

ABSTRACT

LEWIS, MELISSA JEAN. Characterization of a canine model of chronic paralysis. (Under the direction of Dr. Natasha Olby).

Spinal cord injury (SCI) resulting in chronic paralysis is a common and devastating problem in people. Experimental animal models have provided immense information on the pathophysiology of SCI and offer a useful platform on which to test novel therapeutic interventions, but do not capture the complexity and heterogeneity of human SCI, have had limited translational success, and are difficult to maintain into the chronic disability phase.

Acute SCI is common in dogs and a proportion with severe injury characterized by paralysis and loss of pain perception will fail to recover. This results in a readily available population of dogs with permanent impairment in which to study naturally-occurring SCI. Dogs also display heterogeneity at the population level and typically sustain mixed compressive and contusive lesions sharing overlapping characteristics with human SCI. As such, dogs with SCI are being increasingly used as a translational large animal model, but gaps remain in model development including improved recognition and understanding of lesion complexity. We hypothesized that there is a spectrum within the severe injury category and that specific injury features would be associated with post-injury functional status. Our overall aim with this work was to thoroughly characterize naturally occurring, functionally complete canine SCI as a model of chronic paralysis using clinical, gait analysis, electrodiagnostic and imaging techniques. Specific objectives were to develop and refine outcome measures in order to expand the means of evaluating this population, to deepen understanding of the complexity and variability of injury and its relationship to motor recovery in dogs with severe SCI, and to explore potential mechanistic explanations for the variability in response to treatment with potassium channel antagonist, 4-aminopyridine (4AP) in dogs with severe SCI.

Thirty-four dogs were prospectively enrolled that had an incomplete recovery characterized by failure to regain normal pain perception at least 3 months after suffering an acute,

sensorimotor complete thoracolumbar SCI. Using this population, a clinical tool was developed to quantify spasticity called the Canine Spasticity Scale. This scale confirmed that spasticity was common and of variable severity in dogs with chronic SCI similar to humans with SCI. A gait analysis tool focused on center of pressure variability was also developed providing novel information on post-injury alterations in locomotion. Using the spasticity scale as well as magnetic resonance imaging and electrodiagnostic evaluation of long tract function and local circuitry, injury severity was demonstrated to be a spectrum ranging from findings consistent with physical spinal cord transection to evidence of structural and functional trans-lesional connections, highlighting injury complexity. Diffusion tensor imaging also identified microstructural changes associated with the injury not apparent on conventional imaging. Findings also suggested that spared supraspinal influence on reorganized motor circuitry below the level of injury is likely integral to regaining ambulation after severe SCI despite absent pain perception. Exploration into factors underlying the variability in response to 4AP revealed that electrodiagnostic evaluation might be useful to predict favorable response, but that there is unlikely to be a simple relationship between one isolated aspect of injury and functional outcome or response to a specific therapy.

These results enhance understanding of the complexity and heterogeneity of severe chronic SCI in dogs and provide insights into mechanisms of recovery of motor function. This work will expand the ability to utilize dogs as a translationally-relevant large animal model in parallel with rodent and human studies and will facilitate well-designed canine clinical trials with the goal to improve outcomes for both dogs and humans with SCI.

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Characterization of a canine model of chronic paralysis

by
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DEDICATION

To Huxley Lewis, my faithful, furry friend.

BIOGRAPHY

Melissa Lewis received a Bachelor of Science in Biology from Duke University in 2002. She received her veterinary degree from the University of Pennsylvania in 2010. She went on to complete a rotating small animal internship and residency training in neurology and neurosurgery at North Carolina State University, achieving board certification in 2014. She then matriculated into the Comparative Biomedical Sciences graduate program at North Carolina State University.

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CHAPTER 1

Introduction

Human Spinal Cord Injury

Approximately 17,000 people suffer acute SCI every year resulting in approximately 300,000 people in the United States living with chronic paralysis¹. The most common causes are traumatic secondary to car accidents, falls, acts of violence and sports-related injuries.² The average age at injury has been increasing and currently is 42 years.^{1,2} SCI disproportionately affects men, accounting for approximately 80% of new cases, and racial minorities are being increasingly affected.^{1,2} Regardless of underlying cause or who is affected, severe injury impacts all aspects of daily functioning with the permanent physical impairments frequently hindering the ability to live independently. It has also been well-documented that people with chronic SCI commonly suffer from secondary health conditions including pressure sores, urinary tract infections, spasticity, pain, bladder, bowel and sexual dysfunction and reduced overall quality of life.³⁻⁵ Furthermore, people with SCI experience shorter life expectancy compared to the general population.¹ The economic impact of SCI is substantial with estimated annual health care costs in the millions and individual expenses exceeding \$100,000 per year in more severely disabled patients.^{6,7} Current standards of care at the time of injury include performing surgery when indicated to decompress and/or stabilize the spine, addressing concurrent injuries, providing pain relief and meticulous nursing care, and initiating rehabilitation.⁸ There is currently no consensus or definitive recommendation on neuroprotective strategies including corticosteroids or other investigational therapies.⁸ Despite long-standing, active research in animals and humans with SCI, treatment options remain limited and most severely affected individuals can anticipate lifelong impairment. Improved methods to translate promising therapies into meaningful benefits for humans with SCI are needed.

Experimental models of SCI

Experimental models of SCI have provided immense information on the pathophysiology of SCI and offer a readily available platform on which to test novel therapeutic interventions.⁹ While dogs have been utilized in experimental SCI models, they have largely been replaced by rodent models due to reduced cost and availability, amongst other reasons.¹⁰⁻¹⁵ Models can be categorized by the type of induced injury and include contusion, compression, distraction, dislocation, transection or chemical models.¹⁶ Various impactor and compression devices have been developed to produce contusion or compression injury, respectively, each with its own pros and cons regarding ease of use, reproducibility, and variation in pathology and injury mechanics.¹⁶ Models that incorporate both contusion and compression have been developed with the goal to more closely mimic the multifaceted nature of human traumatic SCI.¹⁶ Transection (partial or complete) models are widely used in tissue engineering and neuronal regeneration studies but do not recapitulate the complexities of spontaneous SCI, potentially limiting their clinical relevance.¹⁶

The benefits of experimental models of SCI are numerous. Experimental rodent models have provided invaluable information on the cascade of events that occur following the primary SCI, collectively known as ‘secondary injury’.⁹ This includes a combination of overlapping pathologic features such as hemorrhage, inflammation, excitotoxicity, free radical production, and apoptosis resulting in progressive tissue loss, axonal degeneration and demyelination.¹⁷ Improved understanding of secondary injury might lead to the ability to halt or reverse its effects, which has important therapy implications. In the chronic setting, rat SCI models also depict a reasonable approximation of the lesion seen in human chronic traumatic SCI with an astroglial scar surrounding a cavity, which can be progressive, at the lesion epicenter.¹²

Another benefit of experimental models is that the effects of various types and severity of injury can be studied utilizing a combination of behavioral, electrodiagnostic, histopathologic and imaging techniques. Importantly, the ability to readily obtain spinal cord tissue from experimental animals allows a more comprehensive evaluation of the lesion and offers insight into mechanisms of injury. Despite the assumption of poor regenerative capacity of the mammalian central nervous system, histopathologic evaluation (in the chronic setting) after experimental injury has shown remarkable plasticity of intraspinal neural networks.^{9,18} While this reorganization might have functional limitations, it underscores the dynamic nature of the nervous tissue and provides targets for manipulation to enhance function with potential application to human SCI.

Given the uniformity of the population with regard to age, body weight, genetics/strain, etc., and precise ability to control experimental factors, experimental models can be designed to test very focused aspects of injury in order to understand the contribution of that component to overall impairment, which is not possible in more complex injury.^{9,19,20} Examples include using a demyelination rat model to study the effect of the potassium channel antagonist, 4-aminopyridine, on conduction block secondary to demyelination, and specific rodent models that have been developed to study axonal regeneration after SCI.^{19,20} These models can also be utilized to confirm the mechanism of response to therapy seen in humans with SCI. For example, using a transection rat model to show differences in the effect of epidural stimulation between rats with complete versus incomplete transection would support the hypothesis that epidural stimulation can unmask subtle descending influence in severely injured human patients.^{9,21}

Despite promising results for the treatment of experimental SCI, however, there has been a failure to produce notable therapeutic advancements for humans with SCI.^{13,22,23} This pattern is not confined to SCI but is replicated in other disease processes resulting in neurodegeneration such as traumatic brain injury or stroke.²² There is an increasing need to

find better ways to bridge the gap between experimental injury and spontaneous injury in humans. There are several notable limitations to experimental SCI models that impede their translational success. The homogeneity that characterizes experimental SCI and allows precise targeting of specific aspects of injury is in stark contrast to the heterogeneity of human SCI, both with regard to population and lesion characteristics.^{9,22} As such, experimental models face difficulty in recreating injury that more accurately mirrors typical lesions in human SCI, in which a combination of pathologic events (typically contusion and compression) occur simultaneously. Success in a tightly controlled experimental setting might also be inapparent amongst a wider, diverse human SCI population. The location of the injury to the spinal cord is also constrained by model type (e.g. dorsal lesion for weight drop models) and may be distinct from location of predominant pathology in human SCI.^{16,24} This is further influenced by the small size of the rodent spinal cord and variations in the location (within the spinal cord) and relative importance of the descending motor pathways (such as the corticospinal or rubrospinal tracts) between rodents and humans.^{9,25,26} These anatomic differences might result in different effects of injury on motor function, although there is also anatomic variation in between other species such as dogs and people.²⁶⁻²⁸ Maintaining rodent models into the chronic setting is difficult, further hampering the ability to accurately model chronic paralysis in humans.²⁹

With regard to therapeutic intervention, novel therapies can be applied at the time or at a specified time after experimental injury which differs substantially from human SCI in which there is often a delay between injury and implementation of therapy, perhaps well after irreversible damage has occurred.³⁰ There is also no clear delineation of the transitions between acute, subacute and chronic injury and how that differs between rodents and humans, which has implications for translating therapies that target specific injury settings.^{30,31} Additionally, a major therapeutic target has been promoting axonal regrowth across the site of injury but long distance axonal regeneration in humans is questionable despite successful regenerative strategies in rodents.^{9,32} Furthermore, while experimental

injury studies might detect significant differences in function in response to a particular intervention using purpose designed outcome measures, this does not necessarily translate to functionally relevant or other meaningful changes (e.g. reduction in neuropathic pain) in human SCI patients.^{23,24,31} Expanded ways to study SCI are needed with a distinct opportunity to incorporate large animal clinical models with the hope that the combination will improve human SCI outcomes.^{31,33}

Acute Canine SCI

Acute thoracolumbar SCI is common in dogs due to intervertebral disc herniation (IVDH), vertebral column trauma and vascular events among other causes.^{17,34-38} Dogs with IVDH can be classified into categories based on the type of disc herniation as described by Hansen.³⁹ Hansen's type I disc herniation refers to extrusion of degenerated nucleus pulposus material in chondrodystrophic breeds of dogs while Type II disc herniation occurs in non-chondrodystrophic breeds as a result of fibrocartilage degeneration and protrusion of the annulus fibrosis.³⁹ More recent work has indicated there is overlap between the underlying degenerative processes and breeds affected by each group.⁴⁰ Additionally, other types of disc rupture have been identified including acute, compressive hydrated disc herniation, which primarily affects the cervical region, and acute, non-compressive nucleus pulposus extrusion.⁴¹⁻⁴³ Acute non-compressive nucleus pulposus extrusion has been variably referred to as 'missile disc,' or 'traumatic disc' herniation.^{42,43} As the name implies, this type of injury refers to herniation of relatively normal disc material with little to no residual extradural compression and can occur secondary to blunt force trauma (i.e. 'traumatic disc').^{42,43} Since the insult results in primarily a contusive rather than compressive injury, this cause of acute SCI often presents in similar fashion and shares overlapping features with fibrocartilagenous embolism (FCE).^{34,42-44} FCE is another common cause of acute SCI and occurs when a fragment of fibrocartilage infarcts the segmental blood supply to a section of the spinal cord.⁴⁴ The loss of blood supply results in variable white and gray matter tissue loss within

the region affected. Vertebral column trauma occurs most commonly secondary to being hit by a car or falls.^{36,45-48} Trauma can result in a combination of fractures, subluxation or luxation, traumatic disc rupture and surrounding soft tissue damage in addition to concurrent, non-nervous system injuries such as pulmonary contusions or bladder rupture.^{36,46} Importantly, while there are multiple causes of acute SCI in dogs, the most common, IVDH and trauma, both result in a combination of compression and contusion, comparable to most injuries in human SCI.

The Modified Frankel Scale (MFS), adapted from Frankel et al., is widely used to assign a neurologic grade in dogs with acute SCI.^{49,50} The MFS has various iterations including a 0-5 scoring system where 0 is normal neurologic function, 1 is pain only, 2 is ambulatory para/tetraparesis, 3 is non-ambulatory para/tetraparesis, 4 is para/tetraplegia and 5 is para/tetraplegia with loss of pain perception. While other scales incorporate additional detail or other aspects of neurologic dysfunction such as proprioceptive deficits, the MFS provides broad classification of neurologic status in a manner similar to the A through E categories of American Spinal Injury Association Impairment Scale (AIS A-E), also modified from the original Frankel descriptions and used in human SCI.⁴⁹⁻⁵⁴ Grade 5 SCI in dogs is, therefore, comparable to AIS-A injuries in people where no motor or sensory function is maintained below the level of injury.

The prognosis for recovery from acute SCI in dogs depends on the underlying cause and the severity of neurologic injury. As long as pain perception is maintained below the level of injury, however, the prognosis ranges from fair to excellent regardless of underlying cause.^{42,44,55-57} Even for dogs who suffer major vertebral column trauma, prognosis generally ranges from fair to good as long as sensation remains intact and comorbidities are not severe (DiFazio, Bali, Bruce, Carberry, Patterson, McKee 1990? Selcer?).^{36,45-48,58,59} Functionally complete, acute injury (i.e. grade 5 injury), however, carries a fair to guarded prognosis depending on the underlying cause.^{43,34,60-62} Amongst dogs with acute IVDH without pain

perception treated with surgical decompression, the prognosis for a successful outcome (defined as a return of sensation, independent ambulation and continence) is approximately 50%.^{60,61,63} For dogs with IVDH treated conservatively or SCI due to trauma, non-compressive disc extrusion or FCE, the prognosis is presumed to be guarded when pain perception is lost.^{42,44-46,56,58,61,64} Determining accurate prognosis amongst dogs with absent pain perception secondary to acute SCI is complicated by the high rate of euthanasia at the time of presentation due to presumed poor prognosis leading to only small numbers of dogs in which recovery has been tracked over time. Overall, functionally complete, acute injury frequently results in permanent neurologic impairment that interferes with day to day functioning, overlapping severe SCI in people.

Various prognostic indicators in addition to pain perception have been investigated to help predict outcome in dogs with acute SCI. These include variables associated with advanced imaging including myelography, computed tomography and magnetic resonance imaging (MRI).^{42,44,60,63,65-68} For example, presence and dimensions of a region of intramedullary hyperintensity on T2-weighted images on MRI has been associated with outcome in dogs with IVDH, acute, non-compressive nucleus pulposus extrusion and FCE.^{42,44,66} More recently, diffusion tensor imaging (DTI) and calculation of fractional anisotropy (FA) caudal to the lesion epicenter was associated with early motor recovery in paraplegic dogs but it was not superior to deep pain perception assessment.⁶⁹ Cerebrospinal fluid (CSF) analysis at the time of acute injury has also been explored for potential biomarkers. A higher CSF total nucleated cell count, percentage of macrophages and macrophage to monocyte ratio were associated with a poor outcome amongst dogs without sensation while lower CSF creatinine kinase and myelin basic protein were predictive of long-term ambulatory outcome.⁷⁰⁻⁷² Additional studies have investigated the relationship between prognosis and onset and duration of clinical signs, duration of surgery, changes in the cutaneous trunci reflex, as well as breed, age, body weight, lesion location, prior surgery or administration of corticosteroids.^{55,57,60,63,64,66,68,73-76} Unfortunately, none of the aforementioned variables have

surpassed the prognostic utility and simplicity of the clinical assessment of pain perception below the level of injury. Additionally, prognostic indicators in the acute SCI setting do not necessarily apply to dogs with incomplete recovery months to years after severe, acute injury. However, they do provide a framework from which expanded ways to evaluate injury characteristics in the chronic setting can be adapted.

Chronic Canine SCI

Dogs who suffered an acute SCI and fail to regain pain perception below the injury level are classified as having an unsuccessful outcome. The typical course of a successful recovery starts with regaining pain perception, followed by motor and autonomic function. As such, the failure to recover pain perception is interpreted as an indication of spinal cord transection and complete disconnection from all supraspinal influence. However, comparable to humans with SCI, complete physical transection is uncommon in dogs with spontaneous injuries classified as sensorimotor complete on neurologic examination.⁷⁷⁻⁸⁰ Additionally, despite being classified as ‘unsuccessful,’ the recovery potential amongst this population is not absent. While dogs who do not regain pain perception by one month after acute injury (regardless of cause or treatment), will likely never demonstrate normal sensation and frequently remain urinary and fecally incontinent, a proportion of these dogs demonstrate remarkable, spontaneous changes in motor recovery over time.^{61,81} In Olby et al., 7/19 (38%) dogs regained independent ambulation on average by 9 months after injury despite persistently absent sensation, and all dogs who improved exhibited a voluntary tail wag.⁶¹ In Gallucci et al., 48/81 (59%) acutely paraplegic dogs without pain perception secondary to IVDH, including approximately 2/3 of which underwent surgical decompression at the time of injury, regained the ability to walk independently over time despite no change in sensation.⁸¹ This study found that younger age and lower body weight were associated with regaining locomotion.⁸¹ Despite considering all dogs classified as having an unsuccessful outcome after acute, severe SCI as one uniform group, these variations in recovery potential

reflect that dogs with spontaneous, acute SCI represent a heterogeneous group with regard to population and lesion characteristics.

A more nuanced evaluation is warranted that takes into account the complex and variable lesion pathology amongst dogs classified as having complete lesions. Axons, particularly those of smaller diameter and in a subpial location, have been shown histopathologically traversing the lesion epicenter in dogs and other animals with clinically complete injury.^{77,80,82-84} Additional white matter changes in the region of injury included axonal degeneration and demyelination.⁸⁰ The relationship between residual structure and function has not been established in the dog after spontaneous injury but prior work in rats and cats has shown that as little as 5-10% of the original population of axons can preserve neurologic function after severe injury.⁸²⁻⁸⁴ Intact motor and sensory conduction across the lesion has been reported in dogs with chronically absent pain perception signifying at least some residual axons with spared function.⁸⁵ This is further supported by the finding that some dogs without recovery of pain perception exhibit voluntary tail wagging in response to a positive stimulus, again suggesting functional brain to tail connections.⁶¹ Taken together, these data suggest that some residual connections across the site of injury can be spared after severe injury which might be able to provide some degree of descending, supraspinal input. This has important implications for the nature of spontaneous motor recovery noted in some of these animals.

This is in contrast to the commonly-held belief in veterinary neurology that the motor recovery exhibited by some dogs without pain perception is exclusively reflexive stepping, also known as spinal walking.⁸⁶ The central pattern generator (CPG), an interconnected network of neurons within the lumbar spinal cord, has been attributed with producing autonomous pelvic limb stepping.⁸⁷ The CPG has been identified in multiple species including dogs and people, and spinal walking has been demonstrated in dogs with experimental spinal cord transection.^{10,11,87-93} While the CPG and its plasticity after injury is

no doubt important for return of motor function after experimental SCI, it, by itself, does not necessarily provide adequate explanation for the variability in dogs who do or do not exhibit ambulation after severe, spontaneous SCI.^{10,92} We speculate that regaining independent, functional locomotion amongst dogs with absent pain perception not only relies on a reorganized CPG below the level of injury but also some degree of spared supraspinal influence modulating the activity of the CPG and local reflex circuitry. This is supported by work in sensorimotor complete people in which epidural stimulation aimed at motor networks below the level of injury produced some voluntary control of limb function perhaps by unmasking limited residual supraspinal connections.²¹ Understanding how and why some dogs with chronically absent pain perception after acute SCI recover effective walking while others do not is important for therapy development and can provide important translational insights for humans with SCI.

