitivity on day 28 post-injury. Together, these observations suggests that in SCI-S animals, mechanical allodynia is persistent into the early chronic phase of SCI.

2.4.2. Subjects Characterized As Sensitive Had Mild Impairment Of Motor Function

In the same animals, we conducted grooming assessments for the ipsilateral forepaw for the first 14 days after sham or SCI surgery. Grooming analysis indicated immediate deficits in grooming behavior beginning 1 day post-surgery, including both SCI and laminectomy animals (Fig. 2-1E). By 5 days post-surgery, most animals in the laminectomy group recovered back to a score of 5, indicating a full range of motion. In contrast, animals in both SCI groups improved more slowly and recovered to a score of 4–5 by approximately 11 days post-surgery. A two-way ANOVA revealed significant main effects of time, group, and time x group (all $p < 0.0001$). While SCI-NS animals

exhibited grooming scores that were significantly lower than sham animals at 12 out of 14 time points post-surgery, SCI-NS animals were only significantly lower than sham animals at 3 of the 14 time points. Scores of SCI-S and SCI-NS groups were not significantly different than each other at any time point assessed (Fig. 2-1E). In summary, these findings demonstrate that mechanical allodynia of the ipsilateral forepaw developed in approximately 35% of animals following SCI, and that motor impairment may be more pronounced in animals that did not develop hypersensitivity compared with those that did.

2.4.3. Dorsal Horn Neuronal Loss Negatively Correlates With Mechanical Allodynia After SCI

The observation that only 30% of animals developed at-level allodynia of the ipsilateral forepaw led us to ask whether there were any observable differences in lesion size between animals with and without allodynia. We first employed commonly-used approaches to quantify lesion size [32]; namely, we calculated the volume of the lesion itself using GFAP labeling to define the reactive astroglial layer, and we also calculated percent gray matter sparing by drawing borders around gray matter that contained preserved neuronal cell bodies (Fig. 2-2A). For lesion volume (defined by the volume of tissue contained within the GFAP+ border throughout the entire rostrocaudal extent of the lesion), we did not observe any significant differences between groups (Fig. 2-2B; SCI-S = 0.0248 ± 0.0138 mm3; SCI-NS = 0.0419 ± 0.00991 mm3; $p = 0.344$). Although this method has classically been used as an indicator of lesion volume, we observed that neuronal loss was not contained to the GFAP lesion area; rather, loss of neuronal tissue

extended beyond the astroglial scar. For this reason, we quantified the area of tissue encompassing surviving neurons as another metric of lesion size. For analysis of spared gray matter, we assessed the percent area of tissue containing NeuN+ neuronal cell bodies in the ipsilateral spinal cord, normalized to the contralateral spinal cord, at the lesion epicenter as well as one spinal segment rostral and caudal to the epicenter (Fig. 2-2C).

We found that there was a trend toward reduced gray matter sparing in SCI-NS animals rostral and caudal to the injury epicenter (C4: $p = 0.185$; C6: $p = 0.177$), and significantly reduced gray matter sparing in SCI-NS animals at the lesion epicenter (C5: $p = 0.0426$). Results of this analysis also indicate that the lesion was spread over a greater rostrocaudal extent in SCI-NS animals compared to SCI-S animals. For example, we observed near-complete sparing at spinal segment C4 in all SCI-S animals, and at C6 in most SCI-S animals, but SCI-NS animals had a range of lesion severity at all segments assessed. Together, these data indicate that the development of at-level mechanical hypersensitivity only partially correlates with classical measures of lesion volume and severity.

With any experimental SCI model, injuries are never completely reproducible from animal to animal. Indeed, we observed a high degree of variability in the probe displacement upon impact, with SCI-S animals having a significantly lower probe displacement than SCI-NS animals (SCI-S: 596 ± 116 μm; SCI-NS: 1120 ± 110, $p = 0.0083$) (Fig. 2-7). Anatomically, we observed high variability among the lesions of individual animals in this study, based on GFAP as well as NeuN labeling (Fig. 2-2D).

Qualitatively, we observed that 4 out of 5 SCI-S animals (80%) had near-complete spared ipsilateral dorsal horn tissue, whereas only 3 out of 11 SCI-NS animals (27%) had only partial ipsilateral dorsal horn sparing (Fig. 2-2D). This observation led us to perform a more targeted analysis of neuronal sparing as an indicator of lesion severity. We quantified the number of neurons at C4, C5 and C6 to obtain a precise view of neuronal density. Quantification was performed specifically within the left and right dorsal gray matter quadrants as well as the left and right ventral gray matter quadrants. Notably, we found that there were significant decreases in neuronal density in the ipsilateral dorsal horn of SCI-NS animals, but not SCI-S animals, compared to control laminectomy animals (Fig. 2-3). At the lesion epicenter (C5), SCI-NS neuronal density was significantly lower than both SCI-S and laminectomy animals in the ipsilateral dorsal horn (Fig. 2-3A; SCI-S: 969 ± 110 neurons/mm3, SCI-NS: 548 ± 128, Laminectomy: 1150 ± 105, $p = 0.0033$ SCI-S vs. SCI-NS, $p < 0.0001$ SCI-NS vs. Laminectomy, $p = 0.351$ SCI-S vs. Laminectomy). At spinal segment C6, right dorsal cell counts were significantly reduced in SCI-NS animals versus controls (SCI-NS = 825 ± 121 neurons/mm3, Laminectomy = 1120 ± 71.3, $p = 0.0085$). We did not observe any other significant differences in any other quadrants at these levels, or at spinal level C4. These findings demonstrate that our injury model was inducing neuronal damage at the intended location. Furthermore, we found that neuronal density in the ipsilateral dorsal horn were negatively correlated with paw withdrawal thresholds at 3 out of the 4 time points (Fig. 2-8). Together, these data indicate that neuronal loss specifically in the dorsal spinal cord gray matter is exacerbated in animals that do not develop at-level

mechanical hypersensitivity after SCI, suggesting that increased dorsal horn neuronal sparing is more common in animals that develop pain-associated outcomes.

2.4.4. At-level Nociceptive Fiber Density Does Not Correlate With Sensory Outcomes

Previous work has demonstrated that sprouting of nociceptive afferents is associated with the development of neuropathic pain-like behaviors after SCI [13]. We therefore evaluated changes in peptidergic $CGRP^+$ fiber density at and around the lesion epicenter at 28 days post-injury or sham surgery (Fig. 2-4). Although we did not identify significant differences between ipsilateral dorsal horn $CGRP^+$ fiber density at any of the spinal segments assessed (Fig. 2-4A), we observed a trend toward decreased fiber density in SCI-NS animals compared to SCI-S animals, which reached close to statistical significance in the lesion epicenter (SCI-S: 0.961 ± 0.187, SCI-NS: 0.473 ± 0.162, $p = 0.0713$). This loss of $CGRP^+$ fibers at the lesion epicenter may reflect a loss of local dorsal horn neurons that represent the postsynaptic target population. Despite this, there was no difference between $CGRP^+$ fiber density of SCI-S and sham animals, indicating that the development of mechanical hypersensitivity cannot be attributed to at-level $CGRP^+$ nociceptive fiber sprouting. Indeed, we did not observe a correlation of $CGRP^+$ fiber density with paw withdrawal scores at any time point (Fig. 2-9). We also quantified $CGRP^+$ fiber density in the deep dorsal horn, laminae III-IV (Nees et al., 2016), but failed to observe any significant differences between groups (Fig. 2-4B). Together, these data indicate that nociceptive fiber density is not a reliable predictor of sensory dysfunction after cervical SCI.

2.5. Discussion

In this study, we have characterized distinct parameters of lesion anatomy in individual mice at 28 days post-SCI, exploring how lesion anatomy relates to, and could potentially be used to predict, the development of mechanical allodynia. Cervical spinal cord hemi-contusion is a clinically relevant injury model to assess both sensory and motor function after injury [18, 23-25]. Developing a better understanding of unique aspects of lesion anatomy will be useful to further guide the use of this injury model in preclinical SCI studies, as well as aid in the discovery of new targeted therapeutic approaches to modulate development of allodynia. We found that the subset of animals that developed at-level mechanical allodynia after SCI have a significantly higher degree of dorsal horn neuronal sparing compared to animals that do not develop allodynia. Based on this data, we propose that moderate contusions featuring smaller lesions and a higher percent of surviving neuronal tissue are more conducive to the development of mechanical allodynia versus injuries that ablate most of the dorsal horn at the lesion epicenter.

Allodynia occurs when a non-painful stimulus is perceived as painful, and mechanical allodynia is defined as a painful sensation caused by innocuous stimuli, such as light touch [33]. In humans with SCI, allodynia is a common manifestation of neuropathic pain [34]. However, those who suffer from neuropathic pain do not feel constant pain all the time; rather, the majority of individuals report that pain is intermittent [35]. Hence, in preclinical SCI studies it is important to consider the possibility that pain may also be experienced intermittently by animal subjects. In this study, we defined mechanical allodynia as an individual animal scoring more than 2

standard deviations below its baseline mean score (‘normal range’) for at least three out of the four post-surgical time points. This is a conservative definition of allodynia, and it is possible that more subtle anatomical changes than those we report here might lead to the development of allodynia that is less frequent. Much work is still needed to understand the factors that contribute to variable frequency of neuropathic pain.

Similar to other studies utilizing a cervical hemi-contusion SCI model, our data indicates that animals with sensitivity exhibited significantly decreased ipsilateral forepaw withdrawal thresholds compared to pre-SCI baseline scores; furthermore, we did not see any development of allodynia in the contralateral side of the animal, either at or below injury level [18, 23, 25]. We do report a significant increase in paw withdrawal thresholds of the contralateral forepaw; this could be due to the animal either bearing more weight on the non-injured side or because the animal was using this paw more frequently during normal motor function [36]. Here, we report that only 30% of animals developed allodynia at 28 days post-SCI. This is a modest percentage of animals with allodynia compared to previous studies utilizing a similar SCI model, which reported allodynia in most or all SCI animals [18, 25]. Instead of groupwise comparisons, we established a conservative criterion for categorizing individual animals as “sensitive” based on their post-surgical changes from baseline ranges. This allows us to better understand inter-animal variables that may contribute to changes in sensory function. Indeed, we did not detect any statistically significant differences between SCI and laminectomy groups when SCI animals were grouped together (data not shown). We

separated SCI-S from SCI-NS animals and found that 30% of animals could be classified as having developed allodynia, similar to previous reports [23].

Additionally, behavioral testing methods differ between our study and previous studies in several ways. Instead of manual von Frey filaments and the up-down method of assessing mechanical allodynia [27, 37], we utilized the electronic von Frey (EVF) system in this study. The use of the electronic von Frey system has several advantages; only one probe is used, which provides a continuous readout of the gram force being applied to the plantar surface of the paw rather than using individual monofilaments calibrated to buckle at fixed incremental forces [38, 39]. An important consideration in mechanical allodynia testing is to ensure accuracy in stimulus application to the forepaw. The dermatome innervated by spinal segment C5 is located at the plantar surface of the mouse forepaw, which is the site of von Frey filament application. Accurate placement of the von Frey filaments can be difficult due to the small size of the target region, and because mice are highly exploratory and move frequently. To further promote accurate testing of mouse forepaws, we engineered testing chambers with smaller area and lower celling than commercially available chambers in order to encourage the mouse to plantar place all paws during testing. We used this approach to allow the animal to be unrestrained during testing, as an alternative to gently scruffing the animals [18, 25]. It is possible that these differences in the testing approach may contribute to the observed discrepancies in behavioral outcomes.

Spinal cord lesions continue to develop after the primary mechanical injury, evolving into lesion sites that have high anatomical variability between animals in

preclinical studies [40]. The fully developed lesion is marked by a dense border of GFAP immunoreactivity making up the astroglial scar. These physiological responses to damage alter the composition of residing cells and extracellular matrix; such changes have been reported as a hallmark of SCI pathology and play a critical role in the extent of damaged tissue after mechanical trauma [32]. As expected, in most animals we observed extensive neuronal loss spanning 1-2 mm in the rostral-caudal axis. By analyzing tissue sections spanning the entire length of the injury we were able to determine lesion volume for each animal, defined both by the area contained within the $GFAP^+$ reactive glial border as well as by the area of spared gray matter. Our findings indicate that lesion size is not predictive of pain-associated outcomes, consistent with previous reports [23]. Interestingly, however, animals that did not develop mechanical allodynia had significantly decreased neuronal density in the ipsilateral dorsal horn at all spinal levels assessed (C4-C6), compared to animals that did develop allodynia. Often, we observed that the animals that did not develop mechanical allodynia had lesions in which the dorsal horn was severely damaged and in some cases, totally ablated. This was a surprising finding because we predicted that total ablation of dorsal horn neurons would result in severe sensory dysregulation due to a lack of sensory processing neurons at the spinal level innervating the dermatome being stimulated (C5). Unexpectedly, these animals remained within their 'normal range' of paw withdrawal thresholds, suggesting that some plasticity must occur in order for animals to perceive the evoked stimulus. Ultimately, this data suggests that both size and location of neuronal loss are important physiological characteristics that could be important in processing sensory information

after SCI. Furthermore, our data suggests that there must be some degree of sparing in the dorsal horn in order for the development of mechanical allodynia to occur. Additional work is needed to characterize the aspects of dorsal horn anatomy that are required for neuropathic pain to develop.

The Infinite Horizon spinal impactor system generates real-time readings during the time of contusion about the amount of tissue that was displaced during impact, the actual force of impact, and the velocity at which the probe was traveling upon impact. We anticipated that the observed variability in the lesion sizes of individual animals might be attributed to variation in the surgical parameters during the initial impact; we speculated that higher probe displacements would yield greater mechanical trauma and therefore result in larger lesion volumes. Despite this, we report that impactor probe displacement does not correlate to lesion size. This supports the notion that mechanical trauma is not predictive of the extent of the lesion volume and that secondary injury mechanisms play a large role in propagation of tissue damage. One factor that might lead to variability in contusion size is the precise placement of the impactor probe over the spinal cord. During a hemi-contusion surgery, the surgeon centers the probe over the hemicord, but a lack of precise landmarks makes it difficult to achieve perfectly reproducible probe placement from animal to animal. Because it has been previously shown that differences in white and gray matter mechanical properties lead to variability in spinal cord compression [41], we speculate that small (hundreds of microns) variations in the XY position of probe placement during surgery may underlie inter-animal variability in injury to the dorsal gray matter and overall lesion size.

2.6. Limitations Of This Study

A major limitation of this study is the low number of animals that were categorized as developing mechanical allodynia (N=5 SCI-S out of a total of N=17 SCI animals). As previously mentioned, we used a stringent criterion to define mechanical allodynia as the animal scoring below 2 standard deviations of their 'normal range' in three out of four post-surgical time points. Despite the low sample size, we were able to observe statistically significant differences in dorsal horn neuronal sparing between SCI-S and SCI-NS animals. It is possible that our study was underpowered to detect more subtle inter-group differences in lesion anatomy that might be observable with larger group sizes. Another limitation of this study is that we only used female mice. We recognize that there are differences in how males and females respond to pain, and that there are a multitude of factors to consider in how sex relates to pain processing [42]. For instance, the use of von Frey assay as a measure of mechanical allodynia has been shown to have statistical sex-dependent differences [43]. Current literature suggests that clinically, there is no sex difference regarding the development of neuropathic pain in SCI patients [44, 45]. However, beyond the scope of SCI, several reports have demonstrated sex differences in pain processing [42, 46-49]. Further work is needed to understand whether the findings reported in this study extend to males as well as females.

2.7. Conclusions

We have utilized a clinically relevant mouse model of cervical hemi-contusion SCI that results in neuropathic pain-associated responses in a subset of animals. We found that 30% of animals exhibited mechanical allodynia of the ipsilateral forepaw at 3 of the 4 weekly time points assessed following SCI. These animals had reduced forelimb motor impairment compared to animals that did not develop mechanical sensitivity after SCI. While lesion size was variable in all animals, we did not detect any significant differences in lesion volume, gray matter sparing, or $CGRP^+$ fiber density in the dorsal horn between sensitive and non-sensitive animals. However, we found that dorsal gray matter neurons at the lesion epicenter were spared in animals that developed sensitivity after SCI, but ablated in animals that did not develop sensitivity. Together, these findings illuminate a role for dorsal horn neuronal sparing in the development of mechanical allodynia after SCI, and highlight a need for future work to characterize how changes in local dorsal horn circuitry lead to at-level neuropathic pain.

2.8. Figures

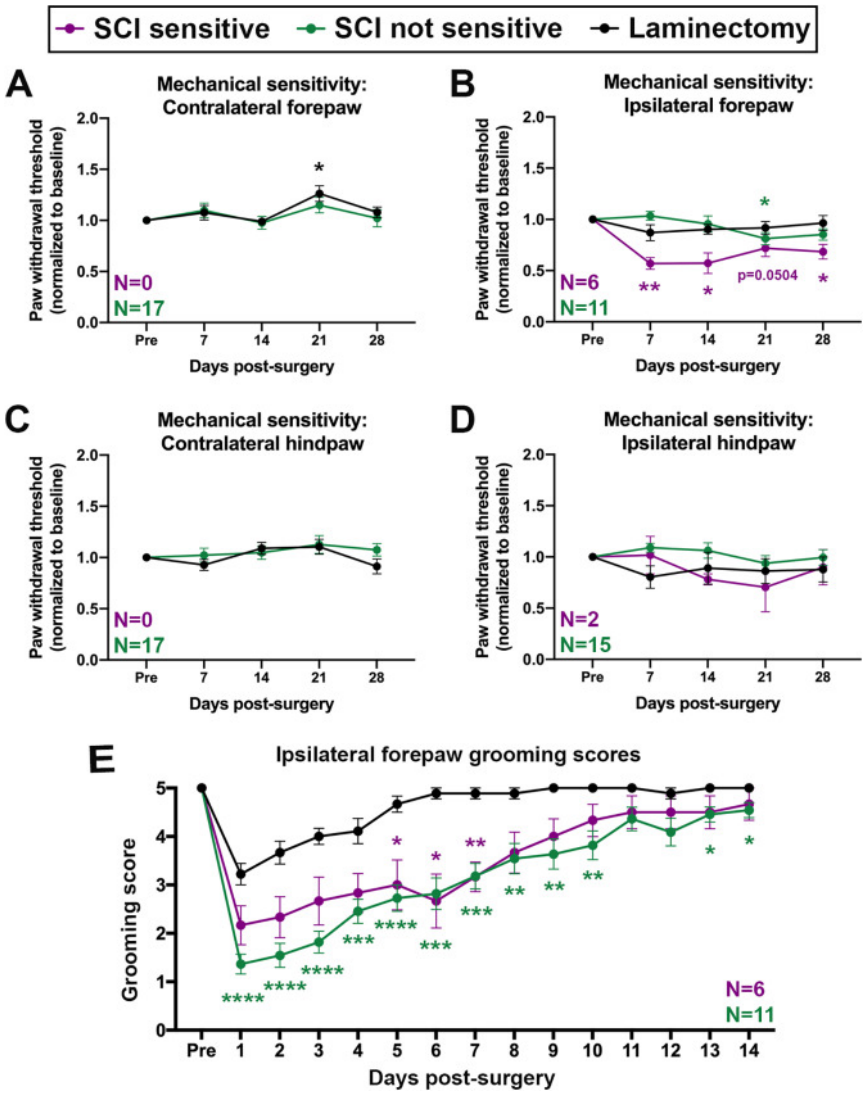

Figure 2-1 Cervical hemicontusion SCI leads to the development of mechanical allodynia of the ipsilateral forepaw in a subset of injured animals.
(A-D) Mechanical withdrawal thresholds for each paw at 7, 14, 21, and 28 days following SCI or sham surgery, normalized to baseline scores. In each graph, animals are categorized into "sensitive" or "not sensitive" based on scores for that individual paw, independently of scores obtained from the other paws. Animals in the laminectomy group never exhibited mechanical sensitivity. (E) Grooming scores over the first 14 days

following sham or SCI surgery; grouping based on mechanical sensitivity of the ipsilateral forepaw. Grooming data were analyzed by two-way ANOVA + Dunnett's multiple comparisons test (every group compared to every other group at each time point); asterisks indicate significant differences versus the laminectomy group. All data are mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. $N = 17$ SCI, $N = 9$ laminectomy.

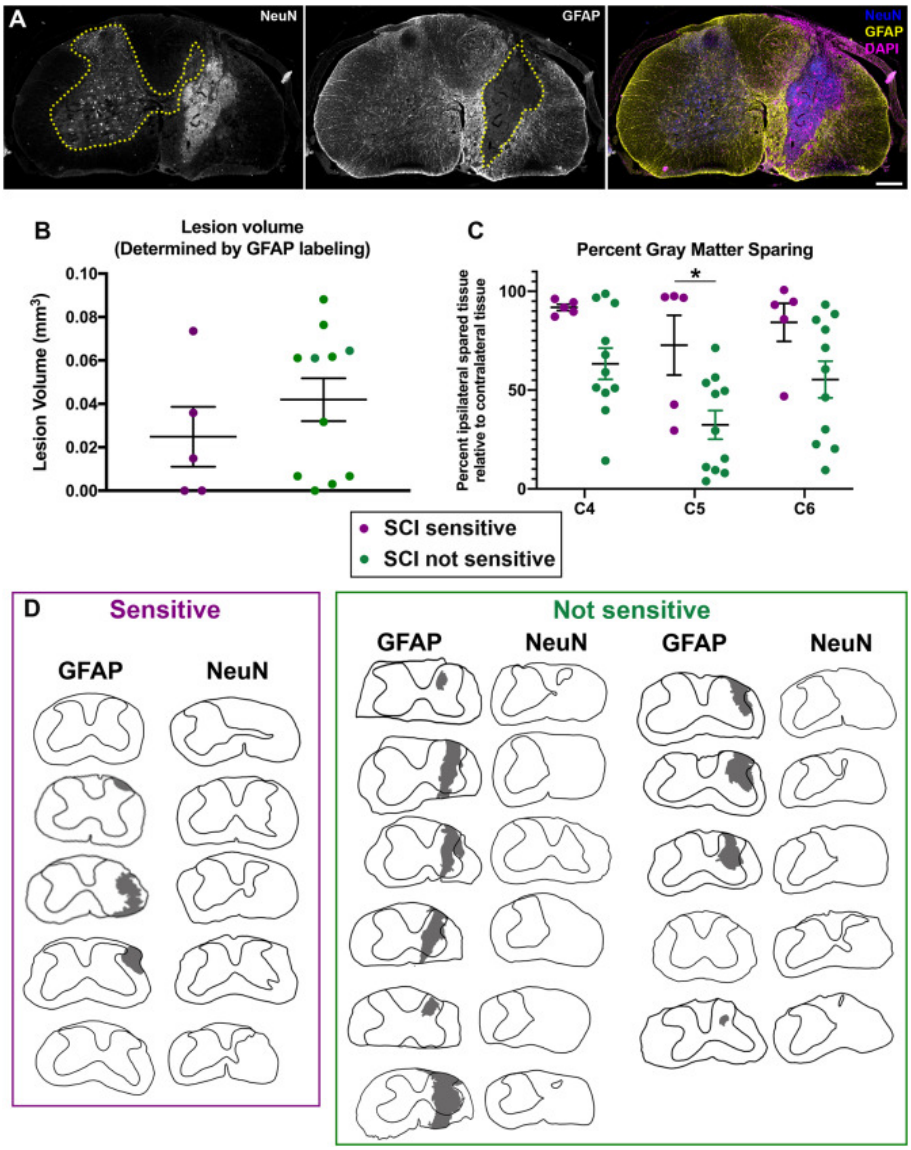

Figure 2-2 Quantification of lesion size at 28 days post-SCI using standard methods.

(A) Representative transverse images of the lesion epicenter at 28 days post-SCI. Dotted lines depict either the border of spared gray matter (NeuN), or the astroglial lesion boundary (GFAP). (B) Volume of tissue contained within the astroglial lesion boundary for animals with ipsilateral forepaw mechanical allodynia (purple) or no allodynia (green) at 28 days post-SCI. (C) Percent gray matter sparing in the ipsilateral spinal cord

relative to the contralateral side, for tissue sections through the lesion epicenter (C5) as well as rostral (C4) and caudal (C5) to the epicenter. (D) Traces of spinal cord tissue sections within the lesion epicenter. Each pair of drawings correspond to an individual animal. For lesion boundaries defined by GFAP (left columns), the shaded gray area represents the lesion contained by the astroglial boundary. For gray matter sparing (right columns), the border is drawn around preserved NeuN-containing gray matter. All data is mean ± SEM. Data in B was analyzed by t-test. Data in C was analyzed by mixed-effects analysis + Sidak's multiple comparisons test. N=4 SCI sensitive, N=12 SCI not sensitive. Scale bar = 250 μm.

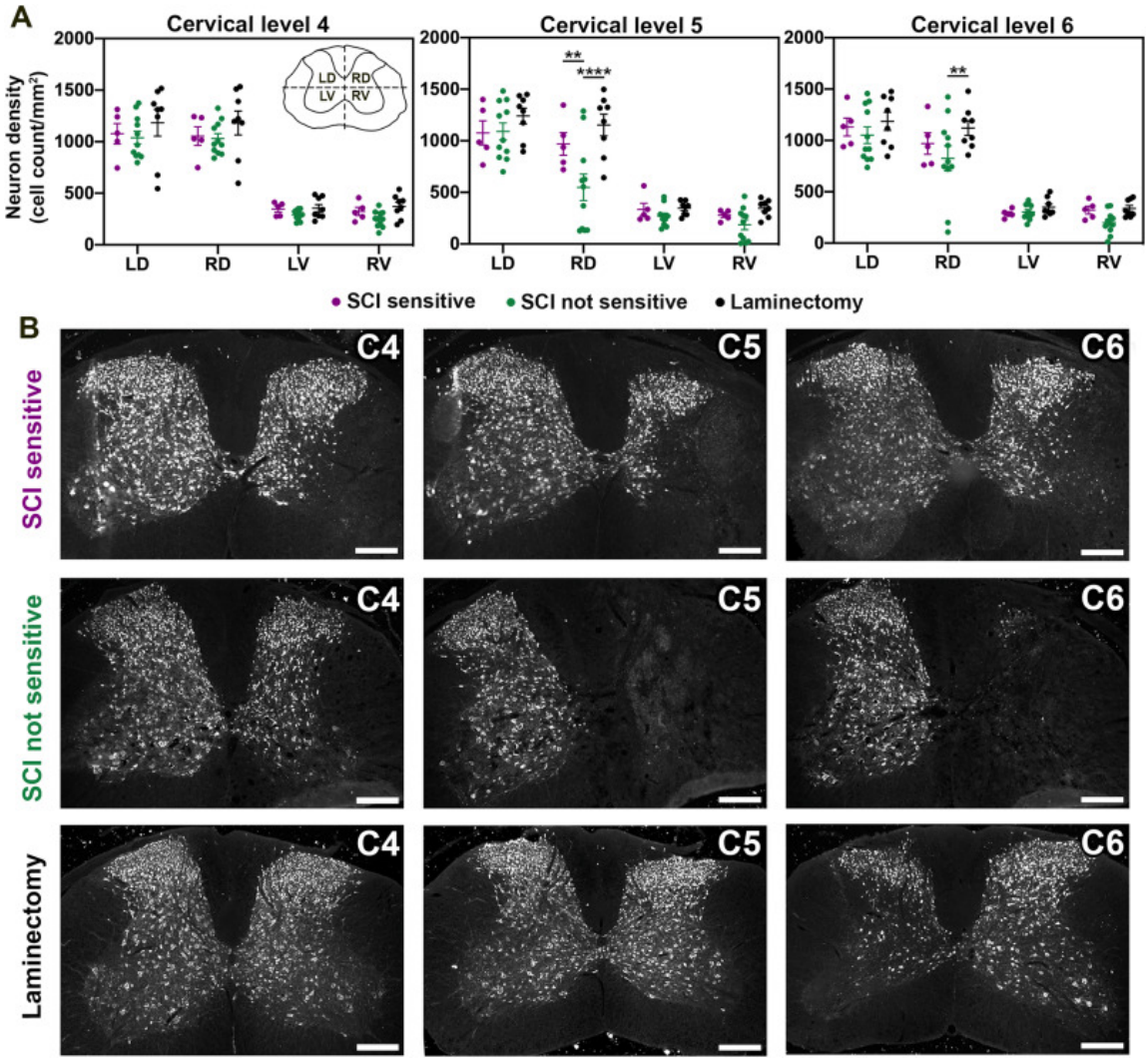

Figure 2-3 Quantification of neuronal density in dorsal and ventral spinal cord gray matter.

(A) Quantification of neuronal density in the left dorsal horn (LD), right dorsal horn (RD), left ventral horn (LV) and right ventral horn (RV). Data are shown for the lesion epicenter (C5), one segment rostral (C4) and one segment caudal (C6); data are quantified for each spinal level independently. Cartoon illustrates the four quadrants analyzed. (B) Representative images of NeuN immunoreactivity. All data are mean ± SEM; **p < 0.01, ****p < 0.0001 by two-way ANOVA + Tukey's multiple

comparisons test (every group compared to every other group for each quadrant). N = 5 SCI sensitive, N = 11 SCI not sensitive, *N* = 8 laminectomy. Scale bars = 250 μm.

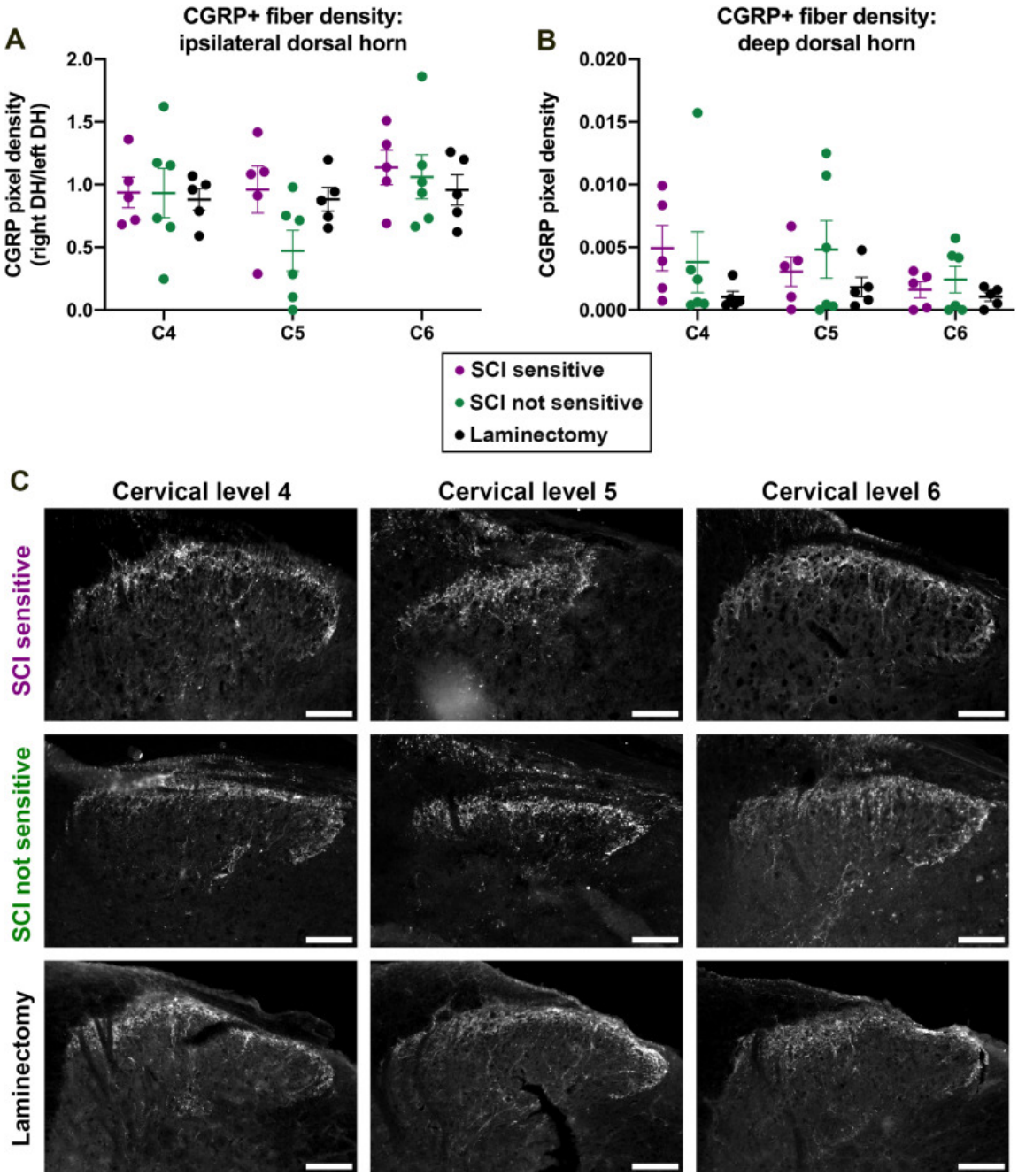

Figure 2-4 CGRP+ fiber density in the dorsal horn does not correlate with the development of at-level mechanical hypersensitivity.

(A, B) Quantification of CGRP^{+} axon density in (A) the entire ipsilateral dorsal horn and (B) the deep ipsilateral dorsal horn at spinal cord segments C4, C5, and C6; data are quantified for each spinal level independently. For both the whole dorsal horn and the

deep dorsal horn, no significant differences between treatment group were identified by two-way ANOVA + Tukey's multiple comparisons test. All data are mean ± SEM. N = 5 SCI sensitive, *N* = 6 SCI not sensitive, N = 5 laminectomy. C) Representative images of CGRP immunoreactivity in the ipsilateral dorsal horn at spinal cord segments C4, C5, and C6. Scale bars = 100 μm.

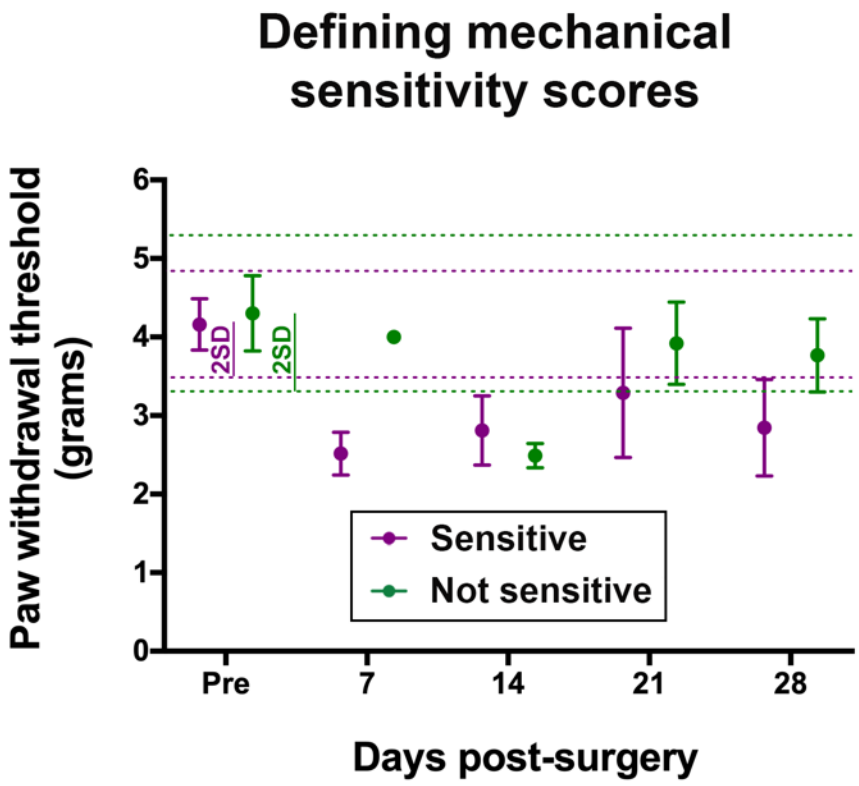

Figure 2-5 Approach used to categorize animals as "sensitive" or "not sensitive". Mechanical withdrawal scores (mean ± SEM) are shown for two representative animals that received SCI. Dashed lines indicate a range of ±2 standard deviations from each individual animal's mean baseline ("Pre") withdrawal score. Post-surgical scores falling below this normal range were classified as allodynic responses. An animal was categorized as "sensitive" (purple) if allodynia was observed at least 3 out of the 4 post-surgical time points; otherwise, the animal was classified as "not sensitive" (green).