Dogs as a model of spontaneous SCI

As mentioned previously, there is a high prevalence of acute, spontaneous SCI in dogs, and for those with IVDH, approximately 15% are reported to present with complete lesions characterized by paralysis and loss of pain perception.^{17,34-38} Among this severely affected group, only a proportion will recover; the prognosis is worse for dogs with complete injuries secondary to vertebral trauma.³⁷ This results in a readily available population of dogs with permanent impairment in which to study naturally-occurring SCI. Dogs also mirror the heterogeneity of human SCI at the population level. While certain breeds such as the Dachshund are predisposed to IVDH and, therefore, overrepresented, dogs of any age, breed and sex can suffer acute SCI, resulting in a mixed population affected.⁴⁰

The most common causes of acute SCI in dogs are IVDH and trauma, both of which cause a mix of contusive and compressive injury, similar to human traumatic SCI.^{17,37,80} While obtaining histopathologic samples is challenging in both canine and human spontaneous SCI,

there are multiple studies reporting many overlapping pathologic features that vary by the severity and duration since the injury.^{78,80,94,95} In human SCI, acute to subacute injury has been characterized histopathologically by edema, hemorrhage, myelomalacia, activated glial and inflammatory cells, early glial scar formation and Wallerian degeneration.⁷⁸ Similarly, histopathologic examination in dogs has identified areas of axonal damage and concurrent demyelination along with necrosis and vascular changes in the early stages after acute SCI.^{77,80} In fact, severe axonal swelling characterizes the acute phase followed by progressive degeneration extending away from the lesion epicenter, myelin loss and a phagocytic response.⁹⁶ Activation of resident microglia has been shown to play a prominent role in the post-injury inflammatory response during the acute to subacute phase in canine SCI which is similar to humans.^{94,96} There is also immunohistochemical overlap with human SCI with widespread expression of non-phosphorylated neurofilament, beta amyloid precursor protein and matrix metalloproteinase 9 in the spinal cords of dogs with spontaneous SCI.⁹⁶

Evaluation in the chronic timeframe in human SCI has revealed gray matter loss replaced by cystic lesions that sometimes coalesced and extended beyond the original region of injury, and the potential for spared long tract axons with variable remyelination located in a peripheral, circumferential location.⁷⁸ MRI findings in chronic SCI in humans have also been reported and describe changes consistent with myelomalacia, syrinx formation and spinal cord atrophy, compression, tethering and disruption which likely reflect corresponding histopathologic changes.⁹⁷⁻⁹⁹ In the chronic stage of canine SCI, demyelination persists in some areas along with other changes including areas of remyelination, axonal degeneration and loss.⁸⁰ While there are no specific reports examining post-traumatic syringomyelia in dogs, cystic cavity formation has been described and syrinx formation has been mentioned as a sequela of chronic canine SCI.^{17,80} Axonal damage varies by size and location with greater loss among larger diameter fibers and sparing along a subpial rim.⁸⁰ Additionally, axon fibers have been noted entering into or traversing the epicenter which could represent axonal regeneration or spared small diameter fibers.⁸⁰ These findings demonstrate the lesion

complexity and heterogeneity in both dogs and humans after severe, spontaneous SCI providing support that injury and the associated pathophysiologic sequelae in dogs is similar to that in people.

Several defined, reliable outcome measures have been developed for use in dogs with SCI.²³ These primarily focus on gait scales with the most extensive use in the acute to subacute phases of injury.^{23,50-53,95,100,101} Other available evaluation methods include magnetic resonance imaging techniques, electrodiagnostic methods, sensory threshold testing, kinematic evaluation, urodynamic studies and ongoing blood and CSF biomarker development.^{23,81,85,95,102,103} The availability of outcome measures allows quantification and tracking of function over time, and allows dogs to serve as an intermediate screening step in evaluating response to a particular intervention.²⁴

There are multiple reports using dogs as a model of acute and chronic spontaneous SCI, most of which evaluated interventions previously tested in rodent models.^{13,85,95,103-109} Borgens et al., reported a beneficial effect on recovery due to an applied oscillating electrical field in a clinical trial of dogs with acute, functionally complete SCI.¹⁰⁵ Levine et al., evaluated a matrix-metalloproteinase inhibitor in dogs with acute SCI that was previously shown to improve recovery in a murine model.¹⁰³ Results of the randomized, blinded, placebo-controlled study in dogs did not show a beneficial effect directly attributable to the inhibitor, however, improved gait scores were noted in the group receiving the vehicle, dimethyl sulfoxide, suggesting it has potential neuroprotective benefits.¹⁰³ Olby et al., performed a placebo-controlled, randomized trial of polyethylene glycol and methylprednisolone sodium succinate in acutely paralyzed dogs with absent pain perception and failed to show a benefit of either treatment in this population.¹⁰⁹ This trial highlights that some canine studies have been performed in advance of human clinical trials while others have been executed concurrent with or after comparable trials in people. Studies of dogs with spontaneous injuries have the ability to inform human SCI research but also to try to replicate work in

human clinical trials to help guide clinical decision-making regarding SCI for veterinarians in parallel with physicians, both of whom routinely encounter SCI cases in practice.

Clinical trials performed in the chronic setting typically recruit dogs with incomplete or absent recovery after prior acute, severe SCI. Since these are pet dogs, they can be maintained for months to many years after injury, overlapping with human SCI with regard to range in injury chronicity. Blight et al., performed a phase I trial in dogs with chronic, severe SCI investigating the potassium channel antagonist, 4-aminopyridine (4AP), that has been shown to improve central conduction in the injured spinal cord.¹⁰⁴ In a blinded, placebo-controlled trial of chronically non-ambulatory dogs, Lim et al., demonstrated 4AP and a derivative, T-Butyl Carbamate, improved hind limb motor function but the response was very variable between dogs highlighting the need to better understand injury differences between individuals.¹⁰⁷ Granger et al., evaluated intraspinal transplantation of autologous olfactory ensheathing cells, previously shown to improve outcome in rodent models, in a population of dogs with absent pain perception and chronic deficits after acute, functionally complete SCI.⁸⁵ While this study and other canine stem cell studies have demonstrated the safety of the transplanted cells and possible beneficial effects on locomotion, meaningful improvement in neurologic function was limited suggesting this therapeutic modality requires further optimization for both dogs with SCI and translational application to humans with SCI.^{13,85,106,108}

Rationale for this work

Dogs with spontaneous SCI are being increasingly used as a translational large animal model, but gaps remain in model development that need to be addressed to maximize their potential utility and to allow more widespread use and acceptance by the SCI research community.^{13,33} Chief among this is improved recognition and understanding of lesion heterogeneity within the severe injury category. Currently, human and canine clinical trials recruit primarily based

on neurologic grade (i.e. using the MFS or AIS grading schemes). This method treats all dogs with grade 5 injury or people with AIS-A injury as if they have the same injury. While dogs who fail to regain pain perception represent a good approximation of people with chronic, sensorimotor complete SCI, this paradigm fails to consider the impact of injury complexity and varying severity on differential outcomes amongst these populations. Improving our recognition of lesion heterogeneity will allow differentiation amongst subpopulations and enhance our overall understanding of SCI. Studying a more targeted population might improve the translation of therapies that appear successful in rodent models but fail in large-scale human clinical trials. Even without sub-dividing dogs with severe SCI, understanding the heterogeneity of the entire population still allows promising interventions to be screened in dogs with a reasonable approximation of expected outcome in a comparable human clinical trial as well as offers mechanistic insights into successes or failures. Key to success with this method, however, is ensuring appropriate clinical trial design and sufficient numbers of dogs to have the power to detect an effect.²³

While specific outcome measures to define injury severity and functional status in dogs with SCI have been developed, there is an ongoing need to refine and expand the available tools. This is especially relevant for evaluation methods that overlap with those available in humans to allow a more direct comparison. These include further investigation of novel imaging techniques, electrodiagnostic and complex gait analysis methods. Additionally, clinical scales (and questionnaires) are commonly used in human SCI patients to address specific aspects of impairment such as spasticity or pain but these are, as yet, mostly untapped areas in canine SCI. Improved ability to assess the structural and functional components of the injury and to determine how lesion characteristics relate to motor function will provide insight into the nature of recovery from SCI. This may identify specific aspects of injury as targets for manipulation and facilitate testing of novel therapies in canine clinical trials to improve recovery amongst all severely affected individuals. While dogs with spontaneous, severe SCI will not replace experimental rodent models, they have the potential

to address some of the challenges and perhaps provide a link between animal and human SCI.^{24,33,95}

We, therefore, aimed to thoroughly characterize a naturally occurring canine model of chronic paralysis using clinical, gait analysis, electrodiagnostic and imaging techniques. Objectives with this characterization were to develop additional outcome measures to expand the available means of evaluating this population and to further understanding of the nature and variability of motor recovery in dogs with severe SCI. As a component of this enhanced characterization, potential mechanistic explanations were explored for the variability in response to 4AP as a treatment for SCI in this population. The specific aims for each chapter are outlined below. Given the high prevalence and profound impact of SCI in both humans and dogs, the ultimate goal is to expand the ability to utilize dogs as a translationally-relevant large animal model in parallel with rodent and human work to improve outcomes for both canine and human SCI.

Chapter 2 – Development of a clinical spasticity scale for evaluation of dogs with chronic thoracolumbar SCI. The aims were to develop a feasible and repeatable scale for assessment of spasticity in dogs with incomplete recovery from severe, acute thoracolumbar SCI, to investigate factors associated with spasticity development and to determine the relationship between spasticity and gait.

Chapter 3 – Development of novel gait analysis tool for evaluation of canine thoracolumbar SCI. The aim was to use an instrumented, pressure sensitive treadmill to analyze the center of pressure and its variability as well as the percentage of body weight support during ambulation in dogs with chronic gait deficits after severe, acute thoracolumbar SCI.

Chapter 4 – The relationship between trans-lesional conduction, motor neuron pool excitability and motor function in dogs with incomplete recovery from severe SCI. The aims

were to characterize the electrophysiologic status of motor and sensory long tracts and local reflex circuitry in dogs with incomplete recovery from severe, acute thoracolumbar SCI and to correlate findings to gait.

Chapters 5 – Magnetic resonance imaging (MRI) features of dogs with incomplete recovery after acute, severe SCI. The aim was to describe the MRI features of chronic SCI amongst dogs with an incomplete recovery after acute, functionally complete injury and to investigate associations between imaging variables and functional status.

Chapter 6 – The relationship between lesion severity characterized by diffusion tensor imaging (DTI) and motor function in chronic canine SCI. The aims were to perform DTI in a population of dogs with incomplete recovery from severe, acute thoracolumbar SCI, to generate fractional anisotropy (FA) and mean diffusivity (MD) values cranial, caudal and within the lesion, to use tractography to determine integrity through the lesion epicenter, and to investigate associations between DTI indices and motor function.

Chapter 7 – Predictors of response to 4-aminopyridine in a population of chronically paralyzed dogs. The aim was to compare spasticity severity, trans-lesional motor and sensory conduction, motor neuron pool excitability, gait scores and lesion characteristics on conventional MRI and DTI between dogs that do and do not respond to 4AP amongst a population with incomplete recovery after severe, acute SCI.

Chapter 8 – The main conclusions are reiterated and discussed along with limitations and future directions for this work.

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CHAPTER 2

Development of a clinical spasticity scale for evaluation of dogs with chronic thoracolumbar spinal cord injury

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OBJECTIVE

To develop a spasticity scale for dogs with chronic deficits following severe spinal cord injury (SCI) for use in clinical assessment and outcome measurement in clinical trials.

ANIMALS

20 chronically paralyzed dogs with a persistent lack of hind limb pain perception caused by an acute SCI at least 3 months previously.

PROCEDURES

Spasticity was assessed in both hind limbs via tests of muscle tone, clonus, and flexor and extensor spasms adapted from human scales. Measurement of patellar clonus duration and flexor spasm duration and degree was feasible. These components were used to create a canine spasticity scale (CSS; overall score range, 0 to 18). Temporal variation for individual dogs and interrater reliability were evaluated. Gait was quantified with published gait scales, and CSS scores were compared with gait scores and clinical variables. Owners were questioned regarding spasticity observed at home.

RESULTS

20 dogs were enrolled: 18 with no apparent hind limb pain perception and 2 with blunted responses; 5 were ambulatory. Testing was well tolerated, and scores were repeatable between raters. Median overall CSS score was 7 (range, 3 to 11), and flexor spasms were the most prominent finding. Overall CSS score was not associated with age, SCI duration, lesion location, or owner-reported spasticity. Overall CSS score and flexor spasm duration were associated with gait scores.

CONCLUSIONS AND CLINICAL RELEVANCE

The CSS could be used to quantify hind limb spasticity in dogs with chronic thoracolumbar SCI and might be a useful outcome measure. Flexor spasms may represent an integral part of stepping in dogs with severe SCI. (*Am J Vet Res* 2017;78:854–861)

Spasticity is a commonly recognized complication following SCI in humans, affecting an estimated 60% to 78% of people living with chronic SCI.^{1,2} Only rare reports^{3–5} exist involving spasticity due to experimentally induced SCI in dogs, and no data are available regarding the frequency and severity of spasticity in dogs with naturally occurring SCI.

In human medicine, the most frequently cited definition refers to spasticity as a motor disorder characterized as a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks due to hyperexcitability of the stretch reflex.⁶ Clinical spasticity can most accurately be described as a combination of abnormalities, including exaggerated reflexes, increased muscle tone, un-

controlled limb movements, and flexor and extensor spasms, any of which may be more or less apparent in an individual patient and may develop secondary to > 1 pathophysiologic mechanism.^{7,8} Proposed mechanisms include a combination of factors related to loss of supraspinal input and maladaptive central processing of afferent sensory input to the spinal cord below the level of the SCI, which together cause an increase in motor neuron excitability and the clinical manifestations of spasticity.⁸

Although clinical findings of spasticity in affected limbs are simple to recognize, the condition represents a complex problem and accurate quantification is challenging. Several clinical scales have been developed in human medicine to quantify the severity of spasticity, including the Ashworth Scale (also the Modified and Modified Modified Ashworth Scale) and SCATS.^{9–14} The Ashworth Scale is used to assess an increase in muscle tone and resistance to passive movement and provides a global measure of spasticity, whereas the SCATS is used to evaluate clonus, flexor spasms, and extensor spasms.^{9,10} Addition-

ABBREVIATIONS

CSS	Canine spasticity scale
OFS	Open field score
RI	Regularity index
SCATS	Spinal cord assessment tool for spastic reflexes
SCI	Spinal cord injury
SS	Stepping score

al evaluation methods include self-reported measures such as the Penn Spasm Frequency Scale as well as biomechanical and electrophysiologic testing.^{2,15-23}

The clinical scales are straightforward to implement in a clinical setting, but no single test provides a comprehensive evaluation and so multiple tests are often combined. Clinical tests for spasticity are also variably correlated with each other, with patient-reported impairment attributable to spasticity, and with electrophysiologic and biomechanical measurements.^{2,10,11,15,19,21,23,24} Furthermore, spasticity varies between and within affected individuals over time.^{14,15,21} Nevertheless, spasticity can cause pain, interfere with daily functioning, and adversely affect quality of life for humans with SCI.^{1,15,21,25-28} The impact of spasticity coupled with the high prevalence in this population warrants its use as the primary outcome measure in clinical trials and development of improved diagnostic testing methods.²⁹

Dogs with severe, chronic thoracolumbar SCI commonly develop signs of spasticity weeks to months after the initial injury.^{3,5,30} However, no clinical assessment tools exist in veterinary medicine for the evaluation of spasticity in affected dogs. The impact of spasticity on affected dogs is also unknown. The purpose of the study reported here was to use the aforementioned human scales to develop a feasible and repeatable scale for assessment of spasticity in chronically paralyzed dogs and to investigate factors associated with spasticity development and the relationship between spasticity and gait.

Materials and Methods

Ethics statement

Informed consent was obtained from all owners of participating dogs. The study protocol was approved by the North Carolina State University Institutional Animal Care and Use Committee (protocol No. 15-004-01).

Dogs

Dogs were prospectively enrolled from among patients of the Canine Spinal Cord Injury Program at the North Carolina State University College of Veterinary Medicine between March and December 2015. For inclusion, dogs were required to have chronic gait deficits with absent or reduced hind limb and tail pain perception (with or without urinary and fecal incontinence) due to an acute thoracolumbar (T3-L3 region) SCI sustained a minimum of 3 months previously. Data were collected for each dog regarding signalment, concomitant medications, diagnosis, lesion location, injury duration, and SCI treatments.

General neurologic evaluation

All dogs underwent an initial neurologic examination that included evaluation of gait, proprioception, spinal reflexes, and pain perception. Gait analysis consisted of watching the dog walk on a nonslip surface as well as on a treadmill, with sling support

provided if it was unable to walk unassisted. The treadmill was adjusted to a comfortable speed for each dog, and dogs were walked for approximately 3 minutes. All examinations were videotaped. Gait was categorized as ambulatory (at least 10 consecutive weight-bearing steps unassisted), nonambulatory with motor function, or paraplegic and was also categorized with previously validated objective gait assessment tools (OFS, SS, and RI; **Supplementary Appendix S1**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>).³¹⁻³⁴

For proprioceptive limb placement and hopping tests, findings were recorded as absent (0), delayed (1), or normal (2). For patellar and withdrawal reflex tests, findings were recorded as absent (0), decreased (1), normal (2), increased (3), or clonus (4). For evaluation of hind limb muscle tone, findings were recorded as decreased or flaccid, normal, or increased. For the cutaneous trunci reflex, the vertebral level of the caudal border where the reflex could still be elicited was recorded. Nociception in the hind limb and tail was recorded as present or absent, and urination was recorded as voluntary or involuntary.

Initial spasticity testing

All dogs received a spasticity evaluation at the time of neurologic examination. Tests of muscle tone, clonus, and flexor and extensor spasms were adapted from human clinical scales (Ashworth Scale and SCATS) and evaluated for feasibility.^{10,11} The Ashworth Scale involves use of resistance to passive movement to assess increases in muscle tone, and responses are scored on an ordinal scale for each muscle group evaluated. The SCATS involves evaluation of plantar flexor clonus, hind limb flexor spasms, and hind limb extensor spasms, with each quantified on an ordinal scale ranging from 0 to 3. Clonus of the plantar flexors is scored on the basis of the duration of clonic activity following rapid, passive dorsiflexion of the ankle joint. Flexor spasms are graded by duration and degree of response following application of pinprick stimulation to the bottom of the foot. Extensor spasms are elicited by passive, simultaneous extension of the stifle and hip joints and scored by duration of the resultant, visible quadriceps contraction.

For feasibility testing, each dog was positioned in lateral recumbency and lightly restrained to ensure it remained still and relaxed, and the uppermost limb was tested. First, resistance to passive range of motion was assessed at each of the hock, stifle, and hip joints (adapted Ashworth Scale). For each joint, observed tone was categorized as normal (no resistance) or mildly, moderately, or markedly increased (passive movement difficult). Following testing of the first hind limb, the dog was rotated in position, and the other hind limb was tested similarly. Results obtained with the adapted Ashworth Scale were extremely variable in all dogs, so this scale was no longer considered for inclusion in the CSS.

While dogs were still positioned in lateral recumbency, patellar and plantar flexor clonus and flexor

and extensor spasms were then assessed (adapted SCATS). The patellar reflex was elicited by striking the patellar tendon with a reflex hammer to assess for the presence of clonus. With the limb relaxed in extension, flexor spasms were triggered by pinprick stimulation of the skin of the bottom of the foot with a 25-gauge needle at the midmetatarsal level. The stifle and hip joints were then flexed to 90°, and extensor spasms were assessed by monitoring for quadriceps contraction following simultaneous extension of the hip and stifle joints by the investigator. Extensor spasms were rarely elicited, so this particular test was eliminated from consideration for inclusion in the CSS. Plantar flexor clonus, tested by rapid dorsiflexion of the hind foot, was never elicited and this test was eliminated from consideration as well.

Final CSS and scoring

Assessment of patellar clonus duration and flexor spasm duration and degree was feasible in both hind limbs of all dogs, and these variables were used to create the CSS (**Supplementary Appendix S2**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>). Each dog was evaluated for patellar clonus and flexor spasm by use of the CSS scoring system. Tests were repeated 3 times (ie, 3 trials) on each hind limb, with at least 30 seconds separating trials.

Duration (as a continuous variable, in seconds) was recorded for each trial of clonus and spasm. If clonus or spasm was still evident 60 seconds after the trial was initiated, that trial was discontinued and a maximum duration of 60 seconds was recorded. The mean duration of the response (mean of the 3 trials) was then calculated for patellar clonus and flexor spasms in each limb. Individual durations for each trial and mean duration for each hind limb were subsequently converted to an ordinal scale, with a score of 0 indicating no clonus or spasm, a score of 1 indicating a duration of ≤ 3 seconds (mild), a score of 2 indicating > 3 but ≤ 10 seconds (moderate), and a score of 3 indicating > 10 seconds (severe).

Continuous and ordinal data for flexor spasm duration averaged across the 3 trials were compared in a subset of dogs for which the data were available ($n = 11$). These data were qualitatively similar. However, the distribution of continuous data was not normal, with most durations < 10 seconds and a broad range in duration among spasms lasting > 10 seconds, up to the 60-second cutoff. Given the skewed distribution but natural separation into categories, ordinal data classification was used for the CSS data in the same manner as for the SCATS data.

Angle of flexion of the hip and stifle joints during flexor spasms was estimated and broadly categorized, with the 2 joints considered as 1 entity. By this system, a score of 0 indicated no flexion, a score of 1 represented $< 10^\circ$ (mild), a score of 2 represented 10° to 45° (moderate), and a score of 3 represented $> 45^\circ$ (severe). The median score for the 3 trials was used

to assign the overall score for degree of flexor spasms for each hind limb.

The mean (clonus and spasm duration) or median (spasm degree) score (0 to 3) was assigned for each hind limb for each scale component, which were then summed for the right and left hind limbs to yield a total score (0 to 6) for each component and an overall score representing all components and both hind limbs (0 to 18). Overall scores were also used to categorize dogs as having absent to mild spasticity (0 to 6) or moderate to severe spasticity (7 to 18).

To evaluate the variability in spasticity (CSS scores) over time within a given dog, a subset of dogs ($n = 10$) was returned for repeated testing by the same rater. The same testing protocol (for both hind limbs) was applied at each testing session. Testing was repeated once for 8 dogs and twice for 2 dogs, with the period between the initial and subsequent testing sessions ranging from 48 hours to 7 months. Interrater reliability was determined by having 2 raters (one of whom had no involvement in scale development) test a subset of dogs on the same day and in the same testing conditions, with 2 to 5 minutes separating testing sessions.

Owner questionnaire development

A questionnaire was developed to collect from dog owners information regarding the presence, frequency, and impact of spastic movements in their dogs at home in a manner comparable to the Penn Spasm Frequency Scale and measures of quality of life for people living with SCI (adapted from the Penn scale; **Supplementary Appendix S3**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>).^{19,20,24,27,28,35} Requested information included presence (yes or no) of involuntary movements, jerking, or muscle spasms (in flexion, extension, or both); frequency of these movements (> 10 times/d, 1 to 10 times/d, > 1 time/wk, or < 1 times/wk); time of day at which these movements were noticed (day, night, or random); asymmetry of these movements (left hind limb, right hind limb, or random); and impact of these movements on daily functioning or quality of life (none, minimal, moderate, or severe). Because of the subjectivity inherent to an owner-reported measure, spastic movements at home were classified as present or not present for comparison with observations recorded for the investigator-administered CSS.