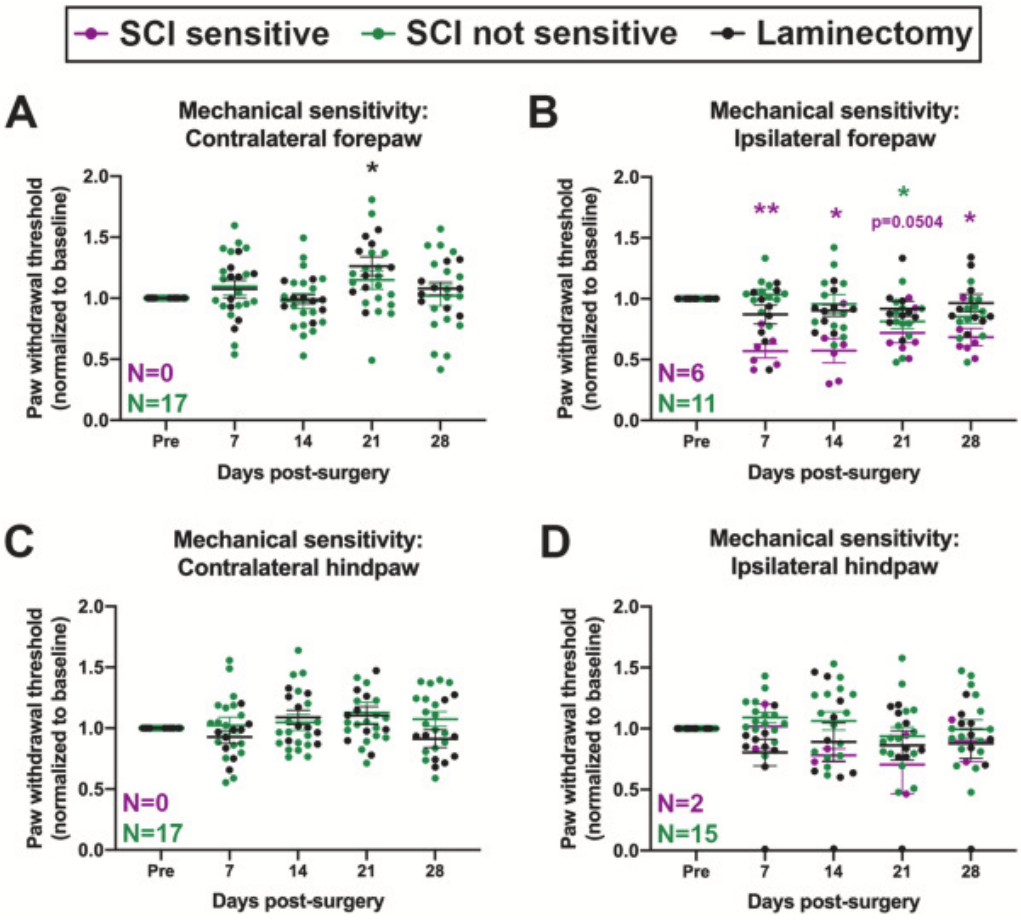

Figure 2-6 Individual data points for mechanical sensitivity scores.
These are the same data that is presented in Fig. 2-1. (A-D) Mechanical withdrawal thresholds for each paw at 7, 14, 21, and 28 days following SCI or sham surgery, normalized to baseline scores. Paw withdrawal data were analyzed by two-way ANOVA + Dunnett's multiple comparisons test (each time point was compared to pre-surgical baseline scores for that group). All data are mean ± SEM. *$p < 0.05$, **$p < 0.01$. $N = 17$ SCI, $N = 9$ laminectomy.

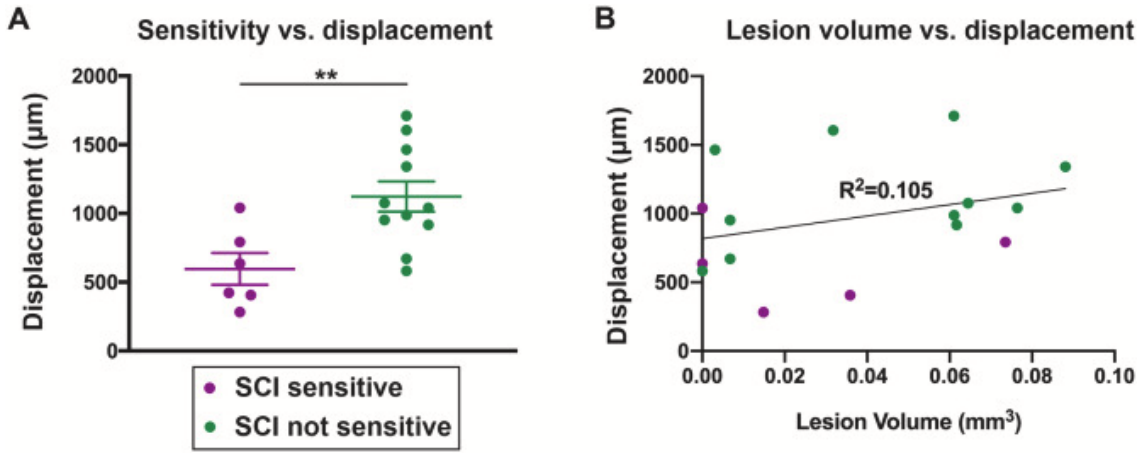

Figure 2-7 Probe displacement during SCI surgery does not correlate with lesion volume or the development of at-level mechanical hypersensitivity.
(A) Mean ± SEM probe displacement during spinal cord impact for animals with at-level mechanical hypersensitivity (purple) or no sensitivity (green); $^{**}p = 0.0083$ by Student's *t*-test. (B) Linear regression analysis of displacement versus lesion volume ($p = 0.220$).

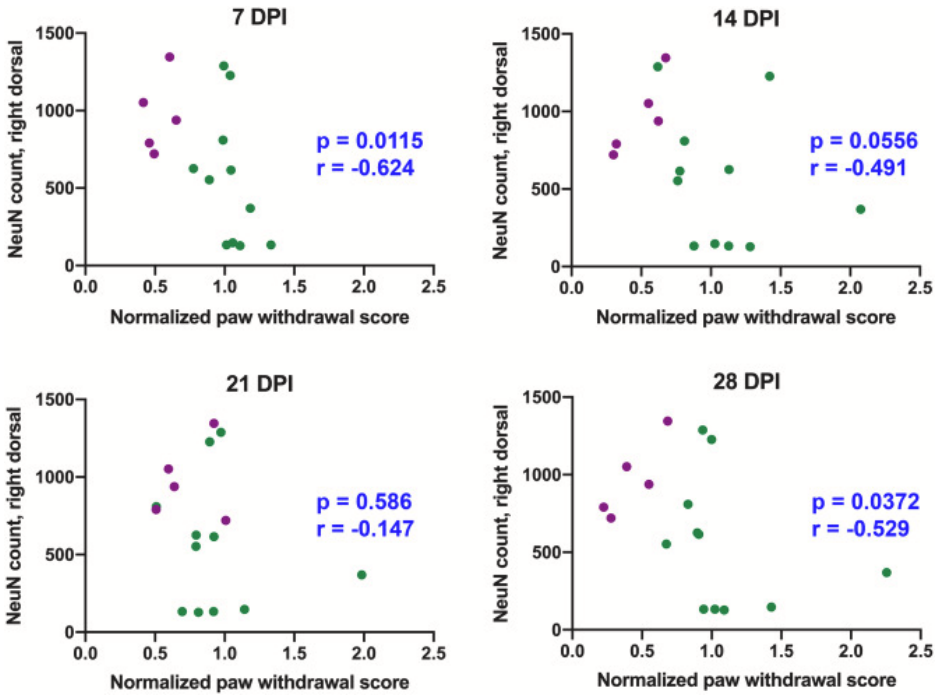

Figure 2-8 Correlation of paw withdrawal scores with dorsal horn neuronal density.
Data are plotted for individual animals that were classified as SCI-S ($N = 5$; purple) or SCI-NS ($N = 11$; green). On the x-axis is the von Frey score (normalized to baseline score for each individual animal) at the indicated time point post-SCI. On the y-axis is the density of $NeuN^+$ neurons in the ipsilateral (right) dorsal horn at spinal level C5 at 28 DPI. *P*-values are shown for Spearman's correlation analysis.

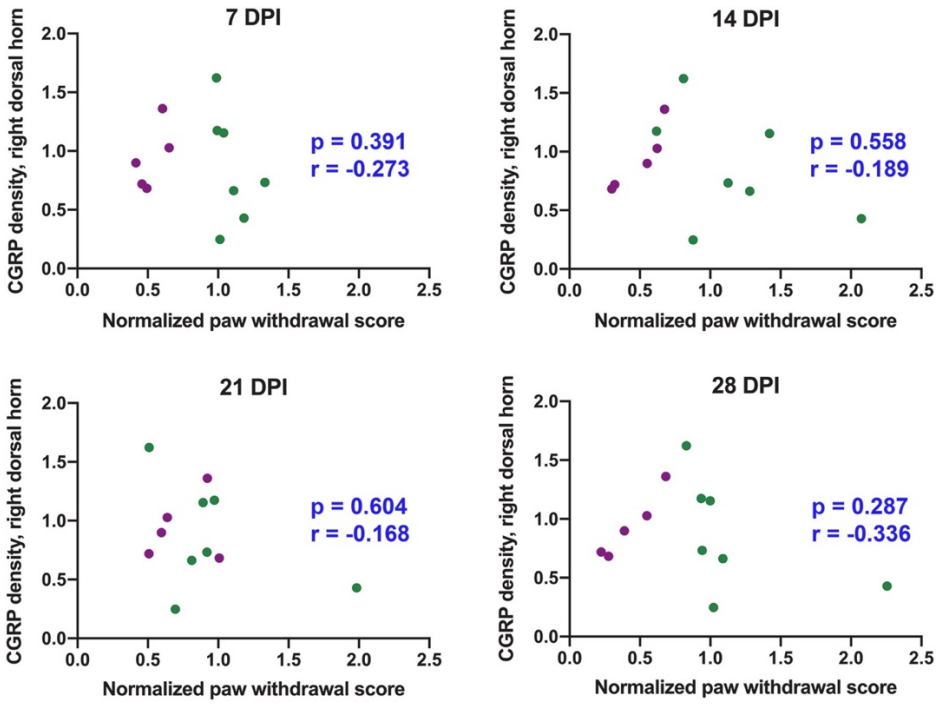

Figure 2-9 Correlation of paw withdrawal scores with dorsal horn CGRP+ fiber density.

Data are plotted for individual animals that were classified as SCI-S (N = 5; purple) or SCI-NS (*N* = 7; green). On the x-axis is the von Frey score (normalized to baseline score for each individual animal) at the indicated time point post-SCI. On the y-axis is the density of $CGRP^+$ fibers in the ipsilateral (right) dorsal horn at spinal level C5 at 28 DPI. P-values are shown for Spearman's correlation analysis.

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CHAPTER III FIGHTING FOR RECOVERY ON MULTIPLE FRONTS: THE PAST, PRESENT, AND FUTURE OF CLINICAL TRIALS FOR SPINAL CORD INJURY*

3.1. Manuscript Contribution To The Field

Decades of preclinical research have advanced our knowledge of spinal cord injury (SCI), paving the way for SCI clinical trials. There are currently 1,149 clinical trials registered in the U.S. National Library of Medicine that are focused on improving outcomes for those living with SCI. We conducted a systematic analysis of these SCI clinical trials based on data extracted from ClinicalTrials.gov, curating basic information about trials such as enrollment, phase, status, and numbers of interventions and outcomes. By categorizing each clinical trial according to the types of intervention being tested and the types of outcomes assessed, we have also identified major focus areas of SCI clinical trials as well as areas of growth and change over time. We also suggest potential areas for improvement with regard to clinical trial reporting, and interpret this data through the perspective of the clinician-scientist as well as the SCI community member. Collectively, our work represents the first systematic review of SCI clinical trials that will be useful for the scientist, the clinician, and the layperson.

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3.2. Abstract

Through many decades of preclinical research, great progress has been achieved in understanding the complex nature of spinal cord injury (SCI). Preclinical research efforts have guided and shaped clinical trials, which are growing in number by the year. Currently, 1,149 clinical trials focused on improving outcomes after SCI are registered in the U.S. National Library of Medicine at ClinicalTrials.gov. We conducted a systematic analysis of these SCI clinical trials, using publicly accessible data downloaded from ClinicalTrials.gov. After extracting all available data for these trials, we categorized each trial according to the types of interventions being tested and the types of outcomes assessed. We then evaluated clinical trial characteristics, both globally and by year, in order to understand the areas of growth and change over time. With regard to clinical trial attributes, we found that most trials have low enrollment, only test single interventions, and have limited numbers of primary outcomes. Some gaps in reporting are apparent; for instance, over 75% of clinical trials with "Completed" status do not have results posted, and the Phase of some trials is incorrectly classified as "Not applicable" despite testing a drug or biological compound. When analyzing trials based on types of interventions assessed, we identified the largest representation in trials testing rehab/training/exercise, neuromodulation, and behavioral modifications. Most highly represented primary outcomes include motor function of the upper and lower extremities, safety, and pain. The most highly represented secondary outcomes include quality of life and pain. Over the past 15 years, we identified increased representation of neuromodulation and rehabilitation trials, and decreased representation of drug trials.

Overall, the number of new clinical trials initiated each year continues to grow, signifying a hopeful future for the clinical treatment of SCI. Together, our work provides a comprehensive glimpse into the past, present, and future of SCI clinical trials, and suggests areas for improvement in clinical trial reporting.

3.3. Introduction

Spinal cord injury (SCI) is a devastating event, typically resulting in lifelong neurological deficits, which affects an estimated 253,000-378,000 persons in the US alone [1]. Individuals living with SCI and their loved ones face physical, emotional, social, and financial strain. It is estimated that the lifetime cost of SCI ranges from $1.2 to $5.4 million USD per person, with 30% of people undergoing re-hospitalizations one or more times during any given year following injury [1]. To date, a large number of clinical trials have been initiated in an effort to improve the lives of individuals with SCI. However, there remain no FDA-approved treatments that can even partially improve neurological dysfunction after injury [2-6]. In recent years, the establishment of various animal models has redefined our understanding of the mechanisms underlying SCI pathophysiology [7-16]. In addition, novel engineering applications ranging from cellular reprogramming [17-19], to the development of sophisticated technology [20-22], have opened new promising therapeutic avenues.

Since 2016, the National Institutes of Health has spent over $530 million on SCI research, and a substantial portion of that has gone toward supporting SCI clinical studies. Indeed, in 2021 more than 25% of NIH-funded projects related to spinal cord injury involved human subjects as reported by report.nih.gov/funding/categorical-

spending#/. While there is still no FDA-approved, proven effective treatment for SCI, some clinical studies have shown great promise, and research priorities of individuals living with SCI have been identified [23]. There have been several excellent reviews published discussing advances in key areas of SCI therapeutics, such as stem cell transplantation and neuromodulation [18, 24-28]. However, these reviews typically focus on outcomes and not general conclusions about the priorities, or evolution, of SCI clinical trials. To address this, we have conducted a systematic review of 1,149 SCI clinical trials using data extracted from ClinicalTrials.org and annotated by a team of investigators. We reviewed clinical trial characteristics including enrollment, phase, results, status, types and numbers of interventions and primary/secondary outcomes, as well as trends over time for the past 15 years. Collectively, this data provides the first comprehensive, systematic analysis of spinal cord injury clinical trials that will be of broad use for researchers, community members, and clinicians. Ultimately, the insights gained from this information highlight the need to continue pushing toward therapeutic interventions in such a way that is more efficient, held to higher reporting standards, and is overall more informative to the broad community.

3.4. Methods

3.4.1. Search Parameters and Exclusion Criteria

On January 10, 2022, a search was performed on ClinicalTrials.gov using “spinal cord injuries” as the keyword under the “Condition or disease” category. This broad search resulted in 1,411 clinical trials. We downloaded and exported all 1,411 studies with all available data columns as tab-delimited text files. The exported ‘raw’ data

included the following data categories: Rank, NCT Number, Title, Acronym, Status, Study Results, Conditions, Interventions, Outcome Measures, Sponsor/Collaborators, Gender, Age, Phases, Enrollment, Funded Bys, Study Type, Study Designs, Other IDs, Start Date, Primary Completion Date, Completion Date, First Posted, Results First Posted, Last Update Posted, Locations, Study Documents, and URL. Data was reviewed, classified, and annotated by a team of six investigators (V.A.D., N.R., K.K., S.M., M.P., J.N.D.), with each clinical trial listing reviewed by at least two independent investigators. Any discrepancies during this process were resolved through consultation between the reviewing investigators and a third reviewer from the team.

Prior to screening, we first excluded listings with Status that was classified as "Withdrawn", "No longer available", or "Temporarily unavailable", as well as trials that were classified as Study Type "Observational" (Fig. 3-1). Clinical trials with the status "Withdrawn" are defined by ClinicalTrials.gov as a trial that ended early before enrolling its first patient. Next, we excluded clinical trial listings that were targeted toward caregivers or healthcare providers, but not individuals with SCI. We removed one listing that was not a clinical trial but rather an expanded access program for an investigational new drug. Finally, we refined the list of clinical trials to exclude those that did not include a therapeutic intervention (intended to have a therapeutic or beneficial effect on patients with SCI), as judged by the investigating team. This led to the exclusion of trials that were focused on generation or validation of a diagnostic tool, identification of biomarkers, or development of an intervention without testing the

effects of the intervention. A total of 262 clinical trial listings were excluded based on these criteria, leaving 1,149 clinical trials used for analysis.

3.4.2. Clinical Trial Annotation and Classification

We generated categories for interventions and outcomes based on common themes that emerged upon reviewing the list of clinical trials. Categories are defined with examples in Tables 1 & 2. For intervention type, we formulated 14 unique categories: Acupuncture/needle therapy, Antibody therapy, Assistive/wearable technology, Behavioral, Biomaterials transplantation, Cell or tissue transplantation, Drug, Implanted/internal medical device, Nerve transfer/tendon transfer, Neuromodulation/electrical stimulation, Radiation therapy/laser therapy, Rehab/training/exercise, Surgical intervention/medical procedure, and Other (Table 1). The "Drug" category was further broken down into 15 subcategories according to the class or group of drug being tested. For types of primary and secondary outcome measures, we formulated 37 unique categories: Activity Level, Autonomic dysreflexia, Biomechanics/kinematics, Bladder function/bladder health, Blood pressure/cardiovascular function, Body mass/composition, Bone health, Bowel function/bowel health, Cognition, Depression/Anxiety, Employment/occupational performance, Fatigue, Fertility/sexual function, Independence, Medical imaging, Metabolism, Motor (lower extremities/locomotor function), Motor (not specified), Motor (trunk), Motor (upper extremities/hand function), Muscle and/or nerve function, Neurological score, Pain, Pharmacokinetics, Pressure injuries/pressure sores/wound healing, Psychological/social, Pulmonary function/breathing/cough, Quality of life,

Safety, Sensory function, Sleep, Spasticity, Survival, Thermoregulation, Usability/feasibility/satisfaction of the intervention, Wheelchair propulsion/mobility, and Other (Table 2).

The 1,149 clinical trials that met our inclusion criteria were then annotated according to the types of interventions used and the types of primary and secondary outcomes assessed (Table 1). For each trial, annotation was performed by at least two independent investigators. Only the information that was listed on the ClinicalTrials.gov webpage for a given clinical trial was used to categorize interventions and outcomes; no outside information (for example, information on other websites or published papers) was used to annotate trials. Interventions, primary outcome measures, and secondary outcome measures were annotated independently of each other, using the information available on the provided URL. If a clinical trial used multiple intervention types, each intervention type was listed once. For a given trial, if multiple outcome measures fell into the same category, that category was listed only once as an outcome for that trial. For example, a trial that lists several different measures of sexual function under Primary Outcomes on ClinicalTrials.gov would have "Fertility/sexual function" listed only once as a primary outcome type in our dataset. Primary and secondary outcomes are independent from one another, so it is possible that, e.g., "Fertility/sexual function" could be listed once under primary outcomes and once under secondary outcomes.

The annotated Excel file containing our classification of the 1,149 clinical trials is available as a supplemental file to this published dissertation.

3.5. Results

3.5.1. General Attributes and Demographics Of Spinal Cord Injury Clinical Trials

Of the 1,411 clinical trial listings identified, we excluded 262 trials that did not meet our eligibility criteria (Fig. 3-1). We identified a total of 1,149 interventional clinical trials for spinal cord injury listed on ClinicalTrials.gov from 1996-2021, which we annotated according to types of intervention and outcome measures (Table 1). We first analyzed general demographics and other attributes of the clinical trial data. We found that the numbers of new clinical trials per year have steadily increased over time, with 50% of all SCI clinical trials initiated between 2016-2021 (Fig. 3-2A). In 2021, 112 new clinical trials were initiated, the most of any year in history.

We next analyzed enrollment. ClinicalTrials.gov lists either estimated enrollment or actual enrollment; however, it is not clear whether estimated enrollments were actually met for most listings, if results are not posted. The majority of clinical trials have low enrollments; 73.0% of trials had enrollment of 50 subjects or less (Fig. 3-2B). Notably, only 9 of the 1,149 clinical trials had enrollment of over 500 participants. Among these were studies examining behavioral community wellness programs on the effects of lifestyle changes and transitions after injury (e.g., NCT03653390, "A Community Wellness Program for Adults Living With Long-term Physical Disability"; NCT02746978, "A Patient-centered Approach to Successful Community Transition After Catastrophic Injury"), as well as prospective studies examining the effects of surgical manipulations on outcomes such as survival rate (NCT01188447, "Evaluation of the Safety of C-Spine Clearance by Paramedics"; NCT03632005, "Negative Pressure Wound Therapy vs. Sterile Dressing for Patients Undergoing Thoracolumbar Spine

Surgery"). Only three clinical trials ranked in the top 20 of enrollment are focused on testing the effects of experimental interventions (methylprednisolone, NCT00004759; minocycline, NCT01813240; methadone, NCT00006448) on neurological outcomes.

There are five phases of clinical trial, defined on ClinicalTrials.gov as "*Early Phase 1 (formerly listed as Phase 0), Phase 1, Phase 2, Phase 3, and Phase 4.*" Some trials were also listed as combined Phase 1/2 or combined Phase 2/3. According to the ClinicalTrials.gov website, "Not Applicable" describes "*trials without FDA-defined phases, including trials of devices or behavioral interventions*", and this category should be chosen if the trial does not involve drugs or biological products (clinicaltrials.gov/ct2/about-studies/glossary). We found that 62.8% of trials were classified as "Not applicable", and the second highest category was Phase 2, at 9.83% (Fig. 3-2C). 50 trials did not have any data listed for the Phase category ("Not listed").

We further analyzed the types of intervention that were represented in each Phase of trial (Fig. 3-5). For trials that were classified as "Not applicable", 42.4% involved rehab/training/exercise, 33.1% involved neuromodulation/electrical stimulation, 19.5% involved assistive/wearable technology, and 18.7% involved behavioral interventions. Surprisingly, 38 of these trials did involve drugs, cells, or biomaterials, so it is unclear how phase classification is not applicable to these trials. One strong trend is that the representation of the Drug category increases with advancing phase. For example, drug-related interventions represent 27.0% of Phase 1 trials, 64.6% of Phase 2 trials, 76.7% of Phase 3 trials, and 84.6% of Phase 4 trials (Fig. 3-5). Other interventions decrease with

advancing phase; for example, cell or tissue transplantation represents 31.7% of Phase 1 trials, 14.2% of Phase 2 trials, but only 2.33% of Phase 3 trials and 0% of Phase 4 trials.

With regard to status, we found that 46.7% of the 1,149 trials were categorized as completed, whereas 23.1% were either recruiting or enrolling by invitation (Fig. 3-2D). 10.1% of the 1,149 trials were not recruiting, and 7.66% were either suspended or terminated. Of the trials that were completed and at least 1 year post-completion date at the time of the search, 75.4% of them (381/505) had no results posted to ClinicalTrials.gov, whereas only 24.6% had results (Fig. 3-2E). Of the 124 completed trials that had results, only 5 of those trials did not meet the primary endpoints; thus, 95.9% of completed trials with results posted were successful at meeting the primary endpoints. This information is indicated in Table 1. When we analyzed gender, we found that the overwhelming majority (95.6%) of 1,149 clinical trials were targeted toward all genders, while 3.57% listed only males and only 0.78% listed only females (Fig. 3-2F). Of the female-only trials, 8/9 of these were focused on women's health; for example, NCT02398331 "Sexual Health of Spinal Cord Injured Females" and NCT04872569 "Pilot Testing a Pregnancy Decision Making Tool for Women with Spinal Cord Injury". Many of the male-only trials were focused on men's health, including reproductive and sexual health (10/41; NCT00223873, "The Use of Penile Vibratory Stimulation to Decrease Spasticity Following Spinal Cord Injury"; NCT00421983, "Efficacy and Safety of Tadalafil in Subjects with Erectile Dysfunction Caused by Spinal Cord Injury), catheterization (8/41; NCT02230540, "Intermittent Catheterization in Spinal Cord Injured Men"), or testosterone replacement therapy (7/41; NCT00266864, "Testosterone

Replacement Therapy in Chronic Spinal Cord Injury"). A subset of male-only trials did not focus specifically on men's health (NCT02703883, "Body Weight Support in Spinal Cord Injury"; NCT01274975, "Autologous Adipose Derived MSCs Transplantation in Patient With Spinal Cord Injury").

3.5.2. Representation Of Intervention and Outcome Types

Types of primary and secondary outcomes were also analyzed. Outcome types are listed in Table 2. We found that the majority of the 1,149 trials (73.0%) examined 1 type of primary outcome, 16.8% examined 2 types of primary outcomes, and 4.96% examined 3; the remaining 5.22% of trials examined 4 or more types of outcomes, with a maximum of 12 types of primary outcomes tested in a single trial (Fig. 3-2G). Inclusion of a single primary outcome in most of these studies is consistent with the goal of addressing a focused research question [29], while inclusion of multiple primary outcomes can inflate the false positive rate [30]. For secondary outcomes, most trials (26.8%) examined only 1 type, though 22.4% did not examine any secondary outcomes (Fig. 3-2H). 34.5% of trials examined 3 or more types of secondary outcomes, with a maximum of 15 types in a single trial.

We next analyzed the numbers of intervention types and outcome types per trial. Intervention types are listed in Table 1. Of the 1,149 clinical trials, 72.1% listed only one intervention, and 24.2% listed two interventions; less than 5% of trials listed 3 or 4 interventions (Fig. 3-2I). Of the clinical trials testing more than one intervention, 74.8% of these featured Rehab/training/exercise as one of the interventions. Top combinatorial interventions included Assistive/wearable technology + Rehab/training/exercise

(25.5%), and Neuromodulation/electrical stimulation + Rehab/training/exercise (34.6%). Four trials had 4 interventions; for example, NCT02136823, "Impact of Persistent Conductances on Motor Unit Firing in SCI", tested the effects of three different drugs plus a stretching exercise on muscle reflex excitability.

We sought to quantify the number of clinical trials according to the types of intervention used, and the types of outcomes assessed. We first quantified the number of the 1,149 trials that used each of 28 classes of intervention, with Drug subcategories collapsed (Fig. 3-3A). We found that the highest-ranking category was Rehab/training/exercise with 386 clinical trials, followed by Neuromodulation/electrical stimulation (284 trials), Drug (all categories; 263 trials), Assistive/wearable technology (172 trials), and Behavioral (155 trials). We further broke down the Drug category into 15 sub-categories and found that neuromodulatory drugs were the most highly represented (70 trials) (Fig. 3-6). In addition to ranking interventions by the number of trials, we also calculated total human subject enrollment in all of the trials utilizing each intervention type (Fig. 3-3B). Using this approach, Rehab/training/exercise and Behavioral ranked highest with 15,824 and 15,650 enrolled, respectively. Drug (all subcategories; 15,753 enrolled) also had among the highest enrollments of any intervention. Some of the lowest categories by enrollment are Biomaterials transplantation (150), Nerve transfer/tendon transfer (237), and Acupuncture/needle therapy (421).

The primary outcomes associated with the greatest number of the 1,149 clinical trials were Motor (lower extremities/locomotor function) with 159 trials, Safety with 136

trials, Pain with 111 trials, and Motor (upper extremities/hand function) with 108 trials (Fig. 3-3C). Among the least-represented primary outcomes were Autonomic dysreflexia (3 trials), Thermoregulation (8 trials), and Sleep (9 trials). Upon calculating total enrollment for primary outcomes, we found that the highest enrollments were associated with Safety with 9236 enrolled, Pain with 6692 enrolled, Motor (lower extremities/locomotor function) with 6147 enrolled, and Neurological score with 5249 enrolled (Fig. 3-3D). Autonomic dysreflexia was still the lowest-ranked outcome by enrollment, with only 77 subjects enrolled in trials that evaluated it as a primary outcome measure. For secondary outcomes, we found that Quality of life was listed for the greatest number of trials (190 trials), followed by Pain with 190 trials, Other with 158 trials, and Motor (lower extremities/locomotor function) with 155 trials (Fig. 3-3E). Upon analyzing actual enrollment associated with secondary outcome measures, we found that there was much greater enrollment represented for secondary outcomes; the highest-ranked categories were Other with 15,115 enrolled, Quality of life with 12,765 enrolled, Usability/feasibility/satisfaction with 11,188 enrolled, and Pain with 10,438 enrolled (Fig. 3-3F). This reflects the finding that trials were likely to have a greater number of secondary outcomes listed compared to primary outcomes (Fig. 3-2G-H).

3.5.3. Trends In Interventions and Outcomes Over Time

We next sought to understand how interventions and outcomes have changed over time. Because of limited data availability for clinical trials initiated prior to 2007, we elected to focus on analyzing trends in data over the past 15 years, from 2007 to 2021. We first analyzed trends in interventions tested over time. In 2007,

drugs/biological compounds were the most represented intervention, with 37.8% of total interventions falling into this category (Fig. 3-4A). However, over time there has been a gradual decrease in the proportion of interventions that are drugs; most recently in 2021, only 8.02% of all interventions were drugs. Figure 3-4B shows the breakdown of different subcategories of drugs comprising the "Drug" category. In most years, neuromodulatory, herbal/natural, and "Other" subcategories represent the greatest contribution to the Drug category. While most types of interventions have remained relatively stable over time, the Neuromodulation/electrical stimulation and Rehab/training/exercise categories have increased over time (Fig. 3-4A). In 2021, Neuromodulation/electrical stimulation represented 27.8% of all interventions, and Rehab/training/exercise represented 25.3% of all interventions. In 2021 alone, 112 new clinical trials were initiated (Fig. 3-2A); of these, 45 utilize Neuromodulation/electrical stimulation, and 41 utilize Rehab/training/exercise. In the past five years (2017-2021), 162 new clinical trials for Neuromodulation/electrical stimulation and 190 new trials for Rehab/training/exercise were initiated.

We did not detect many major shifts in the representation of primary and secondary outcome measures over time (Fig. 3-4C-D). Some general trends emerged; for example, primary outcomes such as lower extremity motor function have stayed relatively steady over time, whereas upper extremity motor function has gradually increased (Fig. 3-4C). Some primary outcome measures, such as autonomic dysreflexia, thermoregulation, and depression/anxiety, have remained consistently underrepresented compared to other outcome measures. For secondary outcome measures, some have

remained consistently high over the past 15 years, such as pain, independence, and quality of life (Fig. 3-4D). Overall, the representation of most secondary outcomes has remained relatively stable. Together, these data reveal that representation of primary and secondary outcomes has remained relatively stable over time.

3.6. Discussion

3.6.1. Emerging Trends In SCI Clinical Trials

Of all the 1,149 clinical trials we reviewed, we observed that the majority of these enrolled less than 100 participants (Fig. 3-2B). The number of participants enrolled in a clinical trial is uniquely based on the design of the trial, phase of the trial and therapeutic being tested. Note that higher recruitment will be needed to sufficiently power the study [31, 32]. Enrollment of clinical trials specifically for SCI present challenges such as low incidence of injury, variable injury/severity among each participant, highly debatable approaches regarding therapeutic intervention and high cost of enrolled participants [33]. Several studies have examined these challenges of recruitment and the difficulties of maintaining recruitment in clinical trials and has opened the discussion for adaptive trial designs [33-42].

Notably, we found that 72% of SCI clinical trials employed only one intervention (Fig. 3-2I). It is a common consensus that to combat the complex nature of SCI, there will be no “magic bullet” single treatment; rather, effective therapies will likely be combinatorial in nature [25, 43-46]. Of the 28% of trials using more than one intervention, almost 75% of these employed rehab/training/exercise as one of the interventions. Furthermore, only 5.1% of these combinatorial trials are either Phase 3 or

Phase 4 studies. Hence, this data indicates a need to progress toward advancement of combinatorial clinical trials to combine the most promising therapies. Scientists and clinicians now face the challenge of figuring out how to incorporate rigor into study design while testing the greatest number of therapeutics in combination.

According to ClinicalTrials.gov, "*Primary and secondary outcomes are required by law to be analyzed and reported if any data was collected for the outcome. The primary and secondary endpoints should be pre-specified*". The primary outcome is the outcome measure of greatest importance and usually the one used in the power calculation during clinical trial design. The highest-ranked categories in primary outcome are motor (lower extremities/locomotion), safety, and pain while the lowest ranked are autonomic dysreflexia, thermoregulation, and sleep (Fig. 3-3C). Similarly, the highest ranked categories of primary outcome also have the highest enrolled participant totals, while autonomic dysreflexia also has the lowest number of enrolled participants (Fig. 3-3D). A natural question, therefore, is, "Does this reflect the priorities of the SCI community" [23]? However, this is a difficult question to answer. It is clear that the expressed needs and priorities change from person to person, and are dependent on a variety of factors such as injury level, severity, and time after injury (*i.e.*, acute or chronic) [23, 47-50].

3.6.2. Trends Over Time

Over the past 15 years, clinical trials have undergone some notable shifts in the representation of intervention and outcome types. It is important to note that clinical trial records may be incomplete prior to September 2007, when registration and submission

of clinical trials and study results with ClinicalTrials.gov first became legally mandated through Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801; clinicaltrials.gov/ct2/manage-recs/fdaaa), with the exception of phase 1 drug investigations, small clinical trials to determine feasibility, and certain clinical trials to test prototype devices (prsinfo.clinicaltrials.gov/ACT_Checklist.pdf). Hence, this could result in artificially low numbers prior to 2008, as there were likely more trials being conducted than were registered to ClinicalTrials.gov. Another consideration is that beginning in 2004, the International Committee of Medical Journal Editors (ICMJE) have required any interventional human trials to be registered at ClinicalTrials.gov as a prerequisite for publication (clinicaltrials.gov/ct2/manage-recs/background).

Beginning in 2007, the most represented intervention category was "Drug", mainly comprised of neuromodulatory drugs; this may explain why most clinical trials in advanced phases are drug-related. As the representation of drug-based interventions has gradually decreased over time, there were concomitant increases in both rehab/training/exercise and neuromodulation/electrical stimulation (Fig. 3-4A). This increase undoubtedly reflects advancements in technology allowing novel engineering of neuromodulation/electrical stimulation and a widely accepted consensus that rehabilitation is fundamental to improved outcomes [51, 52]. An example of this is the combination of assistive technology (*e.g.*, exoskeletons) with rehab/training/exercise. In 2014, the FDA approved the first robotic exoskeleton, ReWalk (ReWalk Robotics, Inc.) [53-55]. As noted above, hundreds of new clinical trials testing neuromodulation- and rehabilitation-based interventions have been initiated in the past few years alone. If this

trend continues, the future of clinical SCI research will be overrepresented with these types of interventions.