Statistical analysis

Statistical software^{a,b} was used for all analyses. The CSS overall score and its 3 individual component mean or median scores were summarized for all dogs and are reported as median (range). Interrater reliability and temporal variation in measurements for dogs that were evaluated more than once were measured by calculation of intraclass correlations. To identify factors that may influence the development of spasticity, associations were examined between overall CSS score and dog age, SCI duration, and le-

sion location (cranial or caudal to T13, to capture the possible effect of greater numbers of intact spinal segments on the development of spasticity) by use of logistic regression (age and injury duration) and 1-way ANOVA (lesion location). Logistic regression and the Wilcoxon rank sum test were also performed to determine whether CSS scores (overall and individual component scores) were associated with the ability to walk (yes or no) and gait scores (derived from the OFS, SS, and RI). The Holm-Bonferroni method was used to correct *P* values for multiple comparisons (denoted as *P*_{adjusted}). For all analyses, values of *P* < 0.05 were considered significant.

Results

Dogs

Twenty dogs with thoracolumbar SCI were included in the study (**Supplementary Appendix S4**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>). Mean (SD) body weight was 13.1 kg (9.9 kg), and mean age was 6.0 years (2.7 years). Breeds included Dachshund (n = 6 [30%]), mix (3 [15%]), Dachshund-Chihuahua cross (2 [10%]), pit bull-type (2 [10%]), Miniature Schnauzer (1 [5%]), Labrador-Retriever-Poodle mix (1 [5%]), German Shepherd Dog (1 [5%]), English Bulldog (1 [5%]), Miniature Poodle (1 [5%]), Shih Tzu (1 [5%]), and Boston Terrier (1 [5%]).

Median SCI duration was 12 months (range, 4 to 84 months). Suspected or confirmed intervertebral disk disease was the most common diagnosis (n = 14). In all dogs, the site of the neurologic lesion was identified between T3 and L3, except for 1 dog with concurrent, less pronounced caudal cervical abnormalities. Magnetic resonance imaging or CT was performed for 15 (75%) dogs, revealing a spinal cord lesion at T13 or cranially in 8 dogs, caudal to T13 in 6 dogs, and diffusely distributed within the thoracolumbar portion of the spinal cord in 1 dog with a suspected inflammatory reaction secondary to an IM (epaxial) melarsomine injection.

At the time of evaluation, 7 (35%) dogs were paraplegic, 8 (40%) were nonambulatory with motor function, and 5 (25%) were ambulatory. Median OFS was 1 (range, 0 to 9), median unsupported SS was 0 (range, 0 to 89), and median unsupported RI was 0 (range, 0 to 46.56). Eighteen (90%) dogs had no

signs of hind limb and tail pain perception, whereas 2 (10%) dogs had a severely blunted response in some digits of the hind limbs. Seventeen (85%) dogs were urinary incontinent, whereas 3 (15%) had consistent urine voiding without the need for manual bladder expression. No dogs had signs of pain on palpation of the vertebral column. No dogs were receiving anti-spasticity treatment when evaluated.

CSS

The CSS assessment was easy to perform and well tolerated by all dogs. Hind limb flexor spasms were extremely variable among dogs and were sometimes dramatic in their intensity and duration (**Supplementary Videos S5 and S6**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>). Patellar clonus was uncommon (identified in ≥ 1 hind limb in 5 [25%] dogs) and relatively brief (median score, 0; range, 0 to 4), compared with the frequency of flexor spasms (identified in all 20 [100%] dogs) and the duration of flexor spasms (median score, 2; range, 2 to 6). Median flexor spasm degree score was 4 (range, 1 to 6). Median overall CSS score was 7 (range, 3 to 11). Ten (50%) dogs were categorized as having absent to mild spasticity (overall score, 0 to 6), and the other 10 (50%) were categorized as having moderate to severe spasticity (overall score, 7 to 18; **Table 1**).

Flexor spasm duration and degree scores were significantly (*P* < 0.001) moderately to highly correlated with overall CSS score (*R*² = 0.57 and *R*² = 0.82, respectively) and were mildly correlated with each other (*R*² = 0.33 [*P* = 0.008]). Patellar clonus duration scores were poorly correlated with flexor spasm duration and degree scores (*R*² = 0.01 [*P* = 0.67] and *R*² = 0.14 [*P* = 0.11], respectively) and with overall CSS score (*R*² = 0.24 [*P* = 0.03]).

Eight dogs had a 1-point difference and 2 dogs had a 2-point difference between total score (all 3 components) for the left hind limb and total score for the right hind limb. Of these 10 dogs, 8 had no detectable asymmetry on clinical neurologic examination, whereas in 2 dogs, the limb with the more severe spasticity (characterized by greater duration or degree of flexor spasms) was the same as the more severely affected side on examination. Subtle to mild clinical asymmetry was noted during neurologic examination of an additional 6 dogs; however, in none

Table 1—Gait scores and clinical characteristics of 20 dogs with chronic thoracolumbar SCI categorized by spasticity severity.

CSS spasticity severity category	Median (range) CSS score	Mean (SD) age (y)	Mean (SD) SCI duration (mo)	No. of ambulatory dogs	Median (range) OFS	Median (range) SS	Median (range) RI
Absent to mild (n = 10)	4 (3–6)	5.2 (3.0)	17.8 (19.5)	1	0.5 (0–6)	0 (0–36)	0 (0–5.88)
Moderate to severe (n = 10)	10 (8–11)	6.7 (2.3)	22.4 (24.7)	4	4.0 (0–9)	48 (0–89)	9.62 (0–89)

Possible range of overall CSS scores was 0 to 18, with scores of 0 to 6 representing absent to mild spasticity and 7 to 18 representing moderate to severe spasticity.

of these dogs did CSS scores differ between left and right limbs.

CSS reliability and temporal variation

Interrater reliability for 2 raters both scoring 8 dogs was high (intraclass correlation coefficient, 0.93). Repeated testing of 10 dogs by the same rater identified some variability between testing times (intraclass correlation coefficient, 0.72). With total CSS score for each limb used, a difference between testing times of 1 point was observed for the left hind limb of 7 dogs and the right hind limb of 6 dogs. For only 1 dog did total scores differ by > 1 point, with a difference of 2 points in each hind limb observed between testing sessions held 3 days apart. Variability between testing times in flexor spasm duration was comparable to that for overall CSS scores.

Associations of CSS scores with other variables

No significant associations were identified between overall CSS scores and dog age ($P_{\text{adjusted}} = 0.29$), body weight ($P_{\text{adjusted}} = 0.28$), SCI duration ($P_{\text{adjusted}} = 0.93$), and lesion location (cranial vs caudal to T13; $P_{\text{adjusted}} = 1.00$; $n = 20$). Data regarding 2 dogs with atypical lesions (1 dog with concurrent injury in the C6-T2 region and 1 dog with diffuse thoracolumbar involvement) were removed from analyses involving comparisons of CSS scores with gait scores because their injuries may have impacted motor function differently than in dogs with focal lesions in the T3-L3 region. For the remaining 18 dogs, no significant ($P_{\text{adjusted}} = 0.26$) association was identified between overall CSS score and ability to walk (yes or no). However, overall CSS score was significantly positively associated with OFS ($P_{\text{adjusted}} = 0.048$), SS ($P_{\text{adjusted}} = 0.042$), and RI ($P_{\text{adjusted}} = 0.048$). To determine which aspect of spasticity was driving this relationship, the scores for each scale component (combined for both hind limbs) were compared with gait scores. No significant ($P_{\text{adjusted}} \geq 0.38$) associations were identified between patellar clonus duration scores or flexor spasm degree scores and any gait scores. However, flexor spasm duration scores were positively associated with the ability to walk ($P_{\text{adjusted}} = 0.01$), OFS ($P_{\text{adjusted}} = 0.008$), SS ($P_{\text{adjusted}} < 0.001$), and RI ($P_{\text{adjusted}} < 0.001$; **Supplementary Video S7**, available at <http://avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.7.854>).

Owner questionnaire

Involuntary hind limb jerking movements at home were reported by owners for 16 (80%) dogs. For 10 of these dogs, spastic jerking reportedly occurred 1 to 10 times/d, whereas for 5 dogs, it occurred ≤ 1 time/wk, and for 1 dog, it occurred > 10 times/d. Four dogs were reported to have spasms, which reportedly occurred 1 to 10 times/d for 3 dogs and ≤ 1 time/wk for 1 dog. When present, spastic movements

of any kind were considered asymmetric between limbs for 5 dogs, and 4 owners reported that spasticity varied with time of day (day vs night).

Owners of 18 (90%) dogs reported that additional spastic movements could be induced by tactile stimulation, such as during urinary bladder expression or touching the digits or hind limbs. Two owners reported a mild, adverse impact of spasticity on their dog's quality of life and daily functioning, whereas no impact was reported for the remaining dogs. No relationships were identified between spasticity observed at home and overall CSS scores categorized by severity. Of the 12 dogs for which owners reported spastic movements (involuntary jerking or spasms) occurred ≥ 1 time/d, 7 were scored as having absent to mild spasticity (overall CSS score, 0 to 6) and 5 were scored as having moderate to severe spasticity (overall CSS score, 7 to 18). Among the 8 dogs in which spasticity at home was reported less frequently or absent, 3 had absent to mild spasticity as measured with the CSS and 5 had moderate to severe spasticity.

Discussion

Results of the present study suggested that spasticity in dogs with chronic, severe SCI can be quantified through assessment of patellar clonus and flexor spasms. Flexor spasms were the most prominent finding and varied widely among dogs, with spasm duration strongly associated with gait scores. The semiquantitative scale we developed may be a useful adjunct to the standard neurologic examination for dogs with incomplete recovery from thoracolumbar SCI as well as an important tool for investigating this largely ignored phenomenon in dogs in more depth.

Spinal cord injury is common in dogs, and the veterinary literature commonly refers to spasticity in dogs concurrent with other signs of upper motor neuron dysfunction.^{36,37} However, no consensus exists on the precise definition of spasticity in dogs and no validated, objective evaluation tools are available in veterinary medicine. We developed a noninvasive test from clinical scales currently used in human medicine,⁹⁻¹⁴ and we focused on a sample of dogs that had sustained acute, functionally complete thoracolumbar SCI because disruption of supraspinal input is considered an important component of spasticity.⁸ To capture a range of spasticity, the included dogs had a wide range in hind limb motor function despite failure to recover normal hind limb and tail pain perception. We elected to include 2 dogs with blunted but not completely absent pain perception, 1 dog with a diffuse thoracolumbar injury, and 1 dog with multifocal injury because these dogs had profound neurologic deficits indicative of severe injury and an incomplete recovery. They were, therefore, at risk for spasticity and were part of the clinical population we wished to investigate. The dog with diffuse thoracolumbar injury and the dog with multifocal injury were removed from statistical analysis of relationships between CSS scores and gait scores because the

impact of their lesions on motor function (compared to the more typical, focal lesion) was unknown.

During development of the spasticity testing protocol in the present study, extensor spasms were elicited rarely from the dogs, which is distinct from similar testing in humans, in whom extensor spasms are more common than plantar clonus.¹⁰ Patellar clonus was also uncommon in the dogs of the present study but was still consistently identified in 25%. In contrast, flexor spasms were elicited to at least some degree in all dogs and were severe and prolonged in some dogs. The reason for these discrepancies from findings in humans is unclear but may reflect positioning differences. Flexor and extensor spasms are assessed in humans while lying supine, whereas many dogs do not appear comfortable or are not stable lying on their backs.¹⁰ Instead, we positioned dogs in lateral recumbency, allowing them to lie comfortably and remain still throughout the testing period, with their hind limbs easily accessible and relaxed in relative extension.

Neuroanatomic differences between species may also explain the differences in frequency of clonus and flexor and extensor spasms between the study dogs and humans. For example, the corticospinal tract is the primary descending motor pathway controlling voluntary movement in humans but is relatively less important in dogs, compared with the rubrospinal and reticulospinal tracts.^{36,38,39} Although overlap in function and compensation after injury between these upper motor neuron tracts has been demonstrated in multiple species, the relative importance of disruption of the corticospinal tract in humans versus dogs, rather than just the general loss of supraspinal input on α motor neurons, may result in variations with regard to when, how, and to what extent spasticity develops in these 2 species.³⁹⁻⁴¹ Despite flexor spasms being the most prominent component and patellar clonus being infrequently elicited and poorly correlated with the other components, we elected to maintain all aspects as a combined scale for the purposes of reporting our initial CSS for dogs with SCI. In this patient population, flexor spasm duration alone might prove most useful and practical in the evaluation of spasticity. However, patients with different SCIs may have different components of spasticity, including extensor spasms.

The final spasticity testing protocol required minimal training to perform, and results were comparable when testing was performed by 2 raters with different clinical backgrounds. Because spasticity within an individual is not a static feature, we assessed the temporal variability in test results for 10 dogs. We noted a mild to moderate difference in scores for most dogs evaluated more than once, consistent with fluctuations of spasticity testing results reported for humans.²¹ However, re-testing was performed only once for 8 of 10 dogs, so this preliminary finding may not represent the true temporal fluctuation in spasticity in dogs. Repetitive testing on multiple occasions may provide further information

about the extent of variation in spasticity in dogs with chronic SCI, the degree to which the CSS captures this vacillation, and how results compare with those for humans with similar injuries.

The owner questionnaire was developed in the present study to capture observations on the manifestation of spasticity in dogs in their home setting. Findings obtained with the veterinarian- or investigator-administered CSS were not associated with owner reports of spasticity in their dogs at home, thereby mirroring the variable correspondence observed between physician- or investigator-administered scales (such as the Ashworth Scale) and patient-reported scales (such as the Penn Spasm Frequency Scale).^{19,24} These findings, considered with the temporal variation in CSS scores, might highlight the importance of assessing dogs with chronic thoracolumbar SCI more than once and might reflect the dynamic nature of spasticity.

In the owner questionnaire, responses were requested in the form of frequency categories, but in working with the owners, we found that spastic movements were not something they were consciously tracking prior to study participation, thereby limiting the reliability and applicability of these preliminary results. Additionally, the dog owners rarely perceived spasticity to have an adverse impact on their dog's daily functioning or quality of life. This may, in fact, indicate that the spasticity was not problematic secondary to the chronic SCI, that owners failed to appreciate the adverse impacts of spasticity, or that the questions failed to elicit information regarding these adverse impacts. Owners whose dogs had the weakest hind limb motor function generally appeared more aware of spastic limb movements than did owners of dogs that could walk (but might still have had spasticity). Further development and validation of the questionnaire, including longitudinal collection of owner observations and CSS measurements from the time of SCI, are warranted to investigate the contribution of such information to the clinical assessment of spasticity in dogs with SCI.

An ordinal scoring scale adapted from human scoring systems was chosen for use in the present study. It is possible that, for patellar clonus and flexor spasm durations, use of continuous data may more accurately depict the degree of spasticity at the point of testing in dogs with chronic thoracolumbar SCI, although increasing the accuracy of angle and duration measurement would need to be addressed, perhaps by careful analysis of video-recorded testing sessions. However, data in continuous and ordinal format were largely similar for the study dogs. Given the fairly small number of dogs and the skewed nature of the continuous data (toward dogs with the most severe spasticity), allocation to limited categories facilitated statistical comparisons between groups and variables. This use of broad scoring categories would also facilitate ease of implementation in the clinical setting.

We focused on the overall CSS score but also examined each of the 3 scale components individually because we believed each component likely reflected different components of what is collectively referred to as spasticity. Flexor spasms appeared to be the most prevalent and useful component in the study dogs. Indeed, in different patient populations, other components of the CSS may be relevant and the scale could be adapted as needed. The positive relationship between flexor spasm duration and ability to step in the dogs with chronic thoracolumbar SCI suggested that development of flexor spasms might reflect an increase in the excitability of the intraspinal circuitry and could be linked to the recovery of stepping in such dogs. Because we focused on dogs with chronic injury and stable neurologic status, the CSS is not intended to be used to predict motor recovery. However, the effect of flexor spasms on functional ambulation warrants further investigation because this might be contrary to the anticipated finding that extensor spasms would facilitate walking. As noted previously, various possible explanations exist for the lack of extensor spasms in the study dogs, but it has also been demonstrated in animals with experimentally induced injury that recovery of standing (which predominantly involves antigravity extensor muscles) can have an adverse impact on stepping.⁴² The relationship between walking and spasms is likely more complex with the development of both flexor and extensor spasticity as well as recovery of stepping, all reflecting overlapping postinjury spinal cord changes.

Given the subjectivity inherent to use of a clinical scale, further validation of the CSS and testing protocol is needed. We demonstrated reliability between 2 raters assessing a small number of dogs, but additional testing of a larger number of dogs, by more raters, and in conjunction with more objective assessment methods such as electrodiagnostic techniques is warranted to determine whether the CSS would be useful for a broader variety of SCI patients and clinical settings. This testing might also provide additional information regarding the relationship, if any, between spasticity and dog and injury characteristics such as age, body weight, injury duration, or lesion location. We specifically evaluated spasticity in a group of chronically injured dogs with static neurologic status. Use of the CSS during the acute phase of injury and repeating it over time, however, might provide additional, indirect information regarding the intraspinal circuitry changes that develop following SCI and that underlie the development of spasticity. Such information may offer insight into the plasticity of the injured spinal cord as dogs transition from the acute to chronic injury phase and may be useful in designing more effective and consistent test components.

Despite any limitations, we believe that the CSS can be readily integrated into routine neurologic evaluation. Dogs with chronic SCI are frequently consid-

ered a uniform population, even though potentially clinically relevant differences exist. For example, all dogs in the present study had a similar severity of initial injury (functionally complete SCI) and none regained normal pain perception but had a highly variable degree of motor recovery (paraplegic to ambulatory) and CSS findings (which varied from mild to severe). Using the CSS, we were able to demonstrate that spasticity and, specifically, flexor spasms were significantly associated with validated measures of gait, including OFS and hind limb stepping and coordination. This suggests that the CSS is a tool worthy of continued development and may be useful to differentiate among dogs with permanent impairment following SCI and guide a personalized medicine approach to treatment strategies with potential application to humans.

Overall, we found that the CSS was a simple tool for quantification of spasticity in dogs with chronic thoracolumbar SCI, and severity of flexor spasms and overall CSS score were associated with gait scores. We conclude that the CSS may provide pertinent clinical information when added to the standard physical and neurologic evaluation of similar patients. Furthermore, the CSS may be a useful outcome measure in clinical trials involving dogs with chronic thoracolumbar SCI, although the impact of spasticity in dogs remains to be determined.

Acknowledgments

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Footnotes

- a. SAS, version 9.4, SAS Institute Inc, Cary, NC.
- b. Jmp 12 Pro, SAS Institute Inc, Cary, NC.

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SPASTICITY ADDENDUM

After an initial recruitment period on which the results presented in the preceding chapter on spasticity are based, additional dogs with SCI were subsequently enrolled, resulting in a total of 34 dogs with chronic SCI who underwent spasticity testing. The following addendum reports repeat analysis for spasticity based on all 34 dogs with a focus on areas of overlap or divergence from the original group analyzed.

Updated signalment and gait scores are presented in Table 1. Seven dogs (21%) were ambulatory and 27 (79%) non-ambulatory including 4 dogs able to take at least some weight bearing steps unassisted (OFS = 4). Results are qualitatively similar to the original evaluation group.

Table 1. Signalment characteristics and gait scores for all dogs evaluated (n=34). OFS: open field scale, SS: stepping score, RI: regularity index.

	Mean (SD) or Median (range)
Age (years)	5.97 (2.55)
Body Weight (kg)	7.65 (3.1-33)
Duration of Injury (months)	17 (3-84)
OFS (0-12)	2 (0-9)
SS (0-100, no support)	0 (0-89)
RI (0-100, no support)	0 (0-46.56)

Spasticity was evaluated in all 34 dogs using the Canine Spasticity Scale (CSS), consisting of the three components: patellar clonus duration, flexor spasm duration and flexor spasm degree. The testing protocol was unchanged. Data for the original group of 20 dogs are presented where indicated for comparison and statistical methods were the same unless specified. Table 2 outlines the summary statistics for the CSS and its component parts. Upon testing a larger number of dogs, findings are very similar with a comparable range in severity

of spasticity using our scale. Flexor spasms remain the most prominent aspect with patellar clonus still an uncommon finding.

Table 2. Summary statistics for CSS and its components in all 34 dogs compared to the original group of 20 dogs. CSS: Canine spasticity scale.

CSS Component	All dogs (n=34)		Original group (n=20)	
	Frequency (%)	Median (range)	Frequency (%)	Median (range)
Patellar clonus duration (0-6)	10/34 (29%)	0 (0-4)	5/20 (25%)	0 (0-4)
Flexor spasm duration (0-6)	34/34 (100%)	4 (1-6)	20/20 (100%)	2 (2-6)
Flexor spasm degree (0-6)	34/34 (100%)	3.5 (1-5)	20/20 (100%)	4 (1-6)
Overall CSS score (0-18)		8 (2-13)		7 (3-11)
Absent-mild spasticity (CSS 0-6)	15/34 (44%)		10/20 (50%)	
Mod-severe spasticity (CSS 7-18)	19/34 (55%)		10/20 (50%)	

To re-evaluate the use of ordinal versus continuous data for flexor spasm duration, we compared the results in 27 dogs for which these data were available. Averaged across the 3 trials, the median duration of flexor spasms was 6 seconds (range: 0.33-60 seconds) for the left hind limb and 4 seconds (range: 0-51.33 seconds) for the right hind limb. The median ordinal score averaged across the 3 trials for each limb in this group was 2, and a score of 2 corresponds to a duration between 3-10 seconds. We, therefore, conclude that using actual seconds for flexor spasm duration (as a continuous variable, up to 60 second cutoff) remains comparable to assigning an ordinal score for each trial and calculating summary statistics from the ordinal scores. The distribution of continuous data remained skewed and, as such, we continue to advocate use of the ordinal categories when scoring.