Although some outcomes—for example, bladder function/health as a primary outcome—appear to be have decreased representation over time (Fig. 3-4C), this is not due to a net reduction in bladder trials. For example, from 2007-2021 there has been an average of 4.2 ± 2.1 clinical trials measuring bladder function/health as a primary outcome per year, with 4 trials in 2007 and 4 trials in 2021 (Table 1). In other words, the total numbers of trials measuring bladder function/health are not decreasing over time, but as the number of total clinical trials grow, bladder outcomes are not keeping up. This is also true for trials measuring pain as a primary outcome; representation of pain appears to decrease over time, but studies have actually increased from 4 trials in 2007 to 11 trials in 2021 (Table 1). It is important to consider these trends in light of the challenges faced by the SCI community; for example, pain was ranked as the #1 most frequently cited challenged faced by those living with SCI according to a recent NASCIC survey [56].

3.6.3. Gaps In Clinical Trial Reporting

ClinicalTrials.gov was developed in an effort to make all ongoing trials accessible to clinicians and patients, combat publication bias, and enhance transparent reporting of clinical trials [57]. This website is a valuable data source, allowing users to track and evaluate the progression of clinical trials in a centralized repository with mandated regulations for reporting results [58]. This database also allows ease of systematic analyses elucidating trends in clinical trial design and in therapeutic

interventions, as others have done previously in different fields [59-61]. Our analyses clearly demonstrate that there are gaps in reporting including a lack of clarity with regard to categorizing trials as “interventional”, reporting the specific characteristics of the SCI itself, or reporting of study results. More broadly, multiple studies have identified areas for potential improvement in reporting and usability for ClinicalTrials.gov [62-64]. In 2021, Warner et al. conducted a systematic analysis on a subset of data extracted from spinal cord injury clinical trials; the authors identified key areas of improvement in reporting of these clinical trials [63]. For instance, only 11.2% of trials correctly identified their study type, provided valid study status and provided sufficient detail about injury characteristics [63].

In our analysis, gaps in reporting became apparent during systematic review of clinical trial characteristics. One of the most noteworthy examples is that although almost half of clinical trials were marked as “Completed”, 75.4% of completed trials have no results available on ClinicalTrials.gov (Fig. 3-2D-E). This is similar to a previous finding that only 23.5% of 344 SCI trials with “Completed” status had results posted on ClinicalTrials.gov [63]. However, we found that the absence of posted results did not necessarily mean that results from the study were not available elsewhere. We performed a PubMed search of 50 randomly selected trials that are listed as “Completed” with “No results available”, and found that 27 of 50 (54%) of these trials had published results associated with the study outcomes. ClinicalTrials.gov denotes that “when results are not available for a study, the results tab is labeled “No Results Posted”. Results of a study may not be posted for the following reasons: the study may not be subject to U.S

Federal requirement to submit results, the deadline for results submission has not passed or the submission of results information has been delayed by the submission of a certification or a request to extend the results submission deadline" as per the FDAAA 801 Final Rule (clinicaltrials.gov/ct2/about-site/history). This issue of reporting is not new and has been observed by authors of other meta-analyses based on ClinicalTrials.gov data [63, 65]. It is crucial that the public, scientific and clinical community be able to see results of clinical trials so that informed decisions can be made moving forward and integrated into the decision of participation, funding and approval of future clinical trials. Working with incomplete datasets leaves individuals unequipped to judge the novelty or innovation of future trials and can directly contribute to redundancy of clinical trials. To remedy this, we join others in suggesting that reporting publications and trial results to ClinicalTrials.gov should be required as part of clinical trial reporting standards (publications.parliament.uk/pa/cm201719/cmselect/cmsctech/1480/148002.htm).

These gaps in reporting underscore a need for better reporting standards and more transparent data sharing. Several studies have demanded that clinical trial results be open access [66] and have recommended that efforts be made to harmonize/standardize data elements so that comparisons between trials can be made [55, 67-71]. Several initiatives have been established to enhance data sharing such as the creation of Open Data Commons-SCI (ODC-SCI) enabling FAIR Sharing practices [72-75], the development of TRACK-SCI (Transforming Research and Clinical Knowledge in SCI) [76], the North American Clinical Trials Network SCI Registry [77], the

International Spinal Cord Society SCI Data Sets [78] and the National Spinal Cord Injury Statistical Center Database [79].

3.6.4. Perspectives From the Clinician-Scientist

In most cases, the burden of reporting falls on the clinician-scientists at the institution conducting the clinical trial [80]. Some institutions have supported the creation of administrative positions dedicated to clinical trials reporting to ease the burden of the primary investigator. However, in our experience, the greater challenge lies in the strict formatting of outcomes required by ClinicalTrials.gov. Whereas an Institutional Review Board can manage a variety of formatting, allowing for investigators to use language directly from a grant application, this is not available in ClinicalTrials.gov. This may directly impact data analysis because results for the funding agency is the priority. Similarly, results for a manuscript may take precedence over the results requested by ClinicalTrials.gov. Another obstacle is that clinicians are often asked to fill out required information in such a way that meets the website's standard but does not necessarily require important information (for example, we observed that several registered clinical trials left fields as "not listed", "unknown status" or "blank", see Fig. 3-2 and Table 1). This lack of "policing" has contributed to this incomplete data set where several trials do not have results posted or have left important information as inaccurately listed. It has become apparent that there needs to be a call for standardizing and updating these reporting standards. It could be beneficial to link IRB permitting with the ClinicalTrials.gov website thereby allowing more accurate reporting of data while also easing the paperwork burden on clinicians. Additionally, having IRB

mandate reporting of results with permit renewal to clinicaltrials.gov could present an avenue to enhance reporting of results.

3.6.5. Perspectives From the SCI Community

SCI research and clinical trials have been conducted for several decades, yet there remains no FDA approved, proven effective treatment for any outcomes associated with SCI; available treatment options are limited, and there is continuing debate about the standard level of care. There has been justifiable frustration and apathy expressed by individuals living with SCI in reaction to the promise of treatments being "just around the corner" fueled by media hype, as well as the slow pace of translation after decades of preclinical research [81].

Individuals with SCI have made clear their desire to be involved in the research process from start to finish [82]. In a 2019 study by the North American Spinal Cord Injury Consortium, community members ranked their highest priorities as receiving research information and serving as advisors to research teams [56]. This brings up two important topics of discussion: inclusion of lived experience consultants and accessibility of research to this population. As a direct result of this continuing call for inclusivity in research, some funding agencies such as the Department of Defense SCI Research Program and the Paralyzed Veterans of America Research Foundation have included individuals living with SCI as peer reviewers on their grant review panels and have required new grant submissions to include SCI consumer advocates or lived experience consultants to partner with research laboratories [83]. Additionally, several

institutions strongly encourage the development of partnership between researchers and SCI community.

With regard to accessibility of research, many barriers remain present. One major example that this review brings to attention is that although 76.5% of SCI clinical trials do not have results posted to ClinicalTrials.gov, it is often the case that if and when published results are posted, they are still inaccessible to general public due to subscription requirements for journal access. This is a major issue because if results are posted on ClinicalTrials.gov they are primarily in tabular format and lack interpretation that is present in peer-reviewed publications. *It is critically important for SCI community members to be able to access and interpret clinical trial data*. They need to be able to understand what types of clinical trials are ongoing, be able to determine whether there are any they are eligible for, and access/look at results so they can interpret results for themselves. Resources such as scitrials.org and scitrialsfinder.net are working toward this goal. It would be useful, for example, if the national clinical trial registry developed a systematic process for suggesting clinical trials tailored to individuals based on profile suitability rather than consumer demand. To date, "*ClinicalTrials.gov is designed to benefit the general public by expanding access to trial information*" [58], yet we found that this dataset was incomplete and will likely be inaccessible to the general public.

Finally, we have identified some actionable items that, if implemented, could be useful for improving the usefulness of clinical trial data to the SCI community. First, a designation labeling interventional SCI trials as "therapeutic" versus "not therapeutic" would be helpful; we found that 2.62% of SCI clinical trials labeled as "Interventional"

were not actually testing a therapeutic intervention (Fig. 3-1), and it would be useful for SCI community members to easily identify trials of therapeutics. Second, some clarification would be useful regarding future planned trials associated with a given intervention, and expectations for future clinical translation. We found that inconsistent or inaccurate application of FDA phase status, as well as the absence of sequential or graduated trial strategies, suggest that most trials do not appear to be designed to progress toward FDA approval. Additionally, it is unclear how much conceptual or programmatic overlap exists among clinical trials testing very similar interventions (e.g., neuromodulatory interventions for locomotor recovery), so some cross-referencing to indicate relationships between trials that are testing the same device, or trials that are otherwise linked in scope, would be useful. Finally, as a future goal, some integration of Clinicaltrials.gov with major data sharing initiatives would be a useful approach to recognize synergies between studies and improve clinical trial design moving forward into the future.

3.7. Conclusion

This systematic review provides a comprehensive view of SCI interventional clinical trials. The number of new SCI clinical trials initiated each year continues to climb. A large proportion of new trials are focusing on interventions such as neuromodulation, electrical stimulation, and rehabilitation. Over time, trials testing drug-based interventions have decreased in representation. These findings should be useful to scientists, clinical researchers, and the SCI community as a resource for understanding the trends in, and evolution of, interventional SCI clinical trials. However, gaps in

reporting to ClinicalTrials.gov may present barriers that will limit the usefulness of this data to the public, scientific, and clinical communities. There is a need for improving reporting standards to ClinicalTrials.gov.

Table 1: Intervention Categories

List of 14 classes of intervention used to classify spinal cord injury clinical trials. Each intervention type is defined and in some cases, examples of interventions are listed. Note that the "Drug" category encompasses 15 subcategories for different types of drugs and biological compounds.

Intervention Type	Definition & Examples
Acupuncture/needle therapy	**Definition**: Puncturing or pricking the skin with needles as a therapeutic practice.
Antibody therapy	**Definition**: Treatment with a monoclonal antibody.
Assistive/wearable technology	**Definition**: Any technology that is worn on the person or used by the person, which does not provide electrical stimulation or directly modulate the nervous system. **Examples**: Wearable garments, robotic gloves, prosthetics, orthoses, vibration/mechanical stimulation devices, CPAP, tongue-control devices, exoskeleton, adaptive robotic devices, adapted furniture, adapted environment.

Behavioral	**Definition**: Interventions that require the individual to modify their behavior, either short-term (during a study visit) or long-term (at home throughout the duration of the study), to produce a desired therapeutic effect. **Examples**: Phone apps, wellness or therapy groups, telemedicine programs, counseling programs, music therapy, educational programs, community programs, modifying diet or exercise routines, self-management routines, cognitive behavioral therapy, hypnosis, virtual reality programs presenting a different environment, visual illusions (*e.g.*, phantom hand).
Biomaterials transplantation	**Definition**: Transplantation of a bioengineered material or biological scaffold, which may or may not contain cells or tissue, into the spinal cord. **Examples**: NeuroRegen scaffold, polyethylene glycol, hyaluronic acid.

Cell or tissue transplantation	**Definition**: Transplantation of living tissue or cells, either into the spinal cord or somewhere else into the body. This excludes biomaterials. **Examples**: Neural stem cells, bone marrow stem cells, mesenchymal stem cells, umbilical cord blood-derived cells, Schwann cells, oligodendrocyte precursor cells.
Drug	**Definition**: A pharmaceutical compound, medicine, supplement, or biological compound that is ingested or delivered into the body. Definitions for some of the subcategories are included below. **Subcategories**: **Adenosine receptor agonist/antagonist**: A compound that modulates activity of adenosine receptors. **Adrenergic receptor agonist/antagonist**: A compound that modulates activity of adrenergic receptors.

	Anti-inflammatory: Non-steroidal anti-inflammatory drugs. **Antibiotic** **Botulinum toxin** **Cannabinoid**: Natural or synthetic compounds within the cannabinoid family. **Growth factor**: Recombinant growth factor such as FGF, EGF, NGF, BDNF. **Herbal/natural/supplement**: Includes vitamins, homeopathic treatments, probiotics, dietary supplements, herbal supplements. **Hormone** **Lidocaine** **Neuromodulatory**: A drug, not falling into the other subcategories, that exerts a direct effect on the nervous system; examples include neurotransmitter reuptake inhibitor or a compound that mimics the effect of a neurotransmitter. **Opioid** **Statin**

	Vasoactive: A drug that exerts effects on blood vessel dilation/constriction and blood pressure. **Other:** Any drug not falling into one of these subcategories.
Implanted/internal medical device	**Definition**: An implanted device that is worn inside the body, but does not provide electrical stimulation. This does not include software or assistive devices that are not worn, or worn on the outside of the body. The implanted device can either be permanent or removable. **Examples**: Indwelling catheters, bowel irrigation devices, recording or monitoring devices, colonic tubes, implanted array to monitor but not stimulate brain activity.
Nerve transfer/tendon transfer	**Definition**: A surgical procedure in which either nerves or tendons are surgically cut and transferred to another nerve or muscle.

Neuromodulation/electrical stimulation	**Definition**: An intervention in which electrical or magnetic stimulation is used to elicit activity of the nervous system. Electrodes or electrical fields can be used. The effect is that some part of the nervous system is stimulated. **Examples**: Functional electrical stimulation, epidural stimulation, peripheral nerve stimulation, transcranial magnetic stimulation, direct current stimulation, transcutaneous stimulation, transcranial stimulation with ultrasound.
Radiation therapy/laser therapy	**Definition**: Treatment with ionizing radiation, UV light, X-ray, or lasers.
Rehab/training/exercise	**Definition**: Any type of intervention comprised of exercise, activity-based training, or physical rehabilitation. **Examples**: Exoskeleton-mediated walking, treadmill training, stepping training, walking training, upper limb cycling, intermittent hypoxia,

	breathing training, high-intensity interval training, exercise regimens, passive motion exercises.
Surgical intervention/medical procedure	**Definition**: Surgical manipulations, surgical interventions, medical procedures, or procedure done during a spinal cord decompression surgery, except for nerve and tendon transfers. The surgery or procedure must be the primary intervention to be performed/evaluated. **Examples**: Surgical decompression, controlled surgical lesions of the nervous system, bladder surgeries, comparing or validating different methods of performing surgery, sustained induced hypertension/hypotension, hypothermia, bronchoscopy.
Other	**Definition**: Any intervention that does not clearly fit into the above categories.

	Examples: Passive heat stress, hypothermia, extracorporeal shockwave therapy, ischemic conditioning.

Table 2: Outcome Measure Categories.

List of 37 classes of outcome measure used to classify spinal cord injury clinical trials. Each outcome type is defined and examples of measurements or scores related to the outcome type are provided.

Outcome Type	Definition & Examples
Activity level	**Definition**: Assessments of physical activity level. **Examples**: Level of physical activity; the Physical Activity Scale for Individuals with Physical Disabilities (PASIPD); Physical Activity Questionnaire for People with Spinal Cord Injury (LTPAQ-SCI), International Physical Activity Questionnaire.
Autonomic dysreflexia	**Definition**: Adverse events resulting from overactivity of the autonomic nervous system in response to stimulation. This does not include

	autonomic function-related outcomes such as autonomic classification, autonomic control of respiratory or cardiovascular function
Biomechanics/kinematics	**Definition**: Measurements of joint position, joint angles, torque, forces, and/or movement of the limbs during motor activity. **Examples**: Torque, resistance to stretching, degrees of flexion/extension of the arm or leg muscles, foot trajectory, propulsion, echogenicity ratio, load, contact time, muscle activity patterns during motion, joint forces.
Bladder function/bladder health	**Definition**: Measurements of bladder function or bladder health. **Examples**: Bladder filling, bladder voiding, bladder emptying, bladder pressure, compliance, leakage, frequency of urination, frequency of catheterization, neurogenic bladder, urinary tract infections.

Blood pressure/cardiovascular function	**Definition**: Measurements of blood flow, blood pressure, or heart function. **Examples**: Blood pressure, systolic blood pressure, hypotension, hypertension, heart rate, cerebral blood flow, arterial stiffness, Cerebral Vascular Resistance Index, VO2peak (peak oxygen consumption), autonomic control of cardiovascular function, head-up tilt test, aerobic capacity.
Body mass/composition	**Definition**: Assessments of body mass or body composition. **Examples**: Body weight, body mass index, whole body skeletal muscle and fat mass, percentage of body fat, fat mass/fat-free mass.
Bone health	**Definition**: Assessments of bone health. **Examples**: Bone mineral density, bone health, bone mass, DXA scanning, osteoporosis, fracture, integral volumetric bone mineral content.

Bowel function/bowel health	**Definition**: Assessments of bowel function or health. **Examples**: Bowel function, bowel emptying, frequency of bowel movements, bowel management, bowel care routine, constipation, Knowles Eccersley Scott Symptom (KESS), Patient Assessment of Constipation Quality Of Life scale (PAC-QOL), Neurogenic Bowel Dysfunction (NBD) score.
Cognition	**Definition**: Assessments of cognitive ability. **Examples**: Memory, d2 Test of attention, any cognitive tests including, verbal learning test, word association tests, Stroop test, Cognitive Functioning as Measured by PASAT, Performance on Cognition Battery Tests, Performance on tests of information processing (WAIS-IV and Digit Span) and working memory (SDMT).

Depression/anxiety	**Definition**: Assessments of depression and/or anxiety. **Examples**: Depression symptoms, Anxiety symptoms, Hamilton Depression Rating Scale, HAM-D, 16-Item Quick Inventory of Depressive Symptomatology - Self Report (QIDS-SR16), Depression Scale of the Patient Health Questionnaire (PHQ-9), Change in Patient Health Questionnaire-9 for measure of patient depression severity.
Employment/occupational performance	**Definition**: Assessments or indices of employment or performance of occupational tasks. **Examples**: Ability to perform occupational tasks, rate or success in employment, perform work-related tasks, Canadian Occupational Performance Measurement (COPM).
Fatigue	**Definition**: Assessments of physical or cognitive fatigue or exertion level.

	Examples: Physical fatigue, cognitive fatigue, exertion level, perceived exertion, muscle fatigue.
Fertility/sexual function	**Definition**: Assessments of sexual function, sexual health, or fertility. **Examples**: Sexual health, sexual function, male sexual function, female sexual function, sexual quality of life, sexual dysfunction, fertility, sperm count, sperm viability, sperm health, ejaculation, erectile function, best method to obtain semen.
Independence	**Definition**: Assessments of the subject's level of independence in daily life. **Examples**: Independence, Spinal Cord Independence Measure (SCIM or SCIM-III), Spinal Cord Independence Measure-Self Reported (SCIM-SR), Craig Handicap Assessment and Reporting Technique (CHART), Functional Independence

	Measure (FIM), Wheelchair independence, performance of daily tasks.
Medical imaging	**Definition**: Noninvasive measurements of brain activity or anatomical parameters. **Examples**: Functional magnetic resonance imaging (fMRI), BOLD signal, MRI, X-ray, CT scan, DXA scan.
Metabolism	**Definition**: Assessments of body metabolism at the molecular level. **Examples**: Metabolic health, metabolism, resting metabolic rate, measurement of metabolites in the blood plasma or other body fluids, expression of gene products or metabolites, fasting insulin, fasting glucose, hemoglobin, insulin or glucose sensitivity, oxygen uptake, lipid measurements, circulating markers, inflammatory markers, blood assays, metabolic panels, energy expenditure.

Motor (lower extremities/locomotor function)	**Definition**: Assessments of lower body motor functions such as walking, ambulation, stepping, standing, or any other motor function of the lower extremities. **Examples**: Ten meter walk test, six minute walk test, WICSI-II, FIM gait score, Spinal Cord Injury Functional Ambulation Index (SCI-FAI), Berg Balance Scale (BBS), Lower-Extremity Motor Scores (LEMS), walking function, stepping function, standing, sit-to-stand.
Motor (not specified)	**Definition**: Assessments of motor function that are not specified as lower body, upper body, or trunk function. **Examples**: Strength, voluntary movement, task completion, physical function, motor function.
Motor (trunk)	**Definition**: Assessments of trunk motor function including trunk stability, trunk coordination, and sitting balance.

Motor (upper extremities/hand function)	**Definition**: Assessments of upper body and arm/hand motor functions. **Examples**: Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) strength subscale, upper extremity muscle strength, Manual Muscle Testing (MMT), Hand Held Dynamometry (HHD), Grasp-Release Test, Activities of Daily Living Test, hand grasp, grip strength, upper motor strength, Disabilities of Arm, Shoulder, and Hand (DASH) scores, Michigan Hand Questionnaire (MHQ), Hand Function Tests.
Muscle and/or nerve function	**Definition**: Physiological assessments of muscle, nerves, and reflexes; not including motor functional outcomes. **Examples**: Muscle area, muscle cross-sectional area, motor evoked potentials (MEPs), H-reflex, nerve conduction velocity, muscle stretch reflexes, reflex activity, excitability, muscle activation, resting motor threshold (RMT), Physiology

	Measurements, electromyography (EMG), Single pulse transcranial magnetic stimulation, nerve action potential latency of nerve conduction studies.
Neurological score	**Definition**: This is a specific terminology that refers to the scores of a neurological exam or the level/degree of neurologic lesion. **Examples**: The ASIA impairment scale (AIS) score, the International Standards for Neurological Classification of Spinal Cord Injury (ISNC-SCI) exam.
Pain	**Definition**: Assessments of pain or pain relief. **Examples**: Pain reduction, Pain severity, Pain interference on quality of life, Mean Pain Intensity, Numeric Rating Scale (NRS), Neuropathic pain scale, International Basic Pain Dataset, mechanical allodynia, Patient-generated Index (PGI), Pain unpleasantness, Wheelchair User's Shoulder Pain Index (WUSPI), musculoskeletal pain.

Pharmacokinetics	**Definition**: Measurements of drug pharmacokinetics. **Examples**: Tolerability, blood serum and cerebrospinal fluid (CSF) levels of the drug, Pharmacokinetic (PK) profile, dosing concentration and drug levels over time, Area Under the Concentration-Time Curve.
Pressure injuries/pressure sores/wound healing	**Definition**: Measurements of pressure injuries, sores, or ulcers, or related parameters. **Examples**: Incidence of pressure ulcers/injuries/sores, wound healing, skin irritation, pressure on skin, bleeding.
Psychological/Social	**Definition**: Assessments of psychological and/or social health and well-being, not related to depression/anxiety. **Examples**: Mood, loneliness, Neuropsychological Tests, social integration, caregiver burden, social

	problem solving, self-esteem, life satisfaction, self-efficacy, social connectedness, perceived stress, The Ways of Coping Scale- Revised (WOC-R), Community Integration Questionnaire (CIQ), Stage of change Scales (SOC), resilience.
Pulmonary function/breathing/cough	**Definition**: Assessments of lung function, breathing, or cough. **Examples**: Pulmonary function, postoperative pulmonary complications, Lung volume, lung capacity, air flow, airway pressure, respiratory motor control, inspiratory/expiratory pressure, inspiratory/expiratory duration, inspiratory/expiratory function, autonomic control of respiratory function, forced vital capacity (FVC), peak inspiratory/expiratory flow, Exhaled Breath Condensate, forced expiratory volume, peak cough flow.

Quality of life	**Definition**: Questionnaires or surveys that allow the patient to self-assess their quality of life (QoL) and/or overall satisfaction with life. **Examples**: Quality of Life Index SCI version (QOLI-SCI), quality of life, satisfaction with life, Satisfaction with Life Scale (SWLS), Life satisfaction Checklist (LiSat-11), World Health Organization Quality of Life (WHQOL), RAND-36 questionnaire to measure health-related quality of life, Quality of Life on the SCI QL-23, EuroQoL.
Safety	**Definition**: This refers to the safety of the intervention being tested. Safety may be assessed by the number or frequency of adverse events (hospital visits, complications, infections, toxicity).
Sensory function	**Definition**: Assessments of sensory function or sensation anywhere in the body, except for pain. **Examples**: Pinprick sensory test (sharp versus dull with a safety pin), touch sensory test (with a cotton

	ball), sensory discrimination, Sensation of urinary bladder filling, sensation in the legs, Thermal sensation, sensory examination, Graded Redefined Assessment of Strength, Sensation and Prehension (GRASSP), Semmes Weinstein monofilament sensation test.
Sleep	**Definition**: Assessments of sleep quality. **Examples**: Sleep quality, sleep apnea, apnea index.
Spasticity	**Definition**: Assessments of spasticity. **Examples**: Participant reported spasticity, severity of spasticity, Modified Ashworth Scale (MAS), Portable Spasticity Assessment Device (PSAD), Modified Penn Spasticity scale, Spinal Cord Injury Spasticity Evaluation Tool (SCI-SET).
Survival	**Definition**: Survival of patients at defined timepoints after treatment.

Thermoregulation	**Definition**: Measurements of body temperature and ability to regulate body temperature. **Examples**: Core Body Temperature, thermal comfort, skin temperature, sweating, thermal sensitivity.
Usability/feasibility/satisfaction of the intervention	**Definition**: Measurements of how well the intervention can be used by the patient. **Examples**: Device usability, level of assistance needed to use the intervention, success rate of task performance, Standardized Usability Questionnaire, any questionnaire that rates the ease of using the device, task completion time, System Usability Scale (SUS).
Wheelchair propulsion/mobility	**Definition**: Assessments of how well the patient is able to use a wheelchair. **Examples**: Wheelchair transfer, wheelchair mobility, Wheelchair Skills Test (WST), wheelchair

	propulsion test, wheelchair independence and mobility, 6-minute Push Test (6MPT), Wheelchair Outcome Measure (WhOM), figure 8 protocol (fatigue intervention).
Other	**Definition**: Any outcome that does not clearly fit into the above categories. **Examples**: Spinal alignment, spinal cord perfusion pressure, expression of genes or gene products, appraisal of disability, nutrition knowledge, skin moisture level.

3.8. Figures

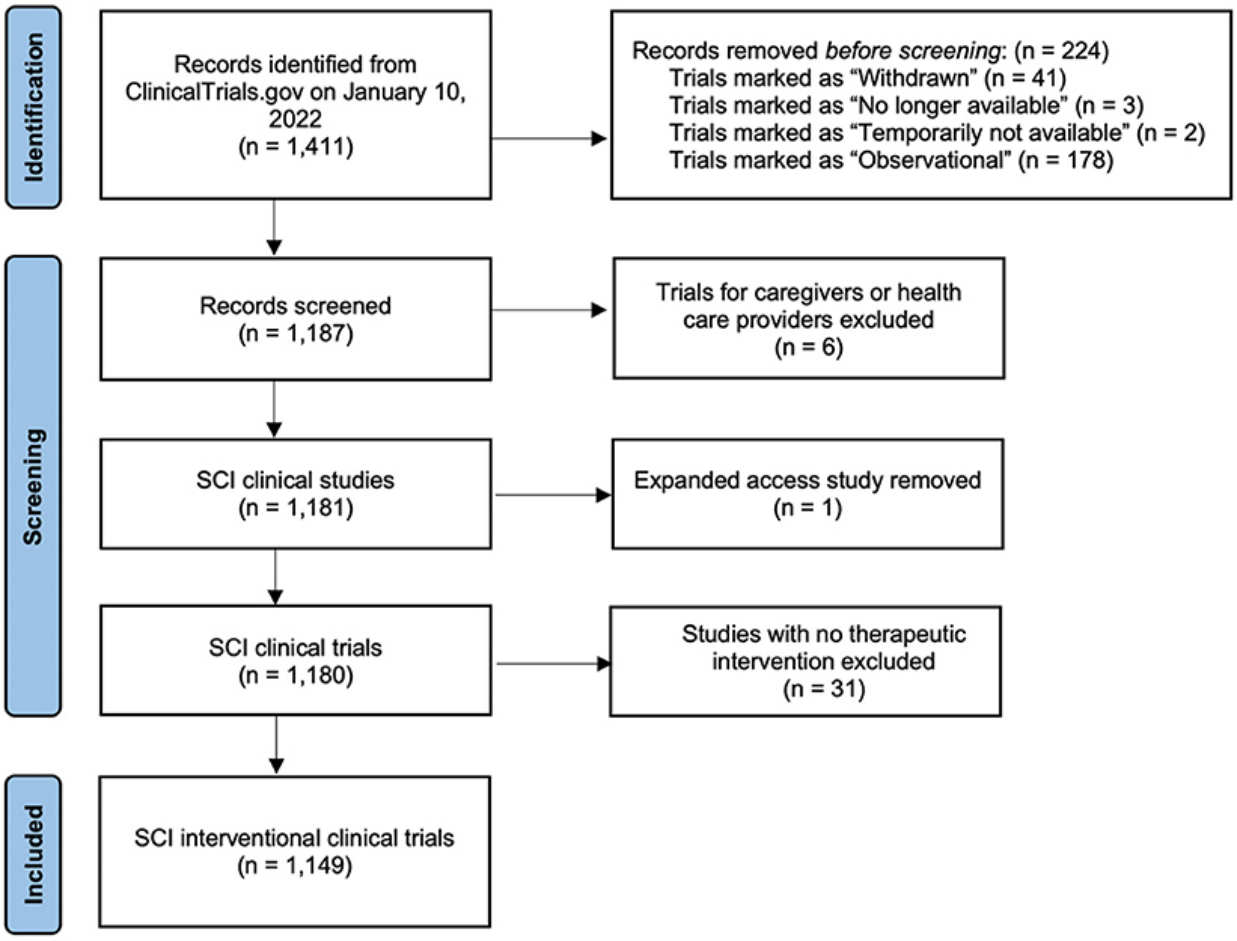

Figure 3-1 PRISMA flow diagram of the search strategy used in this study.
SCI, spinal cord injury.

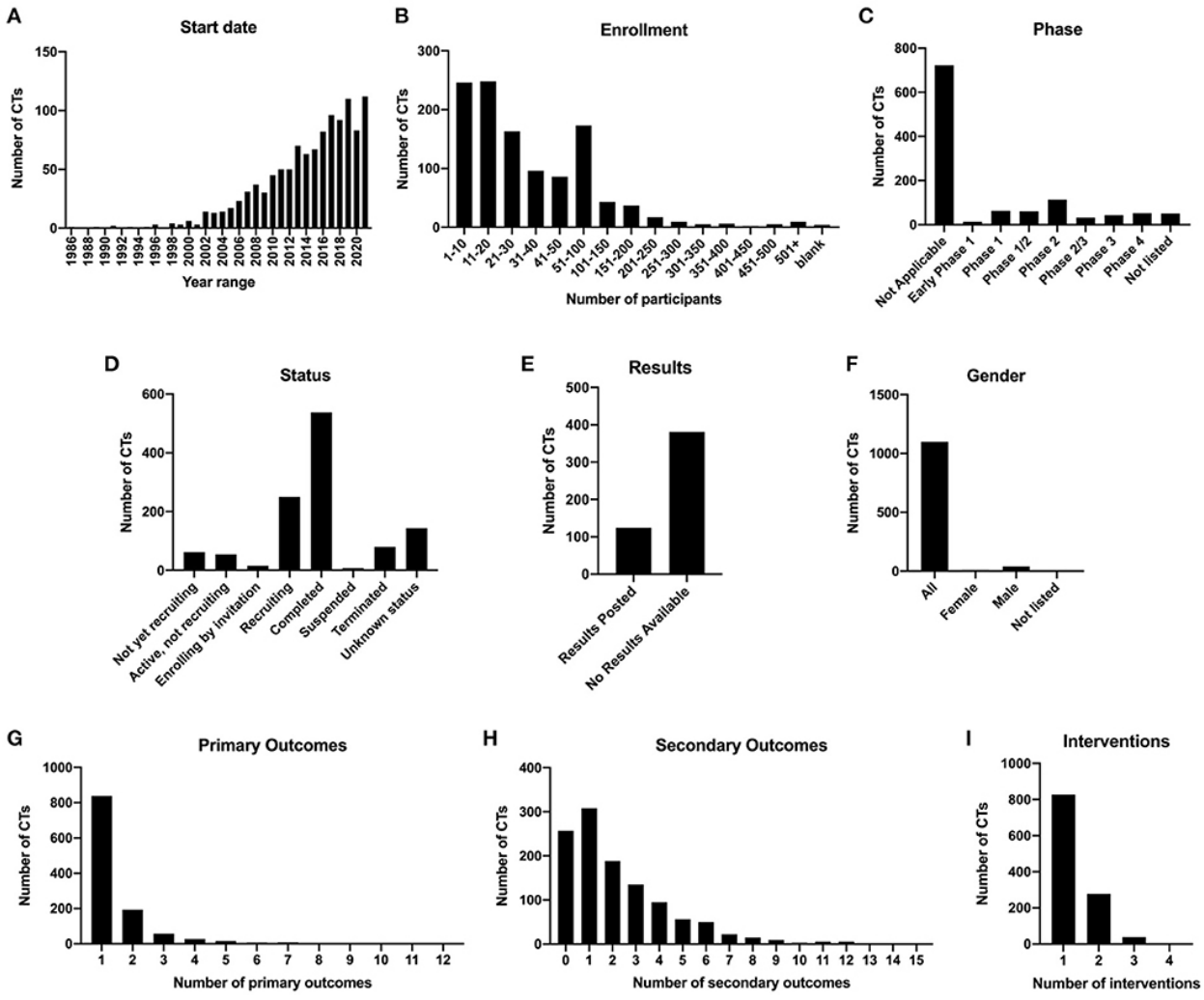

Figure 3-2 Demographics and statistics for 1,149 spinal cord injury clinical trials. (A) Numbers of clinical trials initiated per year from 1986 to 2021. (B) Number of clinical trials binned by actual or estimated enrollment of patients. (C) Number of clinical trials in each phase category. (D) Number of clinical trials in each status category. (E) Clinical trials marked as Completed and at least 1 year past the completion date, with results posted or no results available. (F) Number of clinical trials according to gender of enrolled subjects. (G) Number of clinical trials with 1, 2, 3, or 4

interventions. (H) Number of clinical trials with one or more types of primary outcome. (I) Number of clinical trials with one or more types of secondary outcome.