Correlations between the CSS components are presented in Table 3 and compared to the original group of dogs. R^2 values are generally comparable to original 20 dogs. After testing a larger number of dogs, overall CSS score is more strongly correlated with both flexor spasm duration and degree scores. Patellar clonus duration score remains poorly correlated with overall score and the other components.

Table 3. Correlations between CSS components in 34 dogs compared to the original group of 20 dogs. FS: flexor spasm.

	R^2 (n=34)	R^2 (n=20)
Overall score and FS duration score	0.73	0.57
Overall score and FS degree score	0.82	0.82
Overall score and clonus duration score	0.18	0.24
FS duration score and FS degree score	0.599	0.33
FS duration score and clonus duration score	0.002	0.01
FS degree score and clonus duration score	0.046	0.14

Repeat testing was performed to gather more information regarding the temporal variation in spasticity. The same testing protocol was performed at each session. A total of 12 dogs had repeat testing including 10 from the original group. Repeat testing was performed once (2 total spasticity testing sessions) in 7 dogs, twice (3 total testing sessions) in 4 dogs and 3 times (4 total testing sessions) in 1 dog. There was a range of 48 hours to 19 months between baseline and repeat testing. Amongst new repeat testing sessions, overall CSS score was unchanged in 3, differed by 1 point in 2, differed by 6 points in 1. For the latter, this change represented an increase in CSS score over baseline, and the duration of injury was 3 months at initial testing versus 13 months at the time of repeat testing. Intraclass correlation coefficient was 0.68 across all repeat testing sessions (compared to ICC=0.72 for the original

group with repeat testing). These results continue to show mild variability between testing times and might reflect temporal variation in spasticity severity, which is known to occur in spastic humans.¹ However, repeat testing was only performed in a small number of dogs including only 5 dogs with more than a single repeat testing session and there was a wide range between testing times. Ongoing investigation is warranted in a larger population with more testing time points to capture the true variability and evolution of spasticity amongst dogs with severe SCI.

Dogs were categorized by spasticity severity and compared with regard to age, duration of injury and motor function (Table 4). Associations between CSS scores, clinical variables and gait scores (OFS, SS, RI) showed similar trends between all 34 dogs and the original group of 20 dogs (Table 5). After adjusting for multiple comparisons, overall CSS score remained associated with OFS but not SS or RI, as it was in original evaluation. Flexor spasm duration score remained associated with ambulation and all gait scores but, upon testing a larger number of dogs, flexor spasm degree score was now also associated with ambulation and gait scores. Obtaining similar findings in a larger number of SCI dogs support our prior speculation that spasticity, specifically flexor spasms, likely represents a manifestation of spinal cord changes that occurs post-injury below the level of the lesion and varies between dogs, despite all having severe initial injuries.

Further investigation of spasticity amongst severely injured dogs in the chronic setting might, therefore, offer insight into the nature of such reorganization and its relationship to recovery of motor function and injury severity in this population. Consistent results after almost doubling the number of dogs from the group in which this scale was initially developed suggests that the CSS is robust and worthy of continued development and validation. Given that spasticity remains a prominent issue in humans with SCI, the ability to reliably measure and improve our understanding of spasticity in dogs enhances their utility as a model of chronic paralysis.^{2,3}

Table 4. CSS scores categorized by severity in 34 dogs compared to the original group of 20 dogs. CSS: Canine spasticity scale, Amb: ambulatory, OFS: open field scale, SS: stepping score, RI: regularity index, Orig grp: original group of 20 dogs for comparison.

Spasticity Severity	Median CSS (range)	Mean Age (SD)	Median Duration (range)	# Amb	Median OFS (range)	Median SS (range)	Median RI (range)
Absent to mild (CSS \geq 7) (n=15)	5 (2-6)	5 (3)	12 (3-69)	1	1 (0-6)	0 (0-41)	0 (0-11.4)
Absent to mild (CSS \geq 7), Orig grp (n=10)	4 (3-6)	5.2 (3.01)	8.5 (4-69)	1	1 (0-6)	0 (0-36)	0 (0-5.88)
Moderate to severe (CSS<7) (n=19)	9 (7-13)	6.6 (2.1)	22 (3-84)	6	3 (0-9)	0 (0-89)	0 (0-46.6)
Moderate to severe (CSS<7), Orig grp (n=10)	10 (8-11)	6.7 (2.26)	12 (5-84)	4	4 (0-9)	48 (0-89)	9.62 (0-46.6)

Table 5. Associations between spasticity scores, clinical variables and gait scores in 34 dogs with comparison to the original group of 20 dogs. CSS: Canine spasticity scale, OFS: open field scale, SS: stepping score, RI: regularity index, Cr: cranial, Cd: caudal.

Spasticity score	Parameter	P-value (n=34)	Adjusted p-value (n=34)	Adjusted p-value (n=20)
CSS overall score	Age	0.0367*	0.147	0.292
	Body Weight	0.0657	0.197	0.28
	Duration of injury	0.2802	0.56	0.934
	Lesion cr/cd to T13	0.9638	0.9638	1
	Ambulatory	0.0226*	0.055	0.26
	OFS	0.0106*	0.042*	0.014*
	SS	0.0184*	0.055	0.014*
	RI	0.0234*	0.055	0.014*
Flexor spasm duration score	Ambulatory	0.0247*	0.049*	0.012*
	OFS	0.0258*	0.049*	<0.0001*
	SS	0.0131*	0.039*	<0.0001*
	RI	0.0092*	0.037*	<0.0001*
Flexor spasm degree score	Ambulatory	0.005*	0.02*	0.19
	OFS	0.0062*	0.02*	0.144
	SS	0.011*	0.022*	0.144
	RI	0.0156*	0.022*	0.144
Patellar clonus score	Ambulatory	0.8944	1	0.604
	OFS	0.5793	1	1
	SS	0.9111	1	1
	RI	0.7111	1	1

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3. Skold C, Levi R, Seiger A. Spasticity after traumatic spinal cord injury: nature, severity and location. *Arch Phys Med Rehabil* 1999;80:1548–1557.

SUPPLEMENTARY INFORMATION

Supplementary information (including spasticity videos) available at:
<http://avmajournals.avma.org/prox.lib.ncsu.edu/doi/suppl/10.2460/ajvr.78.7.854>

Appendix S1: Summary of Modified Open Field Score (OFS), Stepping Score (SS) and Regularity Index (RI) gait scales

Modified OFS

Score	Description
0	Paraplegic
1	Minimal non-weight bearing protraction of pelvic limb (movement of 1 joint)
2	Non-weight bearing protraction of pelvic limb with > 1 joint involved < 50% of time.
3	Non-weight bearing protraction of pelvic limb with > 1 joint involved > 50% of time.
4	Weight bearing protraction of pelvic limb < 10% of time.
5	Weight bearing protraction of pelvic limb 10-50% of time.
6	Weight bearing protraction of pelvic limb > 50% of time.
7	Weight bearing protraction 100% of time with reduced strength of pelvic limb. Mistakes > 90% of time (crossing of pelvic limbs, scuffing foot on protraction, standing on dorsum of foot, falling).
8	Weight bearing protraction 100% of time with reduced strength of pelvic limb. Mistakes 50-90% of time.
9	Weight bearing protraction 100% of time with reduced strength of pelvic limb. Mistakes < 50% of time.
10	Ataxic pelvic limb gait with normal strength but mistakes > 50% of time (lack of coordination with thoracic limb, crossing of pelvic limbs, skipping steps, bunny hopping, scuffing foot on protraction, standing on dorsum of foot).
11	Ataxic pelvic limb gait with normal strength but mistakes < 50% of time.
12	Normal pelvic limb gait.

Stepping score (SS) definition: 50 step cycles counted; Hind limb steps/Fore limb steps x 100

Regularity index (RI) definition: 50 step cycles counted; ((Number of normal step cycle patterns x 4)/Total Steps) x 100

Appendix S2: Canine Spasticity Scale scoring form

CANINE SPASTICITY SCALE

 Date: _____ Patient ID: _____

LH Clonus

	Seconds	Score*
Trial 1:
Trial 2:
Trial 3:
Mean:	<input type="text"/>	<input type="text"/>

LH Spasm

	Seconds	Score*	Degree**
Trial 1:
Trial 2:
Trial 3:
Mean:	<input type="text"/>	Median: <input type="text"/>	<input type="text"/>

RH Clonus

	Seconds	Score*
Trial 1:
Trial 2:
Trial 3:
Mean:	<input type="text"/>	<input type="text"/>

RH Spasm

	Seconds	Score*	Degree**
Trial 1:
Trial 2:
Trial 3:
Mean:	<input type="text"/>	Median: <input type="text"/>	<input type="text"/>

	LH	RH	LH + RH#
Patellar Clonus Duration Score*:
Flexor Spasm Duration Score*:
Flexor Spasm Degree Score**:
Total Score^:	<input type="text"/>	<input type="text"/>	<input type="text"/>

OVERALL SCORE***: *0 (none), 1 (mild, $\leq 3s$), 2 (mod, $>3-10s$), 3 (severe, $>10s$),** 0 (none), 1 (mild, $<10degrees$), 2 (mod, $10-45deg$), 3 (sev, $>45deg$)

LH + RH Score (0-6) = sum of LH + RH scores for each measure

^TOTAL SCORE (0-9) = clonus duration + flexor duration + degree scores for each limb

***OVERALL SCORE (-0-18) = sum of left and Right total scores

 Rater Signature: _____

Appendix S3: Owner questionnaire regarding spasticity at home in dogs with chronic thoracolumbar spinal cord injury

1. Do you notice involuntary movements (jerking, kicking) of your dog's back legs at home while at rest?
 - a. Yes ____
 - b. No ____
2. Do you notice sustained muscle spasms (ie cramps) in your dog's back legs at home while at rest? (either with the leg flexed or extended out rigidly)
 - a. Yes ____
 - b. No ____
3. If yes to 1 or 2, how often do they occur?
 - a. ____ >10x/day, ____ 1-10x/day, ____ 1x/week to <1x/day, ____ <1x/week
4. If yes to 1 or 2, do you notice this more in one leg than the other?
 - a. Left ____
 - b. Right ____
 - c. No pattern/random ____
5. Is there a pattern to what time of day these movements are noted?
 - a. During the day ____
 - b. At night ____
 - c. No pattern/random ____
6. Are involuntary movements (jerking and/or spasms) elicited by touching your dog's back half (i.e. lower back, legs, toes, tail, during bladder expression, etc)?
 - a. Yes ____
 - b. No ____
7. If yes, describe: _____
8. Do these involuntary movements interfere with your dog's day-to-day functioning and/or quality of life at home?
 - a. Yes ____
 - b. No ____
9. If yes, to what degree?
 - a. Minimal impact ____
 - b. Moderate impact ____
 - c. Severe impairment ____

Appendix S4

Dog	Age	Breed	BWt	DOI	Dx	LL	Gait	Pain perception	Unassisted urination	CSS Overall Score	Patellar Clonus Score	FS duration score	FS degree score	OFS	SS	RI
1	5	MixB	16.3	24	sIVDD vs Trauma	L1-L2	N	Absent	No	9	1	2	6	1	0	0
2	12	Min Schnauzer	8.0	84	Trauma	T11-T12	N	Absent	No	10	4	2	4	4	0 (68)	0 (23.81)
3	5	Dach-Chi	4.0	8	IVDD	L2-L3	PP	Absent	No	4	0	2	2	0	NA	NA
4	12	Pit Bull	23.4	30	IVDD	T11-T12	N	Absent	No	6	0	2	4	1	0	0
5	4	MixB	29.9	15	Trauma	C7-T1	PP	Absent	No	4	0	2	2	0	0	0
6	5	Labradoodle	29.9	9	FCE/ Missile disc	T12-T13	N	Absent	No	6	0	3	3	1	0	0
7	3	Dach	3.6	7	sIVDD	NA	N	Absent	No	5	0	2	3	2	0 (49)	0 (26.85)
8	4	Dach	6.5	12	sIVDD	NA	N	Absent	No	11	2	4	5	4	74	16.09 (39.02)
9	4	GSD	29.7	15	Immucide	T3-L5	PP	Absent	No	4	0	2	2	0	0	0
10	6	Dach	5.3	16	IVDD	T13-L1	A	Absent	No	5	0	2	3	6	36	5.88
11	8	MixB	8.5	5	sIVDD	NA	PP	Absent	No	10	1	4	5	0	0	0
12	5	Dach	5.4	12	IVDD	T12-T13	A	Absent	No	8	0	4	4	8	69	26.03
13	7	Eng Bull	20.9	7	Missile disc	T7-T9	PP	Absent	Yes	8	0	4	4	0	0	0
14	1	Pit Bull	25.7	5	Trauma	T7-T8, T12-T13	N	Absent	No	4	0	2	2	1	0	0
15	8	Poodle	11.7	69	IVDD	T13-L1	PP	Absent	No	4	0	2	2	0	0	0
16	7	Dach	5.8	42	IVDD	L1-L2	A	Absent	Yes	11	0	6	5	9	89	46.56
17	7	Dach	5.9	5	IVDD	T11-T12	A	Blunted	No	10	0	6	4	7	72	32.56
18	4	Shih Tzu	6.1	4	sIVDD	NA	PP	Absent	No	3	0	2	1	0	0	0
19	5	Boston Terrier	10.7	6.5	IVDD	L1-L2	N	Blunted	Yes	9	2	3	4	4	27	3.15
20	7	Dach-Chi	3.1	26	sIVDD	NA	A	Absent	No	11	0	6	5	6	72	25.58 (41.86)

Appendix S4: Characteristics of dogs with chronic thoracolumbar spinal cord injury used in a study to develop the Canine Spasticity Scale.

CHAPTER 3
Development of novel gait analysis tool measuring center of pressure for evaluation of canine chronic thoracolumbar SCI

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Abstract

Gait evaluation after spinal cord injury (SCI) is an important component of determining functional status. Analysis of center of pressure (COP) provides a dynamic reflection of global locomotion and postural control and has been used to quantify a variety of gait abnormalities. We hypothesized that COP would be located more cranially and COP variability would be greater for SCI versus normal dogs. Our objective was to investigate COP, COP variability and body weight support percentage in chronically paralyzed dogs.

Fourteen dogs with permanent deficits after acute, severe thoracolumbar SCI were enrolled. COP measurements in x (right-to-left, COPx) and y (craniocaudal, COPy) directions were captured while dogs walked on a pressure sensitive treadmill. Root mean square values (RMS_COPx, RMS_COPy) were calculated to assess variability in COP. For non-ambulatory dogs, a hind limb sling was attached to a load cell to determine support percentage. Gait was also quantified using an open field scale (OFS) and treadmill-based stepping and coordination scores (SS, RI). Mean COPx, COPy, RMS_COPx and RMS_COPy were compared between dogs with SCI and previously evaluated normal dogs. RMS measurements and support percentage were compared to standard gait scales (OFS, SS, RI).

Mean COPy was more cranial and RMS_COPx and RMS_COPy were greater in SCI versus normal dogs ($p < 0.0001$). RMS_COP measurements correlated poorly ($R^2 < 0.2$) but support percentage was associated with gait scales ($p < 0.003$, $R^2 = 0.5$). COP offers information about

post-injury locomotion alterations. Further development is needed before consideration as an outcome measure to complement validated gait analysis methods in dogs with SCI.

Introduction

Clinical, neurologic evaluation of dogs with spinal cord injury (SCI) is comprised of examination of gait, proprioception, spinal reflexes and pain perception. Gait analysis is of particular importance since recovery of independent ambulation is considered by many to be part of a successful outcome after injury. There are several currently utilized gait scales in dogs that range from simple to more complex.

Frankel et al. established a classification system separating human SCI into 5 grades from 'complete' (A), 'sensory only' (B), 'motor useless' (C), 'motor useful' (D), and 'recovery' (E).¹ Complete injury corresponded no sensory or motor function detected below the injury whereas 'recovery' implied no residual neurologic symptoms including weakness or sensory loss.¹ This classification scheme has been variably adapted for use in veterinary medicine.^{2,3} One of the more common iterations of the 'modified Frankel score' is a 0-5 scale where 0 stands for normal neurologic function and 5 represents dogs who are paralyzed with absent pain perception. While this scale is simple to use in a clinical setting, requires no specific training or equipment, and differentiates dogs with differing recovery potential (i.e. dogs in which pain perception has been lost versus all other groups), it is of limited sensitivity in discerning smaller changes in gait function during recovery and does not account for dogs who lack pain perception but who regain motor function.

Additional, more comprehensive ordinal gait scales have been developed for use in dogs including the Texas Spinal Cord Injury Score (TSCIS), the open field scale (OFS) and the canine Basso, Beattie, Bresnahan locomotor scale (cBBB).^{2,4-6} The TSCIS gait scale incorporates proprioceptive placing and pain perception into the scoring system and evaluates all four limbs separately offering a more comprehensive analysis of function that can be applied to dogs with two or four limbs affected by injury as well as those with asymmetric dysfunction.² The TSCIS has been shown to have high inter-rater reliability and

to correlate well with the previously reported modified Frankel score.² The OFS gives a 0-12 score ranging from paraplegia to normal hind limb motor function.^{4,5} The TSCIS and OFS, which have been utilized extensively, are simple to perform, reliable and do not require special equipment or extensive training. The cBBB was recently adapted for dogs from the Basso, Beattie, Bresnahan locomotor scale (BBB) used in experimental rodent models of SCI.^{6,7} The cBBB incorporates fore and hind limb coordination and is more complex but is otherwise similar to the OFS. It was reported to work well with modifications to accommodate the normal canine gait (compared to rodents), to be sensitive to detect locomotor recovery over time in dogs with spontaneous SCI and to correlate with the MFS and OFS (Song 2016).⁶ Most importantly, all of these scales offer functionally relevant information (i.e. can the animal walk or not). The relatively simplistic evaluation of gait, however, does not allow the assessment of more subtle locomotion patterns.

Treadmill-based scores have also been developed and validated in dogs with SCI including measures of pelvic limb stepping (stepping score, SS) and coordination (regularity index, RI).^{8,9} The SS and RI are calculated on a 0-100 scale relative to normal fore limb stepping and a normal step pattern (right front-left hind-left front-right hind) in dogs, respectively. They require only a treadmill and video camera and generate useful continuous data on hind limb stepping. The recovery of coordination, in particular, as determined by the RI, might be a useful outcome measure in clinical trials of dogs with SCI beyond just focusing on if a dog is ambulatory or not.^{9,10} The SS and RI can be calculated with and without hind end support but, like the OFS, only provide information on dogs with thoracolumbar SCI (i.e. only hind limbs are affected). Scoring is also performed separately from the time of testing and requires specific training. A walking track gait analysis has also been developed which used paint applied to dogs' feet to produce footprints on the walking track to evaluate locomotion with focus on continuous data on stride length and base of support.¹¹ This method demonstrated differences in locomotion amongst dogs with SCI compared to normal dogs and was simple to implement but limitations include the practicality of testing and difficulty in evaluating

non-ambulatory dogs.¹¹ These methods can provide complementary information to the ordinal gait scales when assessing motor function in dogs with SCI.

While simplicity of testing is important for implementation in the clinic and useful for determining broad functional status, there is also a need for evaluation methods that can detect various differences in gait amongst dogs of differing SCI severity, different phases of recovery as well as dogs with incomplete recovery and chronic impairment. The ability to establish specific gait patterns and precisely quantify even subtle changes in gait over time or in response to a particular intervention could provide insight into the plasticity of motor networks after injury and the underlying mechanisms involved in compensation, recovery or a favorable treatment response. More advanced gait analysis has been performed in dogs with and without SCI using pressure sensitive walkways and kinematic analysis.¹²⁻¹⁷ Parameters of interest in these studies have included a multivariate model incorporating stride length, stride time and swing time, quantification of lateral paw placement, and quantification of fore limb and hind limb coordination.¹²⁻¹⁵ Studies utilizing pressure sensitive walkways have been limited to dogs that could walk and kinematic studies have focused either on just ambulatory or non-ambulatory dogs but not a combination of both populations.¹³⁻¹⁷

Our laboratory has developed a novel gait analysis tool for dogs that provides a global measure of locomotor function by analyzing the center of pressure (COP) and the variability in that COP during ambulation on an instrumented, pressure sensitive treadmill. This method has been used to quantify normal gait and posture as well as gait abnormalities in people and horses.¹⁸⁻²³ Prior evaluation in a cohort of neurologically normal, chondrodystrophic dogs showed that COP data could be obtained reliably in dogs with normal ambulation and that variability in COP was low and consistent within individual dogs.²⁴ We hypothesized that the COP would be located more cranially and the COP variability would be greater for dogs with SCI compared to neurologically normal dogs. Furthermore, we hypothesized that COP variability would be able to discern differing motor ability amongst dogs with SCI. The aim

of the current study was to use this gait analysis method to investigate the COP and its variability as well as the percentage of body weight support in dogs with chronic gait deficits after severe, acute SCI. The ultimate goal is to validate this instrumented treadmill gait analysis as a robust outcome measure in clinical trials capable of discriminating changes in global locomotor function, weight support and gait patterns over time during recovery or in response to a therapeutic intervention.

Materials and Methods

Case Selection: Dogs were recruited prospectively from the patient pool of the Canine Spinal Cord Injury Program at the North Carolina State University College of Veterinary Medicine. All dogs had chronic motor deficits with absent or severely reduced hind limb and tail pain perception (with or without urinary and fecal incontinence). In all dogs, signs were due to an acute thoracolumbar SCI (third thoracic to third lumbar spinal cord segments) causing paralysis with loss of pain perception suffered a minimum of three months previously. Body weight between 3-30kg was required to ensure proper measurements could be recorded by the treadmill. Below 3kg, the dogs' steps would not register and dogs weighing greater than approximately 30kg had trouble maintaining all four limbs on a single belt of the two-belted instrumented treadmill which was necessary for accurate data capture. Dogs were also required to be amenable to walking on the treadmill with only verbal encouragement since tactile manipulation can affect measurements. Informed consent was obtained for all animals and examinations were conducted in accordance with the NCSU Institutional Animal Care and Use Committee (protocol #15-004-01).

Standard gait evaluation: All cases underwent a standard gait analysis utilized in our laboratory consisting of walking each dog on a non-slip surface and on a treadmill for approximately 3 minutes with the speed adjusted to a comfortable pace for each individual. Non-ambulatory dogs were walked with and without sling support. All examinations were

videotaped. Gait was categorized as ambulatory (able to take at least 10 consecutive weight bearing steps unassisted), or not and quantified using the OFS (ranging from 0-12).^{4,5} OFS of ≥ 4 corresponds to taking at least some weight-bearing steps. Treadmill footage was scored to quantify pelvic limb SS and RI, and non-ambulatory dogs received one score with and one without hind end sling support.⁹ For the purposes of this study, the highest SS or RI score obtained with or without support was utilized for comparison to instrumented treadmill data.

Instrumented treadmill gait evaluation: An instrumented, force-plate treadmill (Fully Instrumented Treadmill (FIT), Bertec Corporation, Columbus, OH) designed for bipedal/human gait analysis was utilized. The set up consisted of two independent pressure sensitive belts as well as 6 cameras with infrared sensors (mx-t020, Vicon) mounted on the ceiling surrounding the treadmill to track the location of specific reflective markers. All dogs were outfitted with reflective markers at the lateral aspect of each carpus and tarsus as well as one additional marker placed on midline between the scapulae in line with the point of the elbow when the dog was standing at rest (Figure 1). Elastic tape was used to secure the markers in place without interfering with joint flexion and extension. The interscapular marker was utilized for COP measurements while the additional markers captured kinematic data (data not presented). COP was assessed in the x (lateral) and y (craniocaudal) directions. Refer to Blau et al., for more detailed information on COP computations.²⁴ For non-ambulatory dogs, a standard sling (Walk-a-bout) was used to provide hind quarter support. Height was adjusted such that the spine was parallel to the ground and the dog was in a biomechanically appropriate stance for locomotion. The handles of the sling were attached to a load cell in order to record the percentage body weight support provided by the sling during testing. Dogs were acclimated to the treadmill for several minutes and then walked at a steady, comfortable pace for approximately 5-10 minutes or until at least 5 ‘good’ or ‘excellent’ trials were recorded. Trials were subjectively designated as good to excellent if multiple (more than 2) step cycles were recorded with minimal visible variation in fore limb gait, with all four limbs contained on a single belt of the treadmill, with all markers visible

and no manual intervention by the handler. Anomalous movements such as lunging or stopping or other deviation from a steady stepping (in the fore limbs) were grounds to stop an individual trial. A leash was placed loosely around the neck but trials were only counted if the dog was walking willingly in response to verbal encouragement without pulling or re-direction with the leash. Treadmill speed was recorded for each dog. All trials were also videotaped using a digital video camera (HDR-CX580V, Sony) positioned to capture all four limbs of the dogs as they walked as a reference on dog behavior during an individual trial if needed.



Figure 1. Set up for dogs with SCI on the instrumented treadmill depicting sling support attached to the load cell and reflective marker placement.

All data for each trial in each dog were collected as .c3d files and visually inspected for quality and ability to accurately track marker position throughout the trial. Trials with marker loss were not included in analysis. Data processing consisted of converting .c3d files to .txt files (Visual 3D Software, C-Motion) which were subsequently imported into MATLAB (MATLAB Software, Mathworks) for analysis and calculations using purpose written code (Appendix 1).

Statistical analysis: All analyses were performed using Jmp 12 Pro (SAS Institute, Cary, NC, USA). COP measurements were captured in the X (right to left, COPx) and Y (craniocaudal, COPy) planes for each trial in each dog. COP summary statistics were calculated for each dog (mean and standard deviation) as well as presented collectively as a mean and standard deviation across all dogs (n=8). Due to errors in the MATLAB code after the initial data capture that prevented repeat analysis of mean COP values, COP data is only available in 8 of the 14 dogs. To evaluate the variability of COP, calculations also included the root mean square (RMS) of the COPx and COPy (RMS_COPx, RMS_COPy) for each trial in each dog. Mean RMS-COPx and mean RMS-COPy and standard deviation were also determined for each dog and collectively for all dogs (n=14). The percentage weight support was also calculated for each trial with mean and standard deviation reported across all dogs. Dogs who did not require support were given a value of 0. OFS, SS, RI were each reported as mean and standard deviation or median and range, as appropriate. Associations between age, duration of injury or limb length (greater trochanter to lateral digit) and RMS_COPx or RMS_COPy were investigated using linear regression and an ANOVA. Limb length (distance from the front foot to intrascapular marker) was noted to be associated with RMS_COP measurements in the previously acquired data in our laboratory in neurologically normal, chondrodystrophic dogs using the same protocol on the same treadmill.²⁴ Therefore, a model was constructed incorporating limb length (measured as the distance from the greater trochanter to lateral digit for SCI dogs and from the front foot to the intrascapular marker in a standing position for normal dogs). An ANCOVA was then performed to compare RMS_COPx or RMS_COPy between the SCI and normal dogs. Agreement between RMS_COP calculations and standard gait measures (OFS, SS, RI) was determined by calculating correlation coefficients. For the purposes of comparisons to variability data, the best SS and RI scores were utilized whether obtained with or without sling support. Support percentage was also compared to the standard gait measures using linear regression and a one-way ANOVA.

Results

Twenty dogs met the initial criteria for inclusion, however, only 14 were enrolled in the instrumented gait analysis evaluation with 6 dogs eliminated due to body size, temperament issues or unwillingness to walk without manual correction or restraint on a standard treadmill. Mean body weight was 10.63 kg (8.7), mean age was 6.1 years (2.3). Median duration of injury was 13.5 months (4 to 84 months). Median OFS was 3 (0-9), SS without support was 0 (0-89) and RI without support was 0 (0-46.56). When the best score was used whether obtained with or without support, median SS was 32.5 (0-89) and RI was 4.52 (0-46.56).

The mean number of good to excellent trials per dog was 7.79 (2.0). Treadmill belt speed ranged from 0.3-0.7mph. All dogs walked willingly on the treadmill after an acclimation period of several minutes and in response to verbal encouragement. Three dogs walked independently and did not require sling support; sling support was provided in the other 11 dogs.

Across all dogs in whom data was available (n=8), mean COP position relative to the interscapular reference marker was -0.85cm (SD) in the x-direction (just left of midline) and -3.7cm (SD) in the y-direction (caudal to the intrascapular reference marker) (Table 1). Representative traces depicting the change in COP in the X and Y directions relative to the interscapular marker during walking for a given trial are shown for two dogs of different levels of hind limb function in Figure 2. The change in COP_x and COP_y over time for a single trial is shown for 2 dogs in Figure 3. Mean RMS_COP_x and RMS_COP_y for all dogs (n=14) are presented in Table 1. Since changes to COP while walking on a treadmill might vary widely between dogs with different degrees of motor impairment, mean COP and RMS_COP values in the x and y directions were also calculated for dogs grouped by hind limb function (using the OFS scores) (Table 2). Mean COP_x and mean RMS_COP_x between

dogs with OFS 0-3 versus OFS >3 were not significantly different ($p=0.4657$, 0.4195 , respectively). Mean COP in the y direction was located more cranially and mean RMS_COPy showed less variability in higher functioning dogs but the differences were not significant ($p=0.1821$, 0.2889 , respectively). Mean sling support percentage was 18.24% (11.16); it was 23.67% (5.67) when only the 11 dogs who required sling support were included. Corresponding COP and RMS values for 11 neurologically normal, chondrodystrophic dogs are included in Table 1 for comparison.²⁴

Mean COPx was not statistically different between SCI dogs and the cohort of normal dogs ($p=0.19$). Mean COPy was significantly more cranial in SCI dogs compared to the normal cohort ($p<0.0001$). Age and duration of injury were not associated with RMS_COPx or RMS_COPy ($p>0.05$) but limb length was associated with RMS_COPy ($p=0.0384$). Accounting for limb length, RMS_COPx and RMS_COPy were significantly different between the normal dogs and dogs with SCI ($p<0.0001$). There was greater variability in both the x and y directions in dogs with SCI (Figure 4). When SCI dogs were separated into lower functioning dogs (OFS 0-3) and higher functioning dogs (OFS >3), RMS_COPx and RMS_COPy for both groups remained significantly higher than values in the normal dogs ($p<0.0001$). RMS_COPx and RMS_COPy were poorly correlated with validated measures of gait (OFS, SS, RI) ($R^2<0.2$, $p>0.05$, all comparisons). Support percentage was significantly associated with OFS, SS and RI ($p=0.0007$, $R^2=0.63$, $p=0.0007$, $R^2=0.63$, and $p=0.003$, $R^2=0.5$ respectively) (Figure 5).

Table 1. Summary values for mean COPx, COPy, RMS_COPx and RMS_COPy in dogs with chronic motor deficits secondary to acute, severe SCI compared to neurologically normal, chondrodystrophic dogs previously evaluated in our laboratory. Data for both groups was acquired using the same treadmill and protocol (Blau). $P < 0.05$ is significant. SCI: spinal cord injury, COP: center of pressure, RMS: root mean square.

Variable	SCI dogs (n=14)	Normal dogs (n=11)	P-value
Mean COPx	-0.85cm (0.29) (n=8)	-0.34cm (0.12)	0.19
Mean COPy	-3.96cm (0.44) (n=8)	-8.51cm (5.32)	<0.0001
Mean RMS_COPx	0.0277 (0.0098) (n=14)	0.0138 (0.0047)	<0.0001
Mean RMS_COPy	0.0276 (0.0077) (n=14)	0.0185 (0.0071)	<0.0001

Table 2. Mean COP and RMS_COP values for dogs with SCI grouped by hind limb motor function. OFS: open field scale, scores >4 correspond to independent ambulation. COP: center of pressure in the x (left-right) or y (craniocaudal) directions. RMS: root mean square.

Variable	OFS 0-1 (n=6)	OFS 0-3 (n=7)	OFS >3 (n=7)	OFS >4 (n=5)
Mean COPx	-0.14cm (0.2)	-1.4cm (0.36)	0.001cm (0.67)	0.15cm (0.78)
Mean COPy	-3.96cm (0.25)	-5.26cm (0.44)	-2.03cm (0.37)	-0.58cm (0.44)
Mean RMS_COPx	0.0258 (0.0091)	0.0251 (0.0088)	0.0299 (0.01)	0.0244 (0.0052)
Mean RMS_COPy	0.0312 (0.0082)	0.0304 (0.008)	0.0253 (0.0066)	0.0246 (0.007)

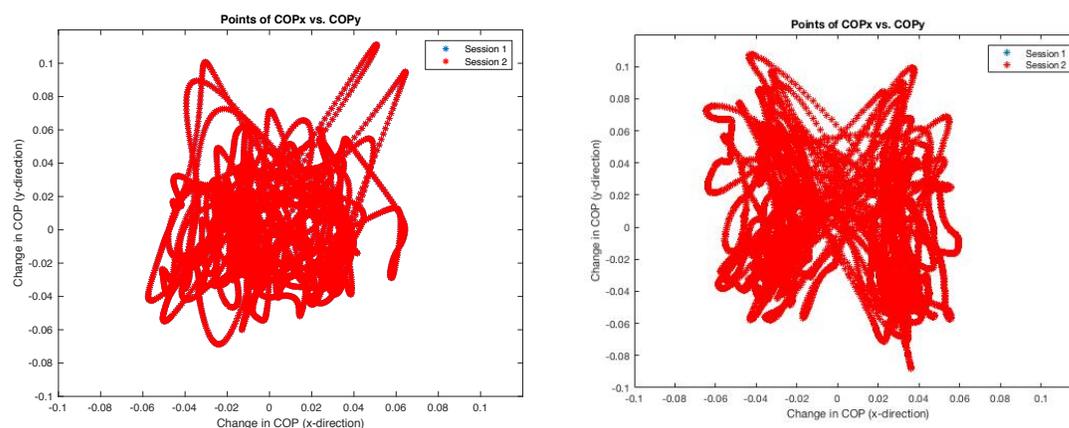


Figure 2. Change in COP in X (left-right plane) and Y (craniocaudal plane) directions relative to the interscapular marker while walking in an ambulatory dog (left) and a paraplegic dog (with hind end sling support) (right). COP: center of pressure.

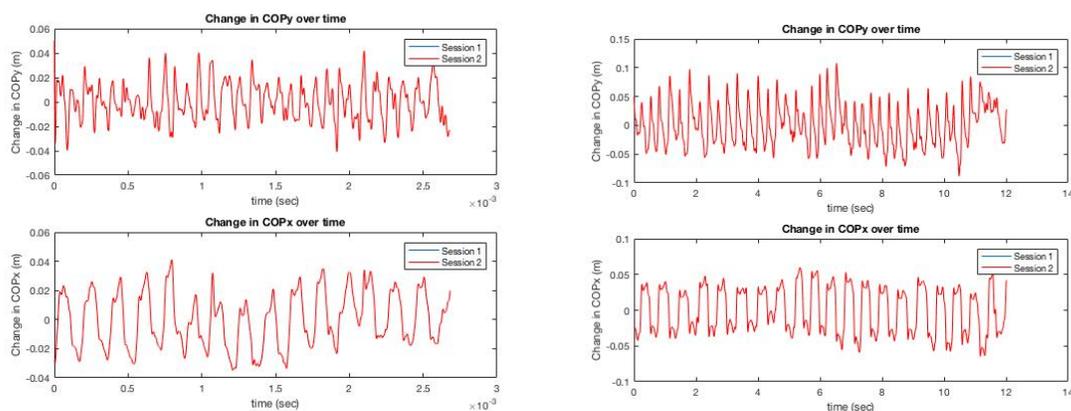


Figure 3. Change in COP over time (seconds) for the X (left-right plane) and Y (craniocaudal plane) directions in an ambulatory dog (left) and a paraplegic dog (with hind end sling support) (right). COP: center of pressure. COP: center of pressure.

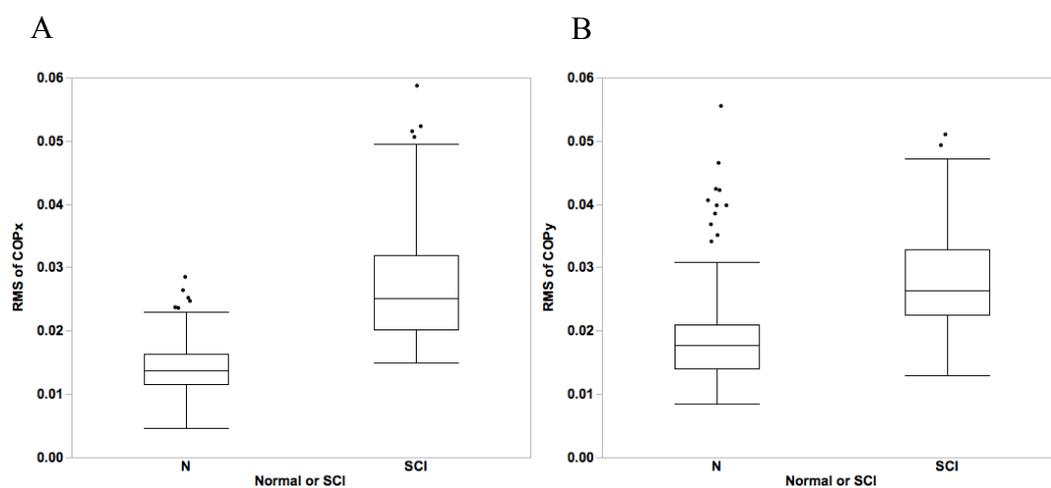


Figure 4. Variability in COP compared between dogs with SCI and neurologically normal, chondrodystrophic dogs. A. RMS_COPx higher is in dogs with SCI ($p < 0.0001$). B. RMS_COPy is higher in dogs with SCI ($p < 0.0001$). COP: center of pressure, RMS_COP: root mean square of COP. SCI: spinal cord injury.

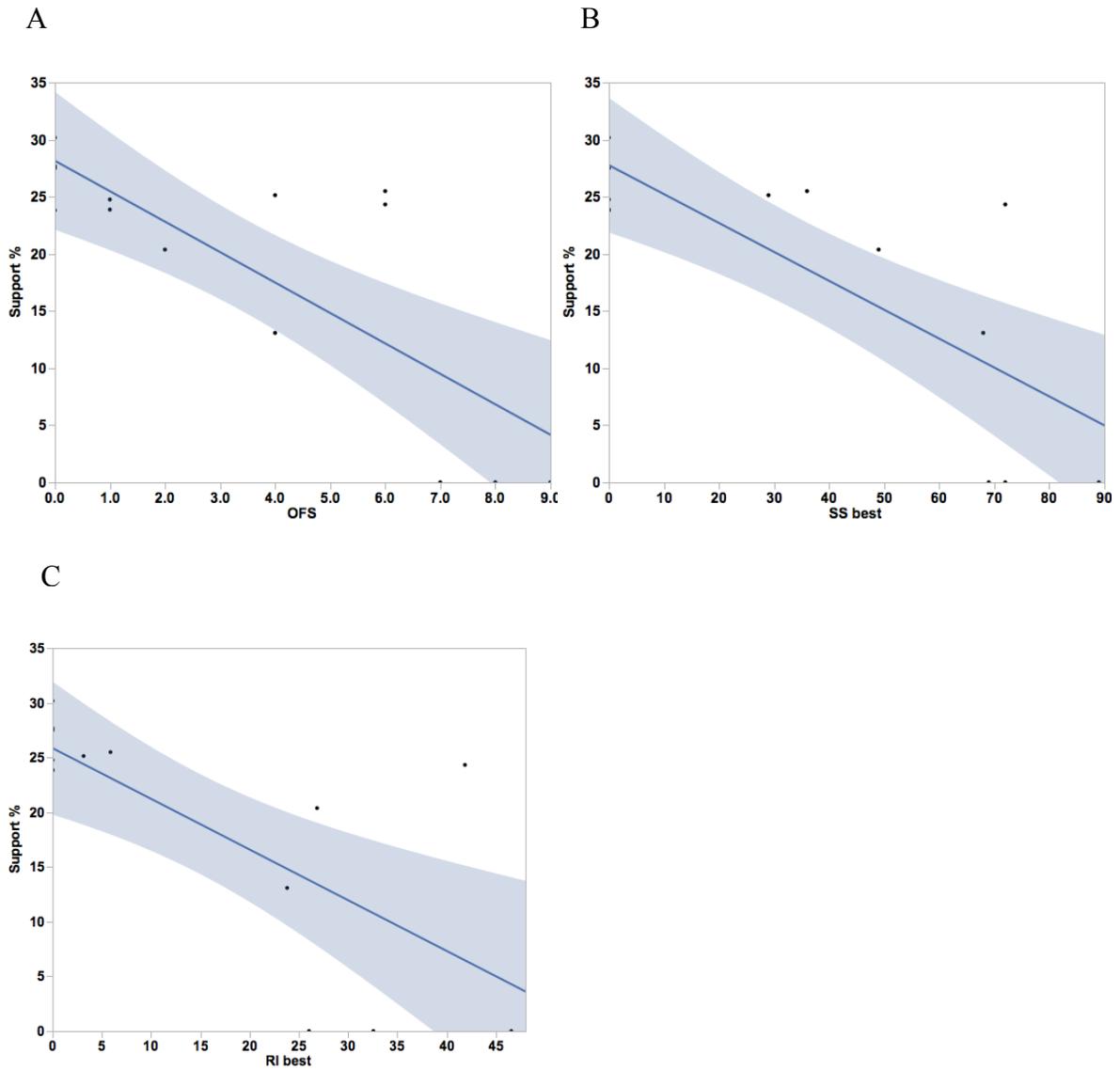


Figure 5. Associations between body weight sling support percentage and (A) OFS, $p=0.0007$, $R^2=0.63$, (B) SS, $p=0.0007$, $R^2=0.63$, and (C) RI, $p=0.003$, $R^2=0.5$. OFS: open field score, SS best: best stepping score obtained either with or without support, RI best: best regulatory index score obtained either with or without support.

Discussion

COP measurements and corresponding variability in COP can be evaluated using a pressure sensitive, instrumented treadmill in dogs with chronic motor impairment secondary to prior acute, severe SCI.

The mean COP in the y direction (craniocaudal) in SCI dogs was located more cranially compared to values in neurologically normal dogs using the same protocol.²⁴ While the two groups were not size or breed matched, this difference likely reflects a forward loading of weight in dogs with chronic, hind limb weakness. Interestingly, the COP was located even more cranially in higher functioning compared to lower functioning dogs with SCI. The decreased forward shifting of body weight in dogs with minimal motor function (compared to those with higher OFS scores) can be attributed to the sling support provided for their hind limbs. This decreased the need to bear more weight on the fore limbs and the sling was also connected to the load cell which was attached to the rigid frame of the treadmill above the level of their hips, perhaps skewing the movement more caudally. The greatest degree of forward weight shifting towards the interscapular marker was in ambulatory dogs. While this difference was not significant compared to lower functioning dogs, it does suggest a notable forward loading of body weight persists even amongst dogs who regain the ability to walk independently.