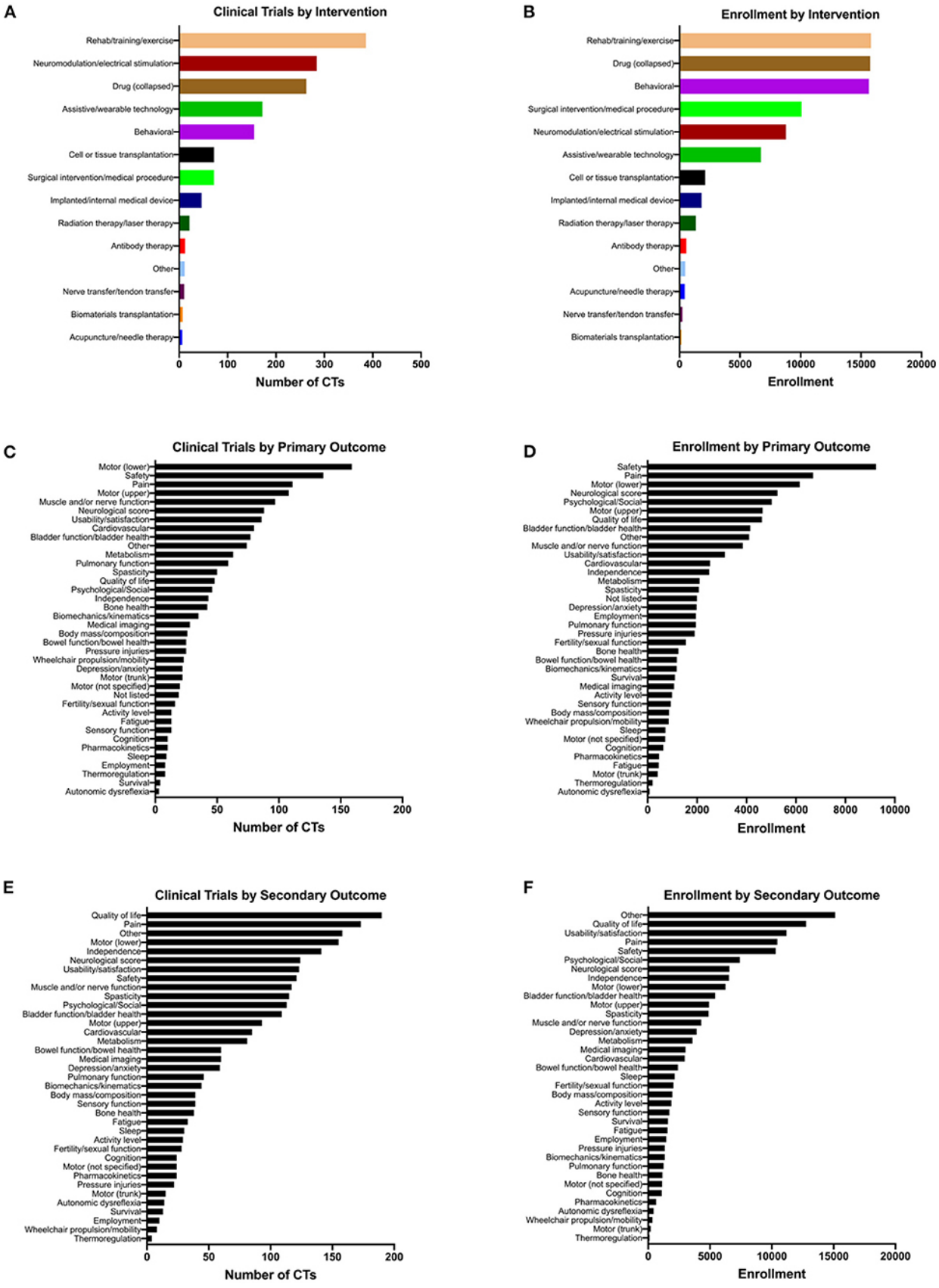
A
Clinical Trials by Intervention
Rehab/training/exercise
Neuromodulation/electrical stimulation
Drug (collapsed)
Assistive/wearable technology
Behavioral
Cell or tissue transplantation
Surgical intervention/medical procedure
Implanted/internal medical device
Radiation therapy/laser therapy
Antibody therapy
Other
Nerve transfer/tendon transfer
Biomaterials transplantation
Acupuncture/needle therapy
0 100 200 300 400 500
Number of CTs
B
Enrollment by Intervention
Rehab/training/exercise
Drug (collapsed)
Behavioral
Surgical intervention/medical procedure
Neuromodulation/electrical stimulation
Assistive/wearable technology
Cell or tissue transplantation
Implanted/internal medical device
Radiation therapy/laser therapy
Antibody therapy
Other
Acupuncture/needle therapy
Nerve transfer/tendon transfer
Biomaterials transplantation
0 5000 10000 15000 20000
Enrollment
C
Clinical Trials by Primary Outcome
Motor (lower)
Safety
Pain
Motor (upper)
Muscle and/or nerve function
Neurological score
Usability/satisfaction
Cardiovascular
Bladder function/bladder health
Other
Metabolism
Pulmonary function
Spasticity
Quality of life
Psychological/Social
Independence
Bone health
Biomechanics/kinematics
Medical imaging
Body mass/composition
Bowel function/bowel health
Pressure injuries
Wheelchair propulsion/mobility
Depression/anxiety
Motor (trunk)
Motor (not specified)
Not listed
Fertility/sexual function
Activity level
Fatigue
Sensory function
Cognition
Pharmacokinetics
Sleep
Employment
Thermoregulation
Survival
Autonomic dysreflexia
0 50 100 150 200
Number of CTs
D
Enrollment by Primary Outcome
Safety
Pain
Motor (lower)
Neurological score
Psychological/Social
Motor (upper)
Quality of life
Bladder function/bladder health
Other
Muscle and/or nerve function
Usability/satisfaction
Cardiovascular
Independence
Metabolism
Spasticity
Not listed
Depression/anxiety
Employment
Pulmonary function
Pressure injuries
Fertility/sexual function
Bone health
Bowel function/bowel health
Biomechanics/kinematics
Survival
Medical imaging
Activity level
Sensory function
Body mass/composition
Wheelchair propulsion/mobility
Sleep
Motor (not specified)
Cognition
Pharmacokinetics
Fatigue
Motor (trunk)
Thermoregulation
Autonomic dysreflexia
0 2000 4000 6000 8000 10000
Enrollment
E
Clinical Trials by Secondary Outcome
Quality of life
Pain
Other
Motor (lower)
Independence
Neurological score
Usability/satisfaction
Safety
Muscle and/or nerve function
Spasticity
Psychological/Social
Bladder function/bladder health
Motor (upper)
Cardiovascular
Metabolism
Bowel function/bowel health
Medical imaging
Depression/anxiety
Pulmonary function
Biomechanics/kinematics
Body mass/composition
Sensory function
Bone health
Fatigue
Sleep
Activity level
Fertility/sexual function
Cognition
Motor (not specified)
Pharmacokinetics
Pressure injuries
Motor (trunk)
Autonomic dysreflexia
Survival
Employment
Wheelchair propulsion/mobility
Thermoregulation
0 50 100 150 200
Number of CTs
F
Enrollment by Secondary Outcome
Other
Quality of life
Usability/satisfaction
Pain
Safety
Psychological/Social
Neurological score
Independence
Motor (lower)
Bladder function/bladder health
Motor (upper)
Spasticity
Muscle and/or nerve function
Depression/anxiety
Metabolism
Medical imaging
Cardiovascular
Bowel function/bowel health
Sleep
Fertility/sexual function
Body mass/composition
Activity level
Sensory function
Survival
Fatigue
Employment
Pressure injuries
Biomechanics/kinematics
Pulmonary function
Bone health
Motor (not specified)
Cognition
Pharmacokinetics
Autonomic dysreflexia
Wheelchair propulsion/mobility
Motor (trunk)
Thermoregulation
0 5000 10000 15000 20000
Enrollment

Figure 3-3 Therapeutic spinal cord injury clinical trials classified according to intervention and outcome types.
Note that a given trial may have more than one intervention and multiple outcomes, so the total numbers of clinical trials in A, C, and E add up to more than 1,149. (**A**) The total number of clinical trials for each class of intervention. (**B**) The cumulative enrollment for all clinical trials that use each type of intervention. (**C, E**) The total number of clinical trials listing each type of (**C**) primary and (**E**) secondary outcome. (**D, F**) The cumulative enrollment for all clinical trials that list each type of (**D**) primary and (**F**) secondary outcome.

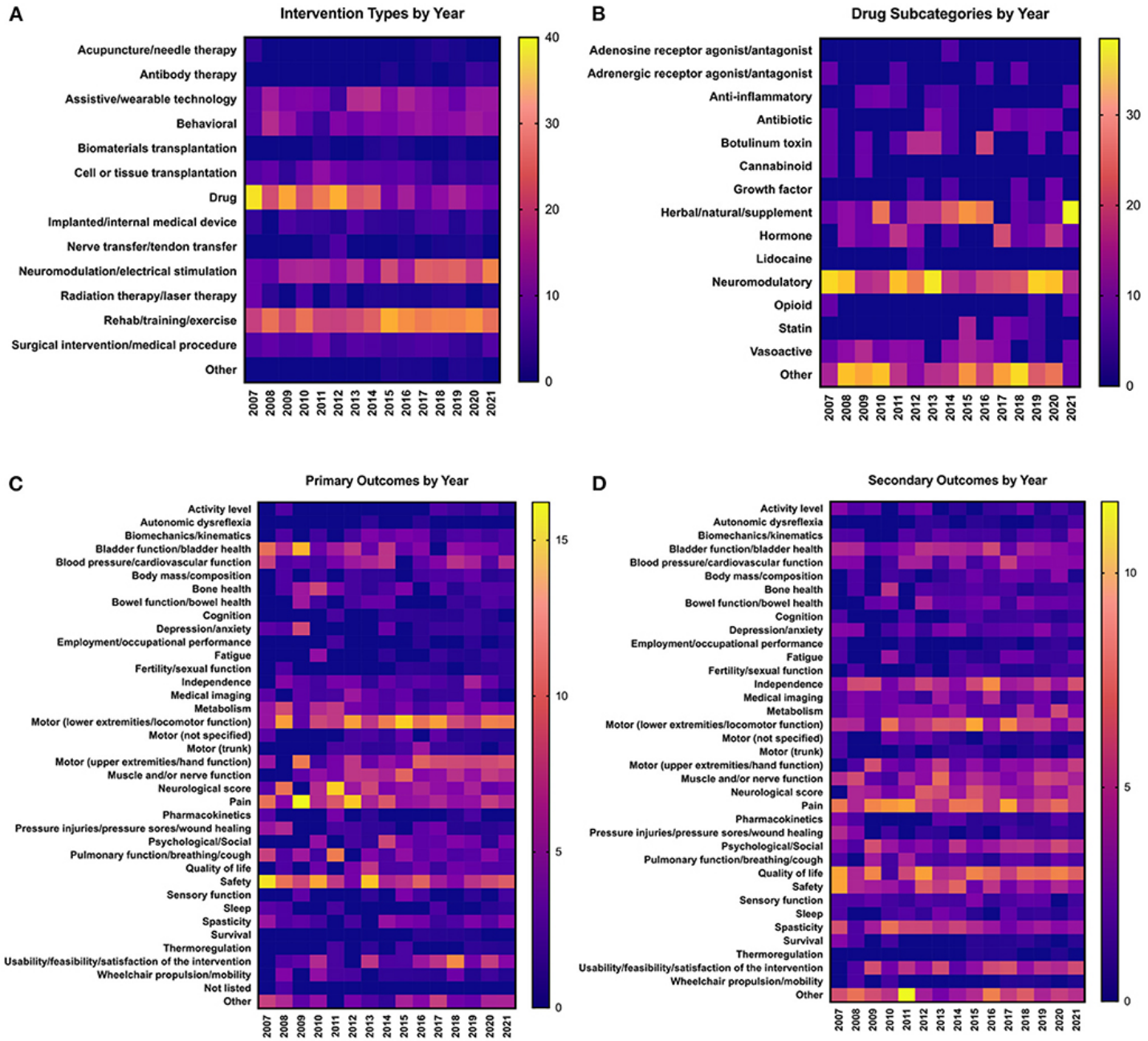

Figure 3-4 Trends in clinical trial interventions and outcomes over time.
Data are from clinical trials initiated between 2007 and 2021. All data are represented as percentages of the trials in a given year that utilize each type of (**A, B**) intervention or (**C, D**) outcome; values in individual columns add up to 100%. (**A**) Frequency of types of interventions used in clinical trials each year. (**B**) Breakdown of the types of drugs that make up the "Drug" category in panel A. Values in individual columns add up to

100% of total drugs in a given year. (**C**) Frequency of types of primary outcome measures assessed each year. (**D**) Frequency of types of secondary outcome measures assessed each year.

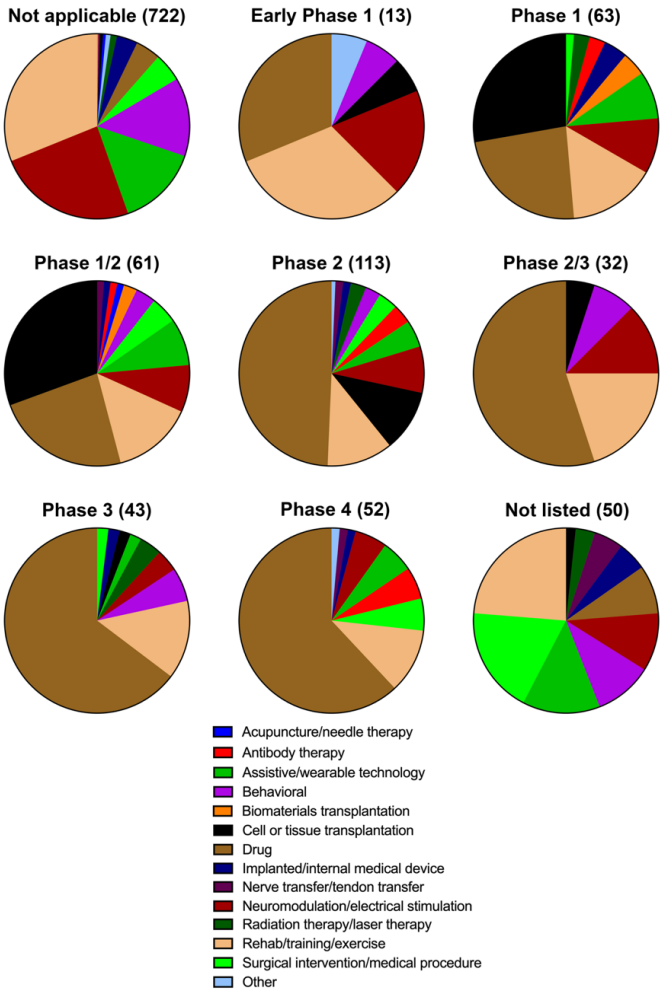

Figure 3-5 Breakdown of trials by phase category.
The total number of trials in each phase are included in parentheses above each pie chart. The pie charts represent the fraction of trials in each phase that utilize the corresponding interventions.

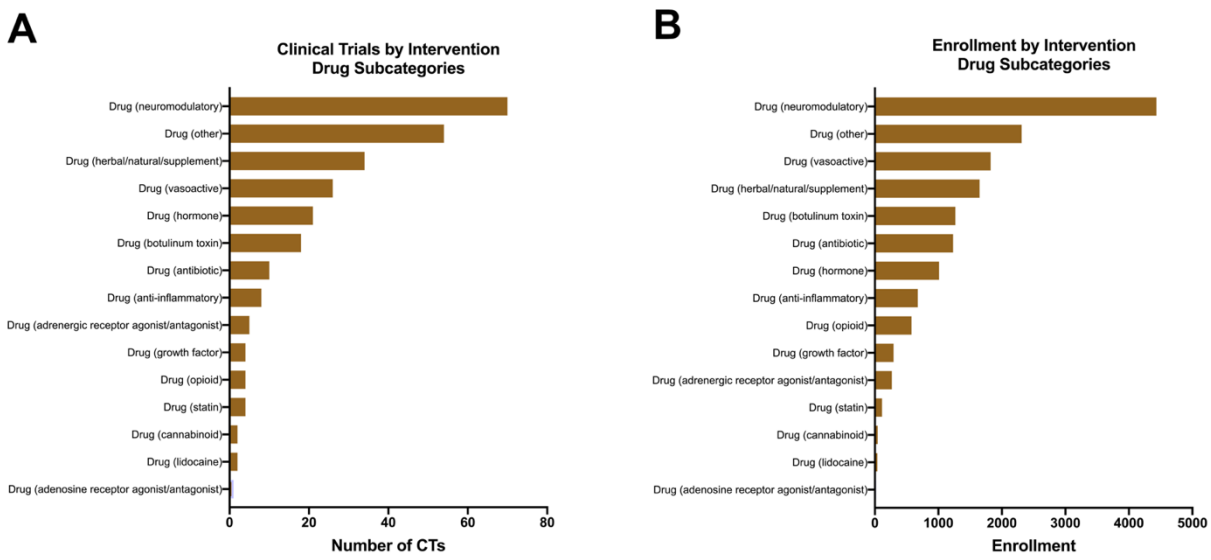

Figure 3-6 These graphs show the expanded data for the Drug subcategories in Figure 3A-B.
(A) The total number of clinical trials for each class of drug-related intervention. (B) The cumulative enrollment for all clinical trials that use each type of drug-related intervention.

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CHAPTER IV CONCLUSIONS

4.1. Overview

SCI induces significant pathophysiological changes within the spinal cord that disrupt sensory circuitry, ultimately leading to dysesthesia. To address the development of neuropathic pain clinically, it is necessary to better understand and characterize how SCI alters the neural circuitry underlying pain processing. Further characterization of specific neuronal cell types involved in pain processing after SCI will be critical for developing novel effective therapeutics for treating chronic neuropathic pain. And more broadly, translating preclinical discovery into clinical trials requires careful consideration and attention to overcoming gaps in reporting that may limit the usefulness of clinical trial data to the public, scientific, and clinical communities. It is imperative that we review the clinical trials that have been conducted thus far, so that we can learn from the past to make the most informed decisions in future trials. The work presented in this dissertation contributes to further characterizing SCI-induced changes in the pathophysiology of the spinal cord in relation to the development of neuropathic pain and presents a systematic analysis of all the past, current, and planned therapeutic clinical trials for SCI. These two projects pose interesting topics of discussion and suggest future directions for research.

4.2. Discussion of Chapter II

In summary, the results presented in Chapter 2 identify distinct patterns of lesion anatomy resulting from a C5 unilateral contusion at 28 days post-SCI. The main purpose of this study was to explore how individual variations in lesion anatomy relates to the

development of mechanical allodynia. Interestingly, we report that only a subset of animals within the experimental cohort developed mechanical allodynia by our conservative standard; this differs from previous reports that the same injury model produces pain in 100% of experimental subjects [1-3]. Furthermore, we report that subjects that developed mechanical allodynia after SCI have a significantly higher degree of dorsal horn neuronal sparing. This finding leads us to conclude that injuries with a higher percentage of surviving neural tissue are more conducive to the development of mechanical allodynia than more severe injuries that ablate most of the dorsal horn.