RMS of COP in both the x and y directions was greater for dogs with SCI compared to the cohort of dogs without gait deficits. Similarly, the change in COP while walking was less predictable in both directions in SCI dogs. In normal dogs, the COP is characterized by craniocaudal movement twice and left to right movement once each step cycle creating a 'butterfly' pattern when the interscapular reference marker is traced.²⁴ These findings support greater variability in the movement of the trunk/body in dogs with SCI walking with or without hind limb support. This is consistent with prior studies using pressure sensitive

walkway or kinematic analysis in which the variations in several different gait parameters were greater for dogs with thoracolumbar SCI compared to non-neurologic controls.^{12,15} There are several caveats to our comparison of the SCI dogs in this study and the neurologically normal, chondrodystrophic controls, notably, the fact that groups were not controlled for differences in dog height or conformation. The prior work using this treadmill demonstrated that limb length impacted variability measurements.²⁴ We also found that limb length was associated with RMS_COPy, specifically that taller dogs displayed greater variability in the y direction. Since the 'control' group we used for comparisons consisted of chondrodystrophic dogs and our SCI study population included a variety of chondrodystrophic and nonchondrodystrophic breeds and body sizes, at least some of the difference in the RMS_COPx and RMS_COPy values can be explained by the SCI dogs being a more diverse group including dogs with longer limb length. However, when we incorporated limb length into our statistical model, RMS values in both the x and y directions remained significantly higher compared to the normal cohort. Our means of measuring each dog's height was not uniform (hind limb length in SCI dogs compared to front limb length to the level of the upper back in the normal dogs), but this approach provided a reasonable way to compare variability during locomotion between the two groups, accounting for the effect of limb length. Repeat analysis using breed and size matched controls and a standard measurement for limb length would be warranted to confirm these findings.

Within our study population of dogs with SCI, RMS calculations also showed differences between dogs with differing levels of hind limb function. There was no consistent trend for variability in the x direction, however, increased variability in the y direction was noted for dogs with less hind limb motor function. A likely contributing factor is the sling support and load cell set up which produced a cranial-caudal pendulum swing effect in some dogs. It is also possible that low functioning dogs sped up or slowed down in their fore limbs more frequently than dogs taking steps on their own. While marked swinging in any direction or visible changes in speed caused a given trial to be rejected, the effect of the sling and

possibly other subtle changes in movement could have contributed to increased variability in the y direction. Prior kinematic evaluations of fore limb-hind limb coordination and lateral paw placement in non-ambulatory dogs with thoracolumbar SCI found no quantifiable impact of weight support provided by an abdominal band when tested in a subset of normal, control dogs.¹⁴ However, distinct from our study, all SCI dogs in these reports received weight support for the hind end with the abdominal band, limiting the ability to determine the effect of support on measurements in SCI dogs or make comparisons to our observations.^{14,15} Focusing future investigations on dogs who are weak and ataxic but do not require sling support might still provide useful information on locomotor patterns in an impaired population without the confounding effects of the sling support.

RMS_COPx and RMS_COPy calculations correlated poorly with validated measures of gait (OFS, SS, RI). This is not necessarily surprising as COP data provides a global assessment of the pattern and variability of locomotion whereas the gait scoring methods provide specific information on functional level. As such, instrumented treadmill analysis is not designed to replace these outcome measures but rather to provide additional information about specific aspects of locomotion. For example, in ambulatory but weak dogs undergoing a rehabilitation protocol, COP analysis might be useful to discern improvements in hind limb and trunk strength, stability and coordination that manifest as decreased cranial weight shifting and decreased variability measurements. It is important to note, however, that our results, along with prior studies utilizing pressure sensitive walkways and kinematic analyses in dogs with SCI, demonstrated marked variability.¹³⁻¹⁵ While these methods appear capable of discerning injured from neurologically normal populations, it is unclear if the variations within dogs with SCI correspond to the level of motor function which may limit the ability to make generalizations across dogs.¹³⁻¹⁵

Unlike COP measurements, support percentage was significantly associated with validated gait scores. Despite challenges with interpreting the effect of sling support on gait

parameters, this suggests that standardized determination of percentage body weight support provided during ambulation might offer complementary data to gait scoring methods in this population and still be worthy of development as an outcome measure. However, challenges were encountered with our sling support and load cell system which might limit its reliability. Further refinement of technique and validation amongst a larger number of non-ambulatory dogs with both acute and chronic SCI will help to determine if this method is capable of tracking changes in motor function over time prior to independent ambulation (such as during an initial recovery period from acute SCI) or in response to a therapeutic intervention.

We evaluated a heterogeneous group with regard to function to determine if this method of gait analysis was feasible in dogs with SCI. While we obtained interesting and useful results, it remains possible that COP analysis is not the most appropriate supplementary means by which to evaluate locomotion in this population. There are substantial limitations on the utility of instrumented treadmill analysis. Data collection and analysis is labor intensive and time consuming. This is further complicated by patient willingness and size limitations (big and small) for dogs using a treadmill designed for human (bipedal) locomotion. This technique also requires relatively clean data and obtaining high quality trials in ataxic and weak animals proved challenging. Furthermore, while using a load cell attached to a commercially available hind end harness provided useful information on support percentage, a more standardized harness and attachment system would facilitate ease of adjustment for dogs of differing body size and ensure greater accuracy and consistency of measurements. Data analysis also requires purpose-designed code, specific software and expertise limiting the widespread applicability of this technique to evaluate gait.

Overall, our findings demonstrate that COP measurements can be obtained in dogs with SCI and might provide unique information about how locomotion is altered post-injury to complement currently available means of gait analysis. Further refinement and adaptation to

dogs with SCI is needed before it can be utilized as an outcome measure or to monitor response to a particular therapeutic intervention.

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CHAPTER 4

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Original Article

The Relationship between Trans-Lesional Conduction, Motor Neuron Pool Excitability, and Motor Function in Dogs with Incomplete Recovery from Severe Spinal Cord Injury

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Abstract

Spontaneous, acute, complete thoracolumbar spinal cord injury (TL-SCI) in dogs frequently results in permanent deficits modeling chronic paralysis in people. Recovery of walking without recovery of sensation has been interpreted in dogs as reflexive spinal walking. To evaluate this assumption, this study characterized the electrophysiological status of motor and sensory long tracts and local reflex circuitry in dogs with absent recovery of sensation after acute TL-SCI and correlated findings to gait scores. Twenty dogs with permanent deficits after acute, clinically complete TL-SCI and 6 normal dogs were prospectively enrolled. Transcranial magnetic motor evoked potentials (MEPs), somatosensory evoked potentials (SSEPs), H-reflex, and F-waves were evaluated. Gait was quantified using an ordinal, open field scale (OFS) and treadmill-based stepping and coordination scores (SS, RI). MEP latency and H-reflex variables were compared between cases and controls. Associations between presence of MEPs, SSEPs, F-waves or H-reflex variables, and gait scores were determined. Pelvic limb MEPs were detected in 4 cases; no case had trans-lesional sensory conduction. Latency was longer and conduction velocity slower in cases than controls ($p_a=0.0064$, 0.0023 , respectively). Three of 4 cases with pelvic limb MEPs were ambulatory, and gait scores (OFS, SS, RI) were each associated with presence of trans-lesional conduction ($p_a=0.006$, 0.006 , 0.003 , respectively). H threshold in cases (mean, $3.2\text{mA} \pm 2.5$) was lower than controls (mean, $7.9\text{mA} \pm 3.1$; $p_a=0.011$) and was inversely associated with treadmill-based scores, SS, and RI ($p_a=0.042$, 0.043 , respectively). The association between pelvic limb MEPs and gait scores supports the importance of descending influence on regaining walking after severe TL-SCI in dogs rather than just activation of spinal walking. The inverse association between H-reflex threshold and gait scores implies that increases in motor neuron pool excitability might also contribute to motor recovery.

Keywords: central pattern generator; disc extrusion; electrophysiology; motor evoked potentials

Introduction

ACUTE THORACOLUMBAR SPINAL CORD INJURY (TL-SCI) IS common in dogs attributed to intervertebral disc herniation, trauma, and vascular events, among other causes.^{1–5} Functionally complete injuries, where no motor or pain perception remain below the level of injury, carry a fair to guarded prognosis depending on the cause and leave many dogs with permanent neurological deficits.^{1,6,7} The typical course of recovery starts with regaining pain perception, followed by motor and autonomic function. The failure to recover pain perception is taken as an indication of spinal cord transection. However, comparable to humans with SCI, complete anatomic transection is uncommon in dogs with spontaneous injuries and some dogs that never regain pain perception in their

pelvic limbs or tail demonstrate remarkable, spontaneous changes in motor recovery over time.^{6,8–10} Reflexive pelvic limb stepping after experimental spinal cord transection, known as spinal walking, has previously been described in rodents, cats, and dogs, but the extent to which reflexive stepping movements develop in dogs after spontaneous injury and the degree to which it translates to functional walking is variable.^{6,11–19} Understanding why some dogs with persistent loss of pain perception recover effective walking while others do not may be important for therapy development and may provide important insights for people with SCI. Axons traversing the lesion epicenter have been demonstrated histopathologically in dogs with clinically complete lesions.^{8,20} In addition, intact motor and sensory conduction has been reported in this population.²¹

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Finally, dogs that recover effective walking without recovery of pain perception may exhibit voluntary tail wagging in response to a positive stimulus.⁶ These data suggest that residual connections across the site of injury might provide some degree of descending, supraspinal input, resulting in enhanced motor recovery potential. Long tract conduction in this population, however, has not been examined in detail, and the role of alterations to local spinal cord circuitry post-injury is unknown. There has also been no attempt to correlate electrophysiological findings with functional recovery.

The aims of this study were to characterize the electrophysiological status of motor and sensory long tracts and local reflex circuitry in dogs with incomplete recovery from acute TL-SCI and to correlate findings to gait. We hypothesized that dogs with intact descending motor tracts would have higher gait scores and independent ambulation whereas evidence of increased motor neuron pool excitability would be associated with stepping movements, but not ambulation.

Methods

Control dogs

Clinically normal dogs were prospectively recruited to establish normal values for evoked potentials and H-reflex testing in our laboratory. Informed consent was obtained and examinations were conducted in accord with the North Carolina State University Institutional Animal Care and Use Committee (protocol #15-150-O). All dogs had to have a normal neurological examination and no history of neurological disease. Laboratory standards were already established for other electrophysiological parameters.

Case selection

Dogs were recruited prospectively from the patient pool of the Canine Spinal Cord Injury Program at the North Carolina State University (NCSU) College of Veterinary Medicine (Raleigh, NC). All dogs had chronic motor deficits ranging from paraplegia to ambulation with weakness and ataxia with absent or severely reduced hindlimb and tail pain perception (with or without urinary and fecal incontinence). In all dogs, signs were attributed to an acute TL-SCI (third thoracic to third lumbar spinal cord segments) based on neurological exam findings causing paralysis with loss of pain perception suffered a minimum of 3 months previously. Advanced imaging (computed tomography or magnetic resonance imaging) or definitive diagnosis were not required for inclusion. Exclusion criteria included muscle contractures or concurrent neuromuscular conditions that alter local reflexes or concurrent systemic conditions that would preclude safe sedation. Data collection on each dog included signalment, concomitant medications, diagnosis, lesion location, duration of injury, and past treatment of the SCI. Past therapy, including participation in interventional clinical trials, were noted, but not utilized, as exclusion criteria for the purposes of this project. Informed consent was obtained for all animals and examinations were conducted in accord with the NCSU Institutional Animal Care and Use Committee (protocol #15-004-01).

General neurological and gait evaluation

All cases underwent a neurological examination, including standard evaluation of gait, proprioception, spinal reflexes, and pain perception (Table 1). Pain perception was tested in standard fashion by squeezing vigorously (using the handle of bandage scissors or blunt-tipped forceps) over the phalanges of the hindlimbs and the base of the tail. Perception was defined as a conscious, behavioral response to the noxious stimulus such as turning around, vocalizing, or trying to bite. Medial and lateral digits were

TABLE 1. NEUROLOGICAL EXAMINATION

<i>Exam parameter</i>	<i>Assessment</i>
Gait	Ambulatory, nonambulatory paraparetic, paraplegic
Proprioceptive placing, hopping	Absent (0), delayed (1), normal (2)
Patellar, withdrawal reflex	Absent (0), decreased (1), normal (2), increased (3), clonus (4)
Hindlimb muscle tone	Decreased/flaccid, normal Increased
Cutaneous trunci reflex	Vertebral level of caudal border recorded
Hindlimb, tail nociception	Present or absent
Urination	Voluntary or involuntary

tested in all dogs. More extensive gait analysis was performed by one of the investigators (M.J.L.) assisted by a veterinary technician and consisted of walking each dog on a nonslip surface for approximately 3–5 min and on a treadmill for approximately 3 min with the speed adjusted to a comfortable walking pace for each individual (range, 0.6–1.0 mph). Dogs were allowed to acclimate to the hospital environment with these two handlers for 1–2 h preceding gait analysis. All examinations were videotaped. Gait was categorized as ambulatory (able to take at least 10 consecutive weight bearing steps unassisted) or not and quantified using an ordinal gait scale (open field score [OFS] ranging from 0 to 12).^{22,23} OFS greater than 4 corresponds to independent walking. Treadmill footage was scored using previously described measures of pelvic limb stepping (stepping score; SS) and coordination (regularity index; RI).^{24,25} Gait scores (OFS, SS, and RI) generated without sling support only were utilized for the purposes of this project.

Electrodiagnostic evaluation

Electrodiagnostic testing included evaluation of long tracts with transcranial magnetic stimulation (TMS) and recording of motor evoked potentials (MEPs) and cortical somatosensory evoked potentials (SSEPs) as well as evaluation of local reflex circuitry by H-reflex, F-waves, and M-waves. All procedures were performed by one of the investigators (M.J.L.) assisted by a veterinary technician. All cases were sedated with 1–4 µg/kg of intravenous (i.v.) dexmedetomidine (Dexdomitor; Orion Pharma, Espoo, Finland) and 0.1–0.2 mg/kg of i.v. butorphanol (Torbugesic; Zoetis, Kalamazoo, MI). The sedation protocol was adjusted, as needed, to ensure that dogs lay calmly. Testing was performed in lateral recumbency and was not initiated until dogs were relaxed and no longer reactive to mild tactile or auditory stimuli. The room was darkened and kept quiet throughout the duration of testing to facilitate continued relaxation through the testing period. Control dogs were sedated with the same protocol for TMS and H-reflex recordings. They were then anesthetized for SSEP recordings, which was required to ensure tolerance of the procedure in neurologically normal dogs (with normal sensation). The anesthetic protocol consisted of propofol induction (Propofol 10 mg/mL; Abbott Laboratories, North Chicago, IL) and inhaled isoflurane for maintenance (VET ONE Fluriso; MWI, Boise, ID).

TMS was performed using a Magstim 200² magnetic stimulator (Version 1.9; The Magstim Company Limited, Spring Gardens, UK) using a 50-mm double coil stimulator with a peak magnetic field strength of 2.5 Tesla. The center of the coil was positioned over the frontal bone of the skull on midline (at the vertex) in contact with the scalp and the current flow within each coil run in an antiparallel direction. Following a single discharge of the stimulator, MEPs were recorded from the left extensor carpi radialis muscle in the thoracic

limb as a positive control and the left cranial tibial muscle in the pelvic limb by active, reference, and ground needle electrodes placed percutaneously in standard fashion and connected to an electromyograph (Nicolet VikingQuest; Natus Neurology Incorporated, Middleton, WI).²⁶ Stimulation was repeated four times at supramaximal intensity (90% stimulus intensity, 1-ms pulse duration, 100- μ s rise time, and 2- to 10-Hz low/high-frequency filters) for each limb. Presence (yes/no) and minimum latency of MEPs were recorded. Amplitude was not measured because of variability and the polyphasic nature of waveforms. MEPs were defined as present if detectable waveforms were identified in at least two of four trials at supramaximal intensity at a sensitivity of 200 μ V/division or less and with a latency of 100 msec or less. Minimum latency was defined as the time interval measured in milliseconds from the stimulus onset to the first deflection from baseline of the resultant waveform. Path length was measured from stimulus site over the cortex following the anatomic neuronal pathway to the active electrode in the extensor carpi radialis or cranial tibial muscle and used to calculate conduction velocity.

SSEPs were recorded after electrical stimulation of the tibial nerve for the pelvic limb and ulnar nerve for the thoracic limb (as a positive control) with an electromyograph (Nicolet VikingQuest; Natus Neurology Incorporated). Active and reference stimulating needle electrodes were placed percutaneously adjacent to the tibial nerve just proximal to the tarsus and adjacent to the ulnar nerve near the carpus. Active recording needles were placed between the fifth and sixth lumbar vertebrae (L5–L6) at the level of the lamina (for recording cord dorsum potential following tibial nerve stimulation) and under the scalp overlying the contralateral somatosensory cortex (for recording of cortical potentials) with reference electrodes placed subcutaneously approximately 2 cm lateral to the L5–L6 recording electrode and just below the opening of the contralateral external ear canal, respectively.^{27,28} A ground electrode was placed subcutaneously between stimulating and recording needles. Stimulation was delivered at a frequency of 3.1 Hz, duration of 0.2 ms, and a stimulus intensity range from 1.2–25.0 mA with at least 200 (range, 200–400) averaged stimulations for the pelvic limb. The ulnar nerve was stimulated at the minimum intensity necessary to elicit a discernible evoked potential (range, 1.0–16.0 mA) in order to ensure patient tolerance. The presence (yes/no) and minimum latency of SSEPs and cord dorsum potentials were recorded. Minimum latency was defined as the time interval measured in milliseconds from the stimulus onset to the first deflection from baseline of the resultant waveform. Path length was measured from the recording site over the cortex following the anatomic neuronal pathway to the site of stimulation of the distal ulnar or tibial nerve. The path length from L5–L6 to the stimulation site for the tibial nerve was also recorded.

M-waves were then generated for sciatic/tibial nerve stimulating at the sciatic notch and just proximal to the tarsus and recording from the plantar interosseous muscles according to standard protocol.^{29,30} The stimulus had a duration of 0.1 ms and intensity was increased to supramaximal, which was defined as the intensity resulting in no further increase in wave amplitude. The maximum M-wave amplitude was measured from the largest negative to the largest positive peak and the motor nerve conduction velocity (MNCV) calculated with greater than 60 meters per second (m/s) considered normal.²⁹ Cathode and anode of the stimulating electrodes located adjacent to the distal tibial nerve were reversed for generating F-waves with active, reference, and ground electrode placement unchanged. The tibial nerve was stimulated repetitively 16 times at a frequency of 2 Hz, duration of 0.1 ms, and supramaximal stimulus intensity producing M-waves followed by F-waves. The minimum latency of the F-waves, F-wave persistence (percentage of F-waves present in 10 stimulations), and the F-ratio [(latency F – latency M – 1) / 2 × latency M] were recorded and compared to reference values.^{31,32} To record H-reflex from the plantar interosseous muscles, the distal tibial nerve was stimulated (duration, 1 ms; frequency, 0.2 Hz or less) starting at low intensity

(machine minimum, 0.1 mA) and increasing gradually according to previously reported methods in dogs.^{33,34} The H-reflex threshold (stimulus intensity at which H-reflex first appeared) and minimum H-reflex latency were recorded. The maximum H-reflex amplitude and maximum M-wave amplitude (defined as the largest negative to the largest positive peak for each waveform) were each recorded during H-reflex testing and used to calculate the H:M ratio (maximum H amplitude/maximum M amplitude). “After discharges,” defined as electrical activity that persisted (sustained activity) or appeared randomly (episodic activity) after the onset of H-reflex and F-waves, were also noted. Sustained after discharge activity was classified into short duration (<10 ms for H-reflex, <20 ms for F-waves) versus long duration (>10 ms for H-reflex, >20 ms for F-waves). Episodic activity was categorized as present or absent for both F-wave and H-reflex recordings. Limb length, measured from the trochanteric notch to the lateral digit, was also recorded.

Statistical analysis

All analyses were performed using Jmp 12 Pro (SAS Institute Inc., Cary, NC). Presence or absence of MEPs, SSEPs, F-waves, H-reflex, after discharges, and ability to ambulate were each recorded and analyzed as categorical data. Summary statistics for continuous data (gait scores, MEP/SSEP latency, F-wave, and H-reflex variables) are reported as mean and standard deviation (SD) if normally distributed or median and range if not using the Wilk-Shapiro test for normality. The Wilcoxon rank-sum test was used to compare means for MEP latency and H-reflex variables between cases with SCI and the cohort of control dogs. Associations between presence of MEPs or SSEPs and ambulation status were established by constructing contingency tables and using Fisher's exact test. Associations between presence of MEPs or SSEPs and gait scores were determined using an ANOVA. Associations between F-wave variables or H-reflex variables and gait scores were determined by linear regression. $p < 0.05$ significant with adjusted p values calculated for multiple comparisons using Holm's correction calculator.

Results

Clinical information for controls

Six neurologically normal adult dogs were enrolled (Supplementary Data 1) (see online supplementary material at <http://www.liebertpub.com>). Median body weight was 12.5 kg (range, 6.3–41.0). Mean age was 6.5 years (SD, 2.7).

Clinical information and gait scoring in cases

Twenty dogs with SCI were enrolled (Supplementary Data 2) (see online supplementary material at <http://www.liebertpub.com>). There were 6 Dachshunds and 2 Dachshund/Chihuahua crosses with 10 additional breeds represented. Median body weight was 8.25 kg (range, 3.1–30.0). The mean age was 5.9 years (SD, 2.75), median duration of injury was 10.5 months (range, 4–84), and suspected or confirmed intervertebral disc disease was the most common diagnosis (14 dogs). In all dogs, neurolocalization was between the third thoracic and third lumbar spinal cord segments based on neurological exam findings. One dog had concurrent, less-pronounced deficits referable to the caudal cervical region. Eighteen dogs had no pelvic limb and tail pain perception whereas 2 dogs (1 ambulatory, 1 nonambulatory) had a severely blunted pain response, 1 with a subtle response in the medial toe of the left hindlimb and 1 with a subtle response in the medial and lateral toes of the left hindlimb. Seventeen dogs were urinary incontinent requiring manual bladder expression. No dogs demonstrated pain on spinal palpation. At the time of evaluation, 6 dogs were paraplegic,

9 were nonambulatory, but with motor, and 5 were ambulatory. The median OFS for all dogs was 1.5 (range, 0–9), median unsupported SS was 0 (range, 0–89), and median unsupported RI was 0 (range, 0.0–46.6). Information on individual dogs is presented in Supplementary Data 2 (see online supplementary material at <http://www.liebertpub.com>). Treatments at the time of acute injury were variable and included surgery (decompression ± stabilization) or medical management with or without formal rehabilitation therapy based on confirmed or suspected underlying cause. Eighteen dogs were not undergoing any specific or formal therapies at the time of enrollment in the reported study whereas 2 dogs participated in intermittent, formal rehabilitation sessions with a rehabilitation certified veterinarian.

Electrodiagnostic testing

Long tract and local reflex testing was feasible in all 20 cases and 6 controls. However, 1 case was removed from analysis of local circuitry and SSEPs because previous self-mutilation altered the anatomy, precluding stimulation and recording from the distal limb. Based on symmetrical signs on neurological examination and the duration of optimal sedation, all dogs were placed in right lateral recumbency and the left limbs were tested.

MEPs were present in all controls and cases when recording from the extensor carpi radialis muscle of the thoracic limb (positive control; Fig. 1A). MEPs were present in all controls and in

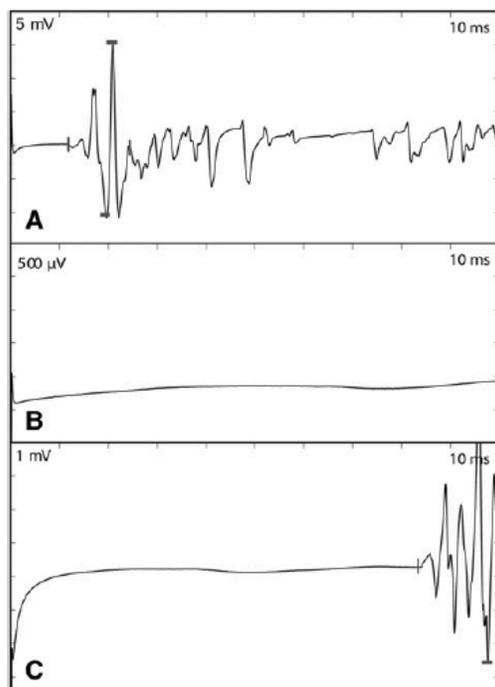


FIG. 1. Representative MEP tracings in dogs with TL-SCL. (A) MEP recorded from extensor carpi radialis muscle; 5 mV/division, 10 ms/division. (B) No MEP recorded from cranial tibial muscle; 500 μ V/division, 10 ms/division. (C) MEP recorded from cranial tibial muscle; 1 mV/division, 10 ms/division. MEP, motor evoked potential.

4 cases (including the 2 with blunted pain perception) when recording from the cranial tibial muscle of the pelvic limb, but were not detected in the remaining 16 cases (Fig. 1B,C). Mean minimum latency and conduction velocity values recorded from the cranial tibial muscle are presented for cases and controls (Table 2). Cases had significantly longer mean cranial tibial muscle MEP latency ($p=0.0064$) and slower mean conduction velocity ($p=0.0023$) compared to controls.

SSEPs and cord dorsum potentials were present following tibial stimulation in all controls under general anesthesia (external positive control; Fig. 2A). Cortical SSEPs were detected after ulnar stimulation in the 6 cases in which testing a forelimb under sedation alone was tolerated (Fig. 2B). Cord dorsum potentials were recorded over L5–L6 after tibial nerve stimulation in 18 cases (internal positive control; Fig. 2C). Testing was not performed in the case with past self-mutilation and in 1 other case because of limited duration of sedation. No cortical SSEPs were detected after tibial nerve stimulation in any cases (Fig. 2D).

Motor nerve conduction velocity (MNCV) was normal in all cases and ranged from 63 to 97 m/s (Table 2). F-waves were elicited in all cases (19 of 19), and F-wave persistence was 100% for each dog (Fig. 3; Table 2).

Three of 6 controls had a discernible H-reflex with amplitudes ranging from 0.5 to 1.2 mV (Table 2). In 2 of the 3 controls, H-reflexes were abolished at 15.4 and 18 mA (which were 9.6 and 0.8 mA, respectively, after the onset of M-waves), whereas they were not clearly abolished in 1 dog. In contrast, H-reflexes were elicited in all (19 of 19) cases with variable amplitudes ranging from 0.2 to 3.3 mV (Fig. 4; Table 2). In 12 cases, H-reflexes persisted and were not abolished at stimulation intensities up to 50 mA. In the remaining 7 cases, the intensity at which the H-reflexes disappeared was variable, but ranged from 6.0 to 34.7 mA (and was between 2.0 and 30 mA after the appearance of M-waves). H:M ratio was higher and H threshold was significantly lower in cases compared to controls ($p=0.3$ and $p=0.011$, respectively).

After discharges were common in cases and noted after F-waves in 17 of 19 cases and after H-reflex in 16 of 19 cases (Figs. 3 and 4). They included sustained activity in which late waves appeared to persist for extended periods of variable durations and episodic, isolated electrical discharges. During F-wave testing, sustained

TABLE 2. SUMMARY VALUES FOR PELVIC LIMB MEP, MNCV, AND F- AND H-REFLEX VARIABLES IN CONTROLS AND CASES

Variable	Mean (SD) or median (range)		p_a value
	Controls (n=6)	Case (n=19)	
MEP latency	28.5 ms (11.7)	63 ms (18.2) (n=4)	0.0064*
MEP CV	39.4 m/s (12)	12 m/s (3.4) (n=4)	0.0023*
MNCV	NA	75.2 m/s (9.7)	
F-wave latency	NA	13.68 ms (3.9)	
F-ratio	NA	1.55 (1.1–5.2)	
H-reflex latency	14.4 ms (5.4) (n=3)	13.5 ms (9.7–21.6)	0.98
H-reflex threshold	7.9 mA (3.1) (n=3)	3.2 mA (2.5)	0.011*
H:M ratio	0.15 (0.1) (n=3)	0.29 (0.2)	0.3

*Denotes significant difference ($p<0.05$) between control and case dogs. p_a refers to p value adjusted for multiple comparisons.

MEP, motor evoked potential; CV, conduction velocity; MNCV, motor nerve conduction velocity; SD, standard deviation; ms, milliseconds; m/s, meters per second; mA, milliamperes; NA, not applicable.

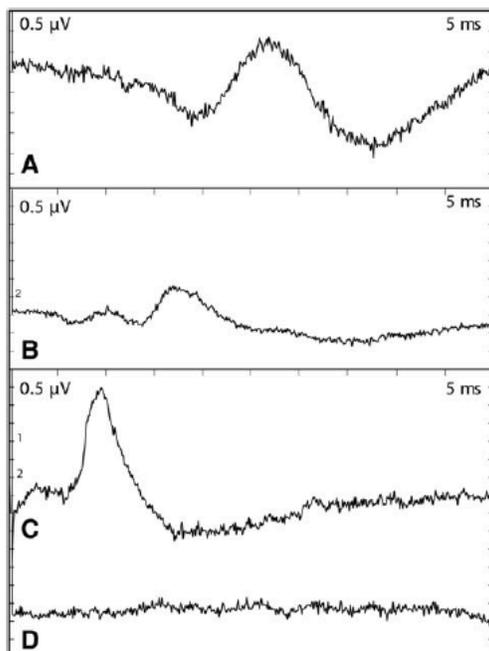


FIG. 2. Cortical SSEP and cord dorsum representative tracings. (A) Positive cortical SSEP following tibial nerve stimulation in a control. (B) Positive cortical SSEP following ulnar nerve stimulation in a case. (C) Cord dorsum potential following tibial nerve stimulation in a case. (D) Absent cortical SSEP following tibial nerve stimulation in the same case as (C). 5 ms/division, 0.5 μ V/division. SSEP, somatosensory evoked potential.

activity was categorized as short duration (<20 ms) in 10 dogs, long duration (≥ 20 ms) in 5 dogs, and not present in 4 dogs. During H-reflex testing, sustained after discharges of short duration (<10 ms) were present in 6 dogs, long duration (≥ 10 ms) in 6 dogs, and absent in 7 dogs. Episodic electrical activity was noted in

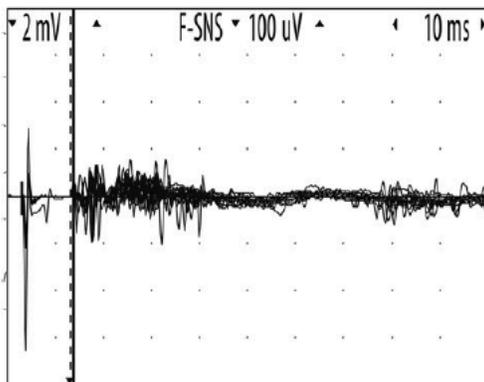


FIG. 3. Superimposed, representative F-wave tracings in a case. Note the prolonged after discharges also visible to the right of the F-waves.

15 and 7 dogs after F-waves and H-reflexes, respectively. Visible limb flexion and spasms were commonly noted in cases with after discharges. After discharges were not noted in controls.

Associations between electrodiagnostic variables, gait scores

Comparison of gait scores between dogs with and without pelvic limb MEPs are outlined in Table 3. Ambulatory dogs were significantly more likely to have detectable trans-lesional spinal cord conduction ($p=0.032$; $p_a=0.032$). Presence of pelvic limb MEPs was also significantly associated with higher OFS ($p=0.0026$; $p_a=0.006$), SS ($p=0.0021$; $p_a=0.006$), and RI ($p=0.0007$; $p_a=0.003$). H-threshold was 1.5 mA (1.6) in ambulatory dogs compared to 3.8 mA (2.5) in nonambulatory dogs and is compared to gait scores in Figure 5. H-threshold was not significantly associated with walking ($p=0.12$; $p_a=0.12$) or OFS ($p=0.056$; $p_a=0.11$), but was inversely associated with SS ($p=0.011$; $p_a=0.042$) and RI ($p=0.014$; $p_a=0.043$). No significant relationships between H:M ratio, H-latency, or F-wave variables (F-latency, F-ratio), and gait scores were identified.

Discussion

This study examined dogs with severe SCI characterized by chronic loss of pain perception in the hindlimbs and tail. In this population, historically described as having clinically complete injuries, recovery of any motor function has been interpreted as exclusively reflexive, termed spinal walking. We demonstrated that trans-lesional motor conduction was present in 20% of the dogs in this study, confirming residual functional motor tract integrity despite permanent loss of pelvic limb pain perception. The presence of pelvic limb MEPs was associated with higher gait scores, implying functional significance of descending influence on the ability to regain walking post-injury in dogs labeled as having clinically complete lesions. H-reflex changes reflective of increased motor neuron excitability were also present in the same population of dogs, providing evidence of reorganization of the local spinal cord circuitry below the level of the lesion. The inverse association between H-threshold intensity and gait scores implies that plasticity of local circuitry, specifically motor neuron pool excitability, might be an additional contributing factor to the motor recovery of these dogs. These data represent a baseline from which to conduct further studies using dogs as a model of chronic paralysis and enhance our understanding of factors impacting functional recovery.

Dogs that present with clinically complete, acute TL-SCI (paraplegic with no pain perception below the level of the injury), have a variable outcome. The most common cause is acute, explosive disc herniation.^{4,5} When the herniation is treated by prompt decompressive surgery, approximately 58% recover pain perception, continence, and the ability to walk over 1–3 months post-operatively.⁶ Of the remaining dogs who never regain pain perception or continence, approximately one third recover ambulation over a more protracted period (mean, 9.5 months).⁶ For dogs suffering a traumatic, complete TL-SCI, the outcome is more guarded, with none recovering pain perception or autonomic function. Approximately 20% can recover ambulation in the absence of pain perception and continence; however, many dogs are euthanized at the time of injury, impacting these numbers.⁶ There is a widely held belief in veterinary neurology that, regardless of the cause of the SCI, delayed motor recovery exhibited by some of these dogs lacking pain perception is exclusively reflexive stepping, also known as spinal walking.

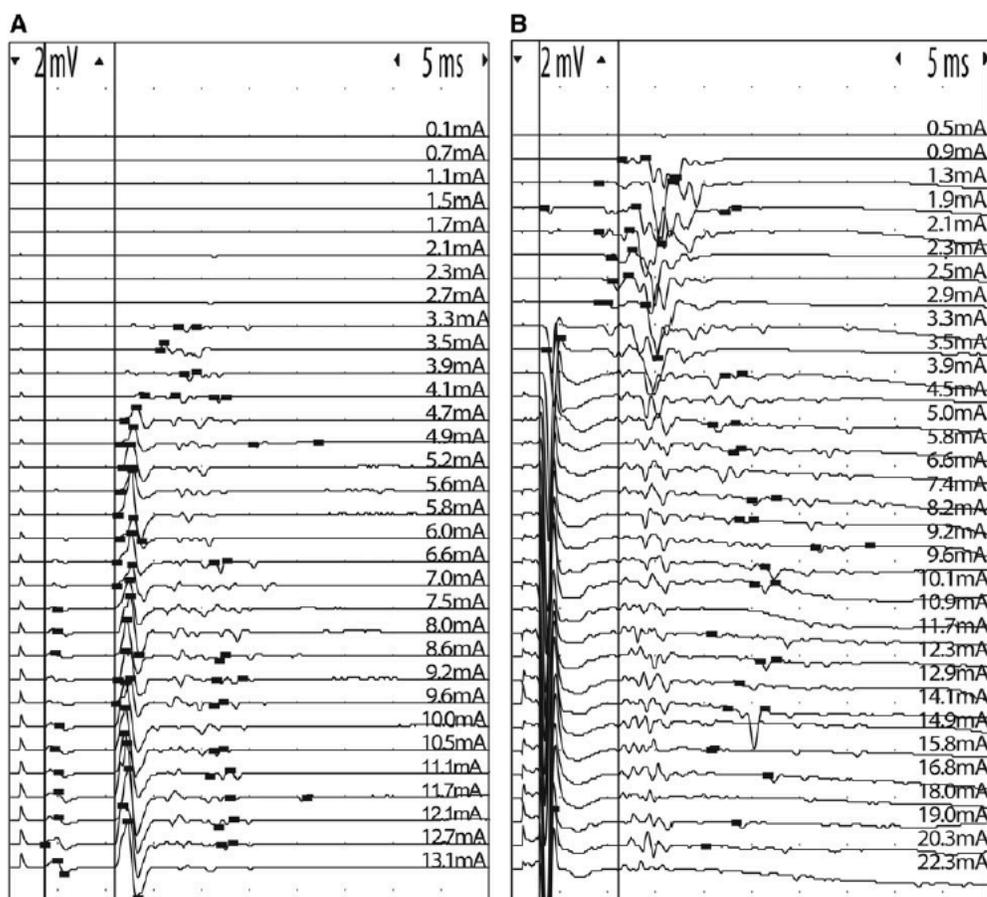


FIG. 4. Representative H-reflex tracings in two cases. In both traces, H-reflexes appear before M waves are apparent, but note the variability of threshold intensity at the onset of H-reflex between the 2 cases. In both traces, H-reflexes are not clearly abolished as stimulus intensity is increased, although the upper limit of stimulation (50 mA) is not displayed.

The interconnected network of neurons that produces such pelvic limb stepping is collectively referred to as the central pattern generator (CPG) and has been identified in multiple species, including dogs and people.^{18,35-40} The CPG is located in the lumbar cord, and the integrity and subsequent reorganization of this region

TABLE 3. ASSOCIATIONS BETWEEN PELVIC LIMB MEPS AND GAIT SCORES

	MEP present	MEP absent	p_a value
No. Ambulatory	3/4 (75%)	2/16 (12.5%)	0.032*
Median OFS	6.5 (4-9)	1 (0-8)	0.006*
Median SS	72 (27-89)	0 (0-74)	0.006*
Media RI	29.1 (3.2-46.6)	0 (0-26)	0.003*

*Denotes significant difference ($p < 0.05$) between cases with present versus absent pelvic limb MEPS. p_a refers to p value adjusted for multiple comparisons. MEP, motor evoked potential; p_a , adjusted p value; OFS, open field score; SS, stepping score; RI, regulatory index.

after experimental injury has been suggested to be crucial and sufficient for regaining locomotion.¹⁸ Indeed, experimental transection of the TL spinal cord resulted in recovery of independent ambulation in 7 of 9 dogs by an average of 4 months after the induced injury and plateaued by 6 months.¹⁵ Subsequent retranssection cranial to the original lesion in 2 dogs did not affect their motor function, providing strong evidence of functional spinal walking in dogs.¹⁵ However, the lack of motor recovery in many dogs with severe, spontaneous injuries suggests that there are important differences between experimental and naturally occurring injury. Understanding the basis of any degree of spontaneous motor recovery in dogs lacking pain perception therefore is integral to maximizing the utility of naturally occurring SCI in dogs as a model of human paralysis.

Our electrophysiological data demonstrated clearly that dogs with no pain perception may have intact motor pathways. Moreover, we demonstrated that the presence of pelvic limb MEPS was significantly associated with independent ambulation and higher

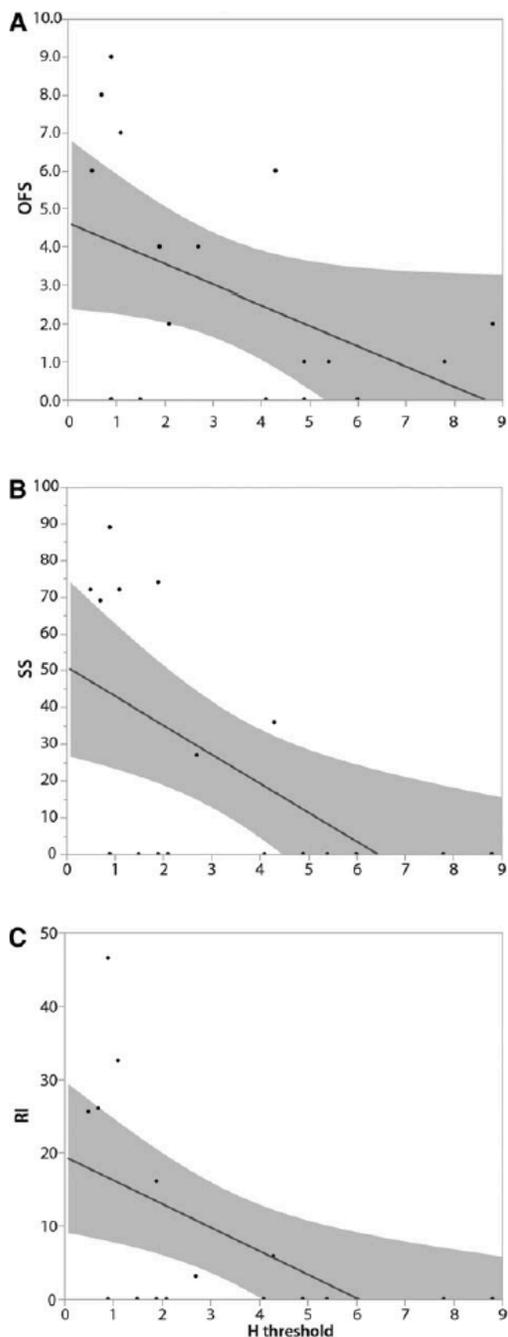


FIG. 5. Associations between H threshold and gait scores in cases. (A) OFS and H threshold, $R^2=0.198$, $p_a=0.11$. (B) SS and H threshold, $R^2=0.343$, $p_a=0.042$. (C) RI and H threshold, $R^2=0.32$, $p_a=0.043$. OFS, open field score; SS, stepping score; RI, regularity index; p_a , corrected p value.

gait scores. This provides strong evidence that intraspinal motor networks located below the site of injury are not completely disconnected from all supraspinal influence, and that this communication might play a role in motor recovery. Intact sensory and motor conduction has been previously identified in some chronically paralyzed dogs, although its relationship to recovery of function has not been specifically evaluated.²¹ The noninvasive methods utilized to evaluate long tract integrity very likely underestimated the number of dogs with intact connections and, among the individual dogs with recordable MEPs, did not allow quantification of the extent of residual trans-lesional connections. Indeed, it has previously been shown that in the acute phase of injury, evoked potentials are abolished by much less-severe SCI, with MEPs present in only 50% of ambulatory dogs with thoracolumbar lesions, consistent with studies in people and rodents.^{26,27,41–45} Although sensitivity appears to increase in the chronic phase, the distance between the site of stimulation (TMS) or recording (SSEPs) and the underlying neural structures is limiting.^{26,27} Cortical evoked potentials were more readily recorded in dogs and monkeys after stimulation at the level of the dura compared to distal tibial nerve stimulation by percutaneously placed electrodes.⁴⁶ This suggests that stimulating or recording evoked potentials by electrodes placed epidurally could facilitate the identification of additional chronic SCI dogs with residual trans-lesional connections.

Motor conduction traversing the site of injury in dogs labeled as having functionally complete injuries shares some overlap with the identification of “discomplete” injuries in people.⁴⁷ In these patients, with injuries designated as motor complete, additional, more-nuanced clinical evaluation shows a degree of volitional control supportive of previously unrecognized residual connections across the site of injury.⁴⁸ People with neuropathologically incomplete, yet clinically complete (by standard clinical examination), SCI have also been demonstrated.^{9,10} Indeed, complete physical transection of the spinal cord is quite rare.^{8,10,20} Our findings underscore lesion heterogeneity with regard to severity and continuity, even among the most severely affected individuals, and raise the possibility that our interpretation of dogs and people with so-called complete injuries might warrant recalibration.