4.2.1. Variability of Neuropathic Pain In Experimental Animals and Humans

Individuals living with SCI report that the development of allodynia is a common manifestation of neuropathic pain that is often intermittent [4, 5]. Furthermore, the study of pain is complicated because it changes over time and can manifest as different sensations ranging from burning, tingling to electric shock sensations [4, 6]. Clinically, it is estimated that the prevalence of chronic pain after SCI ranges from 11%-94% [7, 8]. More specifically, in a community survey of 330 adults living with SCI, 48% reported experiencing allodynia [7, 8]. This wide range likely reflects the subjectivity of pain and the numerous variables that undermine its development, including the injury itself (severity, type, and location), differences in manifestation, and the involvement of multiple mechanisms which have been shown to contribute to the development of pain. It further indicates that there is little congruity in reporting the prevalence, causes, and characteristics of neuropathic pain [8].

There is no consensus on the “best” experimental SCI model to study neuropathic pain [9]. The use of cervical lesion models has emerged due to its clinical relevance to human injuries [8]. Studies utilizing rodent C5 hemi-contusion models have reported variable pain-associated outcomes; for example, in one study, 40% of experimental subjects developed mechanical allodynia [10], while other studies reported 100% [1-3]. Additionally, in our study using this animal model, we found that 30% of SCI subjects developed neuropathic pain [11]. Interestingly, different labs using the same injury model have report different incidences of pain in experimental subjects, which leads us to question what variables may explain these differences.

Such variability in animal models can perhaps be explained in part by intra-lab variability [12]. In acknowledgment of the intermittent nature of pain and its wide range of prevalence based on clinical reporting, we established a conservative definition of mechanical allodynia to best ensure that the animals classified as developing sensitivity responded well outside their normal behavioral range. We defined mechanical allodynia as an individual animal scoring more than 2 standard deviations below its baseline mean score for at least 3 out of the 4 time points evaluated. This differs from studies that utilize a 50% paw withdraw threshold using the up-down method of von Frey [13]. We utilized the electronic von Frey rather than the classical von Frey monofilaments to get a continual reading of the gram force being applied to the plantar surface of the forepaw. Differences between the electronic and classical von Frey filaments have been reported [14, 15].

Another major difference in our analysis is that we did not perform a groupwise comparison by comparing all SCI to sham; instead, we identified individual animals that met our definition of mechanical allodynia based on their post-surgical changes from baseline, regardless of the treatment group. Ultimately, this allowed us to identify and understand inter-animal variables that may contribute to changes in sensory function. This testing approach may be a more accurate representation of "pain," which likely fluctuates over time in humans and experimental animals [10].

4.2.2. The Relationship Between Lesion Anatomy and Allodynia

As discussed in Chapter 1, SCI pathology is complex and extends beyond the initial mechanical trauma. The dynamic evolution of secondary injury results in anatomically unique lesions between individuals, as observed in preclinical studies [16]. Furthermore, the development of unique lesions has been shown to undermine the associated sensory/motor impairments after SCI [16, 17]. Based on this, we predicted that some aspects of lesion pathophysiology might underlie the development of neuropathic pain in some individuals and not others. Experimentally, we observed that 30% of animals developed mechanical allodynia after SCI, and we focused on characterizing and exploring differences in lesion anatomy between individuals in relation to pain.

We first calculated lesion size using GFAP immunolabeling to identify the reactive astroglial border [18]. Based on this method of determining lesion volume, we observed no significant differences in lesion volume between animals that developed pain and those that did not. Previous literature supports the notion that the overall size of

the lesion alone does not directly correlate with pain-associated outcomes [10]. However, we observed that the extent of neuronal loss was often beyond the astroglial border. Interestingly, we observed that one SCI-sensitive animal had increased GFAP staining, but no distinct astroglial lesion border, however, it did have neuronal loss. Based on these observations, we proceeded to evaluate the area of neuronal sparing (spared grey matter) as another metric representative of lesion size. We observed significant differences between sensitive and non-sensitive animals in gray matter sparing at the lesion epicenter. Specifically, we observed that animals that developed mechanical allodynia had less extensive lesions with almost complete sparing at C4 and C6. Taken together, our analysis of lesion volume indicated that animals with smaller lesions and more gray matter sparing were most likely to develop mechanical allodynia.

We next characterized neuronal density in the dorsal and ventral regions of the spinal cord above at and below the level of injury. A great body of work has identified neuronal population-specific changes within the dorsal horn of the spinal cord that contribute to neuropathic pain states [19]. Due to the importance of the dorsal horn in sensory processing, we evaluated neuronal density in this region and compared between animals that developed mechanical allodynia, those that did not develop allodynia, and laminectomy controls. Interestingly, we observed animals that developed mechanical allodynia to have significant neuronal sparing of the dorsal horn. This was a surprising finding, as we predicted severe damage or total ablation would correspond to severe pain states. However, this finding directly corroborates our analysis of percent gray matter

sparing. This raises the question of why less extensive lesions are more likely to produce pain-associated behaviors.

One possible explanation lies in the innate plasticity of the spinal cord, which allows for the rewiring of neural circuits modulating the paw withdrawal response. After SCI, axon regeneration faces challenges that limit its ability to regenerate, such as the non-permissive nature of the SCI lesion [20]. However, it has been shown that in animal models of SCI, reorganization of spinal circuits occurs through several mechanisms including collateral sprouting of spinal tract fibers [21] and through reorganization of long propriospinal pathways [22]. In addition to reorganization, the utilization of parallel pathways and interneuronal connections may allow spared circuitry to compensate, at least to some extent, for lost functions [20, 22]. This intrinsic plasticity plays a critical role in functional recovery and has prompted further investigation to enhance such reorganization (ex., by using an activity-based training [23-25]).

A different possible explanation and future area of study are in further characterizing diverse populations of neurons within the dorsal horn and their contributions to pain after SCI. Recent work by Brown et al. demonstrates that mechanical stimulation in the same injury paradigm induces differential expression of specific populations of neurons in the dorsal horn. Specifically, there was an increase in the activation of interneurons expressing calretinin and PKC γ, a decreased activation of nNOS expressing inhibitory lamina II interneurons, and an altered excitatory-inhibitory balance of interneuron signaling associated with at-level sensation [19]. Perhaps specific

populations of neurons are required, potentially in different amounts, to be spared for the facilitation of pain.

These findings suggest that both the degree and location of neuronal sparing are important physiological characteristics related to the development of pain.

4.2.3. Limitations

While the findings of this study presented in Chapter 2 help elucidate the pathophysiological basis of neuropathic pain, it is important to point out the limitations of this study.

This study did not evaluate specific populations of dorsal horn neurons or projection neurons due to limited tissue availability. There are 30 distinct populations of dorsal horn interneurons, of which several have been molecularly identified to have specific functions [26]. While our study reveals that neuronal sparing is an important factor in the development of mechanical allodynia, further characterization of the differences in specific neuronal populations is necessary to elucidate which spared neuron populations are driving the development of mechanical allodynia.

We did not evaluate white matter tract sparing, so it remains unclear how injury severity and sensitivity may correlate with sparing of ascending and descending tracts known to be implicated in pain-associated behaviors [27]. For example, the corticospinal tract has been implicated in pain-associated behaviors [27, 28]. Additionally, the dorsal column/medial lemniscal or anterolateral spinothalamic tract are important in conveying tactile and temperature information required for pain [10, 29]. It would be valuable to understand how sparing of these tracts correlated to pain behaviors in individual animals.

Additionally, it is known that SCI induces reactivity of glial populations including microglia and astrocytes, which has been linked to the development of neuropathic pain [30-33]. After SCI, astrocytes become activated [marked by upregulated expression of glial fibrillary acidic protein (GFAP)]. During this activated state, astrocytes contribute to neuronal hyperexcitability by releasing proinflammatory molecules, nitric oxide, and through the loss of glutamate transporters [34, 35]. Although we did not characterize the contributions of glial activation to mechanical sensitivity in this study, recent work in the same injury model has characterized significant glial activation in the superficial dorsal horn and concluded that microglial activation extended beyond the lesion epicenter in caudal ipsilateral locations [3].

Lastly, our study is limited by the inclusion of only young female subjects. The biological variables of age and sex are sexually dimorphic in relation to neuropathic pain. While biological sex does not correspond to the incidence of pain clinically between male and female [36], preclinical reports have indicated that biological sex influences pharmacological inhibition [37-41]. It has also been reported that sexual dimorphism exists between injury severity (when measured by displacement of impactor probe at the time of SCI) and behavioral outcomes [42]. With regard to age, clinical reports have indicated that neuropathic pain is positively correlated to increasing age at the time of injury [36, 43-46]. While it was outside the scope of this particular experimental design, we acknowledge the importance of validating these findings in the presence of such biological variables including age and sex.

4.2.4. Future directions

4.2.4.1. Global Characterization of Neuronal Activation Profiles

To better understand the development of neuropathic pain, it is important to characterize SCI-induced changes within the neuronal populations that are pathologically integrated into nociceptive signaling pathways. Utilizing a transgenic mouse line will allow for the visualization of neurons with high levels of activity in response to peripheral noxious or innocuous stimulation. The Fos-GFP mouse line (B6.Cg-Tg(Fos/EGFP)1-3Brth/J, Jackson Laboratories, #014135) expresses a gene product consisting of the green fluorescent protein (GFP) fused to the immediate early gene Fos, a marker of neuronal activity. This mouse strain has been validated in multiple studies to report changes in neuronal activation [47-51]. Utilization of this transgenic line in a C5 hemicontusion SCI model would allow for the detection of differential activation of neuronal populations between animals that develop neuropathic pain and those that do not. Recent work by Brown et al. has utilized a similar approach using a transgenic line called TRAP2, in which Fos is fused to a TdTomato reporter in a Cre-recombinase-dependent manner [19, 52, 53]. This study reported increased activation of interneurons expressing calretinin and PKCγ, decreased activation of nNOS-expressing inhibitory lamina II interneurons, and altered excitatory-inhibitory balance of interneuron signaling associated with at-level sensation [19]. However, further characterization of the 30 distinct populations of dorsal horn interneurons may be required to obtain a global appreciation of SCI-induced neuronal changes [26]. Then, further analysis between these distinct populations should be explored to identify how

these global changes in neuronal populations are related to the development of neuropathic pain.

4.2.4.2. Transsynaptic Tracing of Pain-Responsive Neurons

In addition to determining the changes in the distributions of spinal neurons that are active in response to mechanical stimulation, it would be beneficial to characterize if the presynaptic inhibitory inputs onto dorsal horn neurons are altered after SCI. To evaluate this, we would utilize monosynaptic rabies tracing through the Capturing Activated Neuronal Ensembles (CANE) technique [54-56]. This strategy would allow for transsynaptic tracing from neurons with high activity levels in response to mechanical stimulation of the forepaw, and combined with the use of modified rabies virus, would enable assessment of the synaptic connections of descending inhibitory projections onto these pain-responsive neuron populations. Ultimately, this would allow us to generate an unbiased map of the presynaptic neurons that synapse onto pain-responsive neuron populations and compare how these presynaptic inputs change in animals that develop allodynia.

The data generated from these future studies will provide a foundational understanding of how alterations in local signaling and changes in inhibitory input converge to influence sensory dysregulation in pain circuitry. Furthermore, this detailed characterization could have implications in influencing what types of cells would be necessary to be replaced after SCI to modulate pain circuitry.

4.2.5. Implications To Current Knowledge

After SCI, neuropathic pain develops in approximately 60-80% of individuals, and pain management has been considered a high priority in the SCI community [6-8, 57]. The mechanisms underlying the development and maintenance of neuropathic pain remain incompletely understood, and further characterization is required to develop new therapeutic strategies.

The findings from Chapter 2 elucidate a role for dorsal horn neuronal sparing in the development of at-level mechanical allodynia following a cervical-level contusion injury. We established a conservative criterion to determine if experimental subjects developed mechanical allodynia, and related that to differences in lesion anatomy between animals. Ultimately, our findings suggest that both the size and location of neuronal loss are important physiological characteristics that could be important in processing sensory information after SCI. Further work is necessary to pinpoint which populations of neurons are differentially activated after SCI and which are responsible for modulating pain processing.

Importantly, we acknowledge that pain is highly variable- in humans and in animal models of SCI. It is important to continue studying the pathophysiological differences between lesions that lead to the development of pain compared to lesions that maintain normal sensation. Analyzing inter-animal variability rather than performing groupwise comparisons between SCI and laminectomy may help elucidate why some, but not all individuals with SCI develop neuropathic pain.

4.3. Discussion of Chapter III

In summary, the systematic review of SCI clinical trials in Chapter 3 provides an overview of the past, present, and future of clinical trials focused on improving outcomes after SCI. This collaborative effort included perspectives from research scientists, clinician scientists, and individuals living with SCI.

Decades of preclinical research have guided and shaped the landscape of clinical trials in the field of SCI. We utilized publicly accessible data downloaded from ClinicalTrials.gov and extracted all available data using a keyword search for "spinal cord injuries." This data was then categorized according to the type of intervention being tested and the outcome measures assessed. With regard to clinical trial attributes, we report that most trials have low enrollment, test only a single interventional therapeutic, and examine only a few primary outcomes. In evaluating the types of interventions assessed, the largest representation in trials was testing rehab/training/exercise, neuromodulation, and behavioral modifications. The most highly represented primary outcomes include motor function of the upper and lower extremities, the safety of the intervention, and pain management. Furthermore, we identified gaps in clinical trial reporting in reviewing this large dataset.

4.3.1. Limitations In Translation From Preclinical To Clinical Success

In the field of SCI, there is a disconnect between preclinical success in animal models and success in clinical trials. Despite the 1,149 clinical trials that have tested or are currently testing therapeutic interventions, there remains no FDA-approved treatment. Many variables may play a role in this disconnect that will be discussed

below, ranging from reproducibility, the appropriateness of preclinical models, and differences in experimental design between preclinical and clinical trials.

The failure to translate treatments from the bench to the bedside is often traced back to shortcomings in the reproducibility and reliability of preclinical evidence [58-61]. The Facilities of Research Excellence – Spinal Cord Injury (FORE-SCI) initiative by the NIH called for experimental replication of several studies that produced positive results for therapeutic interventions in SCI [12]. In this effort, experiments were replicated as initially described, but the majority could not replicate the initial results [59]. While it is speculated that the lack of replication is likely due to slight deviations from the initial experiment, this results in a disappointing false start to therapeutic development. This failure of replication in this initiative led to a call for more rigorous preclinical research processes and reporting standards within the field of SCI. A direct result of this was the establishment of Open Data Commons for Spinal Cord Injury (ODC-SCI). A data commons can serve as a repository for Big Data analysis, data exploration, and data sharing to improve reproducibility between different laboratories [59].

Using animal models to study SCI has greatly advanced our understanding and ability to test potential therapeutics. However, there are many limitations in the use of animal models. While a wide variety of injury paradigms exist, capturing the clinical variability of SCI in animal models is difficult. It is possible that preclinical laboratory settings may not be reproducible in clinical settings due to the existing heterogeneity in human injuries and limited measurable outcomes in animal models [62, 63]. Ultimately,

scientists depend on reliable, reproducible behaviors in animals to measure outcomes that are limited in scope. Another compounding factor that limits the translation of preclinical research to the clinic in SCI research is that preclinical SCI models predominately utilize young adult rodents and typically only use females, which does not represent the diverse demographic pool of individuals seen in the clinic [58]. Additionally, there is concern about the pathogenesis and pathological differences between animal models and humans. For example, a rat develops cystic cavitation similar to a human; while mice, on the other hand, develop fibrous lesions filled with extracellular matrix [64]. Strategies to improve the translatability of animal models include increased rigor in experimental design, utilizing a larger effect size, and validation of favorable findings by different laboratories and in larger animal models [65].

Another factor that complicates the translation of preclinical results into clinical settings lies in the innate design of human clinical trials versus the preclinical experimental design [66]. Traditionally, the clinical trial study is designed to have inclusion/exclusion criteria such as motor score, neurological level, and severity of the injury and often includes both sexes; however, preclinical SCI models frequently utilize young adult rodents and focus on females [58]. It may not be feasible for a preclinical animal model to power SCI trials to test multiple variable designs such as different ages, genders, time points after injury, etc. However, these variables are important considerations and should be kept in mind when analyzing the translational potential of results.

4.3.2. Gaps In Clinical Trial Reporting

As the community of individuals supporting SCI research continues to improve communication, several barriers remain in place that plays an important role in the translational pipeline from lab bench to bedside. In particular, barriers such as insufficient reporting of clinical trial data and the accessibility of this data to the broad community.

One of the most significant gaps in reporting that we observed was that over 75% of trials with the status “Completed” had no results posted on ClinicalTrials.gov. Upon further investigation by conducting a PubMed search of 50 randomly selected trials listed as complete, we found that 54% of these trials had published results associated with the study outcome. However, these publications often are inaccessible to the general public as several journals have paywalls. This directly limits the ability of the broad community to access the results from the clinical trial. The results of a clinical trial should be publicly available and easy to interpret so that individuals seeking this information may interpret it for themselves. In this effort, two curated websites have been established to serve as valuable resources, scitrials.org, and scitrialsfinder.net. However, these websites focus on current or recruiting trials, leaving ClinicalTrials.gov as the primary source of historical clinical trial data.

4.3.3. Implications To Current Knowledge

This systematic review provides, for the first time, a comprehensive view of interventional clinical trials focused on improving outcomes after SCI. As the number of

new SCI clinical trials initiated each year continues to climb, it is important that we reflect on past trials to learn from them. These findings serve as a resource for understanding the trends in and evolution of interventional SCI clinical trials.

4.4. Overall Conclusions

SCI is a devastating and debilitating injury that results in a wide array of neurological dysfunction, including the development of chronic neuropathic pain. The work presented in this dissertation contributes to our understanding of neuropathic pain as well as holistically reviews the state of interventional clinical trials for SCI. We have shown that lesion size negatively correlates with the manifestation of at-level mechanical sensitivity and our data suggests that sparing of dorsal horn neurons may be required for the facilitation of neuropathic pain. Attaining a more detailed understanding of the mechanisms driving the development of neuropathic pain is essential for the development of novel therapeutics for pain management. Furthermore, we report characteristics of SCI clinical trials and trends in therapeutic interventions and outcome measures allowing us to take a look at the past, present, and future of SCI clinical trials. This work highlights the need to continue pushing toward therapeutic interventions in such a way that is more efficient, held to higher reporting standards and is overall more accessible to the broad community.

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