The lack of sensory recovery in our cases is mirrored by the absence of electrophysiological evidence for intact ascending tracts in any of the dogs in spite of positive internal and external controls. Granger and colleagues reported cortical SSEPs in 12 of 34 (35%) of dogs labeled as clinically complete.²¹ The difference in results could reflect technical differences or simply that their population of dogs had less-severe injuries than the population examined here.

F-waves and H-reflexes have been reported in normal dogs and have been used to evaluate spasticity in experimental SCI.^{31,33,34,49} However, they have not been previously reported specifically in dogs with chronic disability after spontaneous SCI. Both of these tests evaluate overlapping aspects of local reflex circuitry and, assuming normal peripheral nerve function based on normal motor nerve conduction velocity studies, they specifically provide information at the level of the spinal cord on alpha motor neurons. H-reflex and, to a lesser extent, F-waves have been used in humans with SCI as measures of motor neuron excitability, most often in the context of post-injury spasticity.^{50–57} F-wave variables in the dogs of this study fell within the normal range for our laboratory and published reference values.^{31,32} However, H-reflexes were elicited in all cases, but only 50% of controls. This is consistent with testing in people with upper motor neuron dysfunction secondary to SCI in which H-reflexes are more readily elicited and present in more widely distributed muscles affected by the injury

compared to testing in healthy controls.⁵⁸ H:M ratio is the primary variable analyzed for H-reflexes, with an increasing ratio (attributed to increasing H-reflex amplitude) suggestive of greater motor neuron pool excitability.^{50,55,59} The threshold to elicit the H-reflex has also been shown to be reduced in humans and cats with chronic SCI, again supportive of increased excitability.^{53,56}

The trends were similar in our study where the H:M ratio was higher and the threshold for H-reflexes was significantly lower in cases compared to controls. These results provide indirect support for reorganization of local circuitry post-injury in dogs, which is consistent with the histologically confirmed plasticity in spinal cord connections below the level of a lesion in a rodent model of SCI.⁶⁰ The inability to demonstrate a significant increase in H:M ratio in our cases is consistent with reported overlap between normal and SCI humans and could reflect the low number of dogs tested, prevalence and severity of spasticity, muscle group tested, and influence of patient relaxation.^{50,61} Low stimulus frequency, sub-maximal intensity, and long stimulus duration were utilized to ensure H-reflexes and not F-waves were being recorded. However, it is possible that H-reflexes were contaminated by F-waves or muscle artifact at higher intensities, especially given that the H-reflex was not clearly abolished in 12 dogs even at supramaximal stimulation, further complicating consistent amplitude measurements and H:M calculations.

H-threshold intensity was inversely associated with treadmill-based stepping and coordination scores, suggesting that dogs with increased motor neuron pool excitability have greater stepping ability. The presence and severity of after discharges when recording F-waves and H-reflexes also might provide information on excitability of the reflex circuitry post-injury, but the exact neural generators and relevance of this activity require further study. Recent work in people using epidural stimulation to generate volitional movements in clinically complete patients has demonstrated that increasing the excitability of the motor neuron pool apparently increases its responsiveness to residual descending influence.⁶² The fact that cases with more stepping movement had lower H-reflex thresholds suggests that the same phenomenon may be at work in these dogs. The ability to noninvasively quantify altered excitability of local circuitry in dogs after severe injury might provide a useful baseline for interventional studies warranting additional investigation of the H-reflex in a larger number of chronically paralyzed dogs.

Overall, our findings describe the descending motor tract connectivity and motor neuron excitability in dogs, providing a more complete description as a model of SCI. Further understanding of the complex interactions and plasticity between long tracts and local circuitry post-injury and their relationship to functional recovery is indicated. The ability to subcategorize electrophysiologically might facilitate choosing appropriate candidates for testing specific interventions aimed at manipulating long tracts or local circuitry with the goal of improving outcomes in severe SCI.

Author Disclosure Statement

No competing financial interests exist.

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ELECTRODIAGNOSTICS ADDENDUM

After an initial recruitment period on which the data presented in the preceding chapter on electrodiagnostics are based, additional dogs with SCI were subsequently enrolled resulting in a total of 34 dogs with chronic SCI who underwent electrodiagnostic testing. Long tract function was evaluated by performing transcranial magnetic stimulation (TMS) and recording motor evoked potentials (MEPs) as well as via recording of somatosensory evoked potentials (SSEPs). Motor neuron pool excitability was evaluated by performing tests of local reflex circuitry, the F-wave and H-reflex. One dog was removed from analysis of SSEPs and local reflex circuitry (n=33) due to prior distal limb self-mutilation precluding reliable data capture. The testing protocols were unchanged. Data for the original group of 20 dogs are presented for comparison where indicated and the same statistical methods were used unless specified.

Updated signalment and gait scores are similar to the original evaluation group (Table 1). Seven dogs (21%) were ambulatory and 27 (79%) non-ambulatory including 4 dogs able to take at least some weight bearing steps unassisted (OFS = 4).

Table 1. Signalment characteristics and gait scores for all dogs evaluated (n=34).

	Mean (SD) or Median (range)
Age (years)	5.97 (2.55)
Body Weight (kg)	7.65 (3.1-33)
Duration of Injury (months)	17 (3-84)
OFS (0-12)	2 (0-9)
SS (0-100, no support)	0 (0-89)
RI (0-100, no support)	0 (0-46.56)

Long tract evaluation: MEPs were recorded from the extensor carpi radialis muscle of the front limb following cortical stimulation in 34/34 (100%) dogs (positive control). MEPs were recorded from the cranial tibial muscle of the hind limb in 6/34 (17.6%) dogs. This compares to 4/20 (20%) with positive hind limb MEPs in the original group of 20 dogs. Mean latency and conduction velocity are presented in Table 2 and were comparable between the 4 dogs with positive hind limb MEPs from the original group and the 6 dogs (including the original 4) with positive MEPs from all 34 dogs. MEP latency and conduction velocity amongst the SCI dogs remained significantly longer and slower, respectively, compared to the cohort of neurologically normal control dogs previously described ($p=0.0045$, $p=0.0005$).

Cord dorsum potentials were present in 32/32 (100%) dogs (positive control). SSEPs were not performed in 1 dog due to duration of sedation and 1 dog was removed from analysis due to prior distal limb self-mutilation. SSEPs were present following distal ulnar nerve stimulation in 17/17 (100%) dogs in which front limb testing was tolerated (positive control). No SSEPs were recorded following distal tibial nerve stimulation, which mirrors findings in the initial group of 20 dogs.

Table 2. Hind limb MEP latency and conduction velocity amongst dogs with recordable MEPs. HL: hind limb, MEP: motor evoked potential, CV: conduction velocity

Variable	Mean (SD) (n=6)	Mean (SD), Original group of 20 (n=4)
HL MEP latency	53.35 (11.86)	63 (18.2)
HL MEP CV	13.96 (2.95)	12 (3.4)

Motor neuron pool excitability evaluation: H-reflex and F-waves were present in 33/33 (100%) dogs. One dog was removed from analysis due to prior self-mutilation. F-wave persistence was 100%. Summary values for F-wave and H-reflex variables are presented in Table 3. H-reflex amplitude ranged from 0.2-5.1mV compared to 0.2-3.3mV in the original

group. When all dogs (n=33) were compared to controls, no differences were identified in median H-reflex latency or mean H:M ratio (p=0.98, 0.818, respectively). Mean H-reflex threshold intensity remained significantly lower in cases compared to controls, after correction for multiple comparisons ($p_a=0.011$).

Sustained or intermittent after discharges were noted in 31/33 (88%) and 26/33 (79%) dogs after F-waves and H-reflexes, respectively, compared to 17/19 (89%) and 16/19 (84%) in the original group. Short duration sustained after discharges (<20ms) were the most common after F-waves (23/33, 69%) while short duration (<10ms), long duration (≥ 10 ms) or absent after discharges were more evenly distributed after H-reflexes (10/33 (33%), 15/33 (45%), 8/33 (24%)). After discharges remained common in this population of dogs with SCI with a slightly higher occurrence after F-waves than H-reflexes but overall demonstrating comparable frequency and duration compared to the original group of dogs.

Table 3. Summary statistics for motor nerve conduction velocity, F-wave and H-reflex variables in all dogs (n=33) compared to the original group of dogs (n=19). MNCV: motor nerve conduction velocity.

Variable	Mean (SD) or Median (range)	
	All dogs (n=33)	Original group of 20 (n=19)
MNCV	78.06 (10.2)	75.2 (9.7)
F latency	13.18 (3.16)	13.68 (3.9)
F ratio	1.72 (1.13-5.17)	1.55 (1.1-5.2)
H-reflex latency	12.5 (9.2-20.7)	13.5 (9.7-21.6)
H-reflex threshold	2.5 (2.4)	3.2 (2.5)
H:M ratio	0.27 (0.18)	0.29 (0.2)

Associations between electrodiagnostic variables, ambulation status and gait scores: Five out of 6 (83%) dogs with recordable hind limbs MEPs were ambulatory compared to 1/28 (3.6%) dogs with absent MEPs ($p_a=0.002$, original group $p_a=0.032$). The presence of hind

limb MEPs were positively associated with gait scores (Table 4). The mean H-reflex threshold was lower in ambulatory (1.9mA +/-1.7) versus non-ambulatory dogs (2.7mA +/- 2.6) but the difference was not significant. Mean H:M ratio was similar between ambulatory (0.23 +/- 0.14) and non-ambulatory dogs (0.28 +/- 0.19). H-reflex threshold and H:M ratio were not associated with gait scores which is distinct from the original group of dogs in which the threshold intensity was inversely associated with treadmill-based SS and RI (Table 5). To investigate potential relationships between motor neuron pool excitability and motor conduction after injury, H-reflex and MEPs were also compared. We did not identify any associations between H-threshold and presence/absence of hind limb MEPs, MEP latency or conduction velocity ($p=0.5586, 1733, 0587$, respectively).

Table 4. Association between presence or absence of MEPs and gait scores in 34 dogs. HL: hind limb, MEP: motor evoked potential; OFS: open field scale, SS: stepping score, RI: regularity index.

	HL MEP Present	HL MEP Absent	p_a -value	Original group p_a -value
Median OFS	6 (2-9)	1.5 (0-8)	0.004*	0.006*
Median SS	58.5 (0-89)	0 (0-75)	0.003*	0.006*
Median RI	16.93 (0-46.56)	0 (0-34.29)	0.004*	0.003*

Table 5. Associations between H-reflex threshold and H:M and gait scores in 33 dogs. OFS: open field scale, SS: stepping score, RI: regularity index.

	H threshold p -value	H:M p -value	Original group H threshold p_a -value
Median OFS	0.2554	0.9435	0.11
Median SS	0.0876	0.5991	0.042*
Median RI	0.0883	0.4505	0.043*

Overall, our electrodiagnostic findings in a larger number of chronically impaired dogs are comparable to our results in the original group of dogs. Intact MEPs remained uncommon and no dogs had recordable SSEPs which likely reflects the severity of injury in this

population. However, this further underscores the low sensitivity of these tests to detect potential connections across the lesion compared to more invasive recordings at the level of the dura.¹⁻³ Despite this limitation, the presence of MEPs recorded from the cranial tibial muscle of the hind limb remained significantly associated with ambulation, OFS, SS and RI. This provides growing evidence that at least some supraspinal influence traversing the site of injury is crucial to functional motor recovery in dogs after spontaneous SCI.

Consistently present H-reflexes, higher H:M ratio and significantly lower H-reflex threshold intensity further supports increased motor neuron pool excitability in dogs with chronic SCI compared to neurologically normal dogs. While H-reflex threshold for all dogs (n=33) was lower in ambulatory compared to non-ambulatory dogs, it was no longer inversely associated with treadmill-based SS and RI as it was in the original group of 20. There continued to be no relationships identified between H threshold and ambulation status or OFS, and H:M ratio and ambulation, OFS, SS or RI. It remains likely that reorganization of local intraspinal circuitry after injury, including changes to motor neuron pool excitability, plays a role in recovery of function but more work is needed to determine how plasticity facilitates or impedes motor function in individual dogs.⁴ The interplay between residual trans-lesional connections and motor neuron pool excitability after injury and how that affects function also needs to be elucidated in this population. We did not identify any associations between H-reflex threshold intensity and MEPs but this was limited by the small number of dogs with the presence of hind limb MEPs. Our results enhance our understanding of injury complexity amongst this population, provide clues about the necessary components for functional recovery after severe injury, and highlight the potential utility of electrodiagnostic tests as outcome measures in clinical trials of chronically paralyzed dogs.

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SUPPLEMENTARY INFORMATION

Supplementary Data 1. Clinical information in controls.

Dog	Age	Breed	Body Weight
1	4	Doberman mix	39
2	6	Shepherd mix	41
3	9	Whippet	9
4	10	Mixed breed	16
5	3	Terrier mix	7.3
6	7	Dachshund	6.3

CHAPTER 5
Magnetic resonance imaging features of dogs with incomplete recovery after acute, severe spinal cord injury.

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Abstract

Study Design: Retrospective case series

Objectives: Describe the magnetic resonance imaging (MRI) features of dogs chronically impaired after severe spinal cord injury (SCI) and investigate associations between imaging variables and residual motor function.

Setting: United States of America

Methods: Thoracolumbar MRI from dogs with incomplete recovery months to years after clinically complete (paralysis with loss of pain perception) thoracolumbar SCI were reviewed. Lesion features were described and quantified. Gait was quantified using an ordinal, open field scale (OFS). Associations between imaging features and gait scores, duration of injury (DOI) or SCI treatment were determined.

Results: 35 dogs were included. Median OFS was 2 (0-6), median DOI was 13 months (3-83) and intervertebral disc herniation was the most common diagnosis (n=27).

Myelomalacia was the most common feature followed by cystic change; syringomyelia and fibrosis were uncommon. Lesion length corrected to L2 length (LL:L2) was variable (median LL:L2=3.5 (1.34-11.54)). Twenty-nine dogs had 100% maximum cross-sectional spinal cord compromise (MSCC) at the lesion epicenter and the length of 100% compromised area varied widely (median length 100% MSCC:L2=1.29 (0.39-7.64)). Length 100% MSCC:L2

was associated with OFS ($p=0.012$). OFS was not associated with any qualitative features. DOI or treatment type were not associated with imaging features or lesion quantification. Conclusions: Lesion characteristics on MRI in dogs with incomplete recovery after severe SCI were established. Length of 100% MSCC was associated with hind limb motor function. Findings demonstrate a spectrum of injury severity on MRI even amongst severely affected dogs which is related to functional status.

Key words: magnetic resonance imaging, chronic spinal cord injury, canine, intramedullary hyperintensity, malacia, model

Introduction

Spinal cord injury (SCI) is common in dogs and magnetic resonance imaging (MRI) has become widespread in the diagnosis of acute canine SCI.¹⁻⁷ However, the appearance of severe injury on MRI in the chronic setting amongst dogs with an incomplete clinical recovery has not been well-documented, and the relationship between specific imaging features in this population and functional status is unknown.

The MRI appearance of acute myelopathies in dogs has been documented with various qualitative and quantitative features.^{2,3,5,6,8-16} Among these, the presence and dimensions of intramedullary hyperintensity on T2W images and length of cord compression have been associated with outcome for dogs suffering from acute intervertebral disc herniation.^{6,10,13,17} The maximal cross-sectional area of intramedullary hyperintensity on T2W images has been associated with outcome in acute, non-compressive nucleus pulposus extrusion.³

Regardless of functional outcome, dogs that suffered an acute, spontaneous SCI are not commonly imaged in the chronic phase unless there is an abrupt change in neurological status suggestive of a new injury. The spinal cord has been reported to be normal on MRI by 6-weeks post-injury for dogs who recover clinically following intervertebral disc herniation.¹⁸ Dogs have been evaluated with MRI up to 16 weeks following experimental traumatic SCI and demonstrated MRI changes in the spinal cord (areas of relative hypointensity on T1W images and hyperintensity on T2W images) that mirrored histopathologically-confirmed formation of a glial scar surrounding a fluid-filled cavitation at the lesion site.¹⁹ Studies describing imaging features in the chronic setting amongst dogs left with permanent neurologic deficits after severe, spontaneous injury are lacking.

Specific MRI changes in acute and chronic SCI have been described in humans.²⁰⁻²⁵ Increased frequency of quantitative and qualitative abnormalities at the lesion site in acute cervical SCI in people has been associated with worse neurologic recovery.²¹ Syring formation was more common in chronic patients when there was a functionally complete SCI.²⁰ More recently, cross-sectional spinal cord area measured above the level of injury has demonstrated that structural atrophy distant to the site of injury was associated with worse motor recovery after SCI.²⁶⁻²⁸

Dogs with SCI share many overlapping features with human SCI making them an attractive large animal model capable of capturing the heterogeneity (of both population and injury) that is lacking in experimental models.²⁹ The full spectrum of MRI features of this population needs to be described, and identification of imaging features that predict functional recovery would enhance their use in clinical trials on chronic paralysis. Our objective with this study was to describe the MRI features of chronic SCI amongst dogs with an incomplete recovery after acute, functionally complete injury and to investigate associations between imaging variables and functional status. We hypothesized that smaller spinal cord lesion dimensions would be associated with greater motor function.

Methods

Case Selection: The medical records of dogs that suffered an acute, severe thoracolumbar SCI in which a thoracolumbar MRI was performed at least 3 months after injury were reviewed. All dogs were participants in one or more clinical trials for chronic paralysis as part of the North Carolina State University College of Veterinary Medicine Canine Spinal Cord Injury Program. MRIs were performed as part of these studies but prior to initiation of any therapy associated with the particular study. Prior therapies were noted but not considered exclusion criteria. To be included, the SCI needed to be severe enough to cause acute paralysis and loss of pain perception followed by incomplete recovery characterized by absent or minimal recovery of pain perception and chronic motor deficits. Data collected

from the medical records included signalment, diagnosis, treatment(s) of the SCI, spinal cord lesion location and duration of injury (DOI, interval from injury to chronic imaging). Results of neurologic examination and gait status at the time of imaging were also obtained. Gait was quantified using an ordinal gait scale (open field score, OFS, ranging from 0-12).^{30,31} OFS of <4 corresponds to taking no weight bearing steps, a score of 4 corresponds to taking weight bearing steps less than 10% of the time and scores ≥ 5 correspond to taking weight bearing steps between 10-100% of the time.

Imaging acquisition: All MRIs were performed using a 1.5T scanner (Siemens Medical Solutions USA Inc., Malvern, PA) with acquisition of standard transverse and sagittal sequences (T1W pre- and post-contrast, T2W, STIR, half-Fourier acquisition single-shot turbo spin-echo (HASTE)).³² Additional sequences utilized in some, but not all, scans included proton density and GRE/T2*.

Imaging analysis: MRIs were reviewed for the presence or absence (Yes/No) of the following qualitative lesion features: extended spinal cord atrophy (associated with the injury site), myelomalacia, syrinx, cystic change and intramedullary fibrosis (defined in Table 1).²⁵ Gradient echo sequences were not commonly performed in this population, however, when available, they were evaluated along with the standard sequences for the presence of hemorrhage as an additional qualitative feature. Calculations characterizing the dimensions of the lesion were performed as defined in Table 2. Lesion length (LL) and length of the region within the lesion with 100% cross-sectional abnormal signal intensity (Length of 100% MSCC) were recorded as raw values and normalized to the length of vertebral body L2 to adjust for dogs of different sizes (LL:L2, Length of 100% MSCC:L2).¹²

Table 1. Qualitative imaging features

Feature	Definition
Extended atrophy	Narrowing of the spinal cord in the mid-sagittal plane spanning at least 2 vertebral bodies in length; spinal cord height subjectively compared to adjacent normal areas cranial and caudal to the lesion
Myelomalacia	Ill-defined area of T1 hypointensity and moderate T2 hyperintensity (less intense than cerebrospinal fluid)
Syringomyelia	Well-defined region of T1 hypointensity, T2 and HASTE hyperintensity, isointense to cerebrospinal fluid extending > 1 vertebral body in length; tubular or loculated in shape
Focal cystic change	As for syringomyelia but extending < 1 vertebral body in length; round or oval in shape
Intramedullary fibrosis	Area of T1 and T2 hypointensity on sagittal images
Intramedullary Hemorrhage	Area of T1 and T2 hypointensity with susceptibility artifact on T2*GRE images

Table 2. Quantitative imaging variables

Feature	Definition
Lesion length (LL)	Length (in cm) of the abnormal spinal cord signal intensity visible on T2W sagittal images with cranial and caudal extent corroborated on T2W transverse images; also expressed as a ratio compared to the length of the L2 vertebral body
Maximum spinal cord compromise (MSCC)	Area of abnormal spinal cord signal intensity at the lesion epicenter on T2W transverse images expressed as a percentage of the total cross-sectional area of the cord at that location (0-100%)
Length of 100% MSCC (if MSCC=100%, i.e. an area with no apparent normal tissue present)	Length (in cm) of the region with 100% MSCC on T2W sagittal images with the cranial and caudal extent corroborated on T2W transverse images; also expressed as a ratio compared to the length of the L2 vertebral body

Statistical analysis: All analyses were performed using Jmp 12 Pro (SAS Institute, Cary, NC, USA). Qualitative features were treated as categorical data and reported as frequency of occurrence. Summary statistics for continuous data (quantitative variables) were reported as mean and standard deviation if normally distributed or median and range if not using the Wilk-Shapiro test for normality. Associations between presence of specific qualitative features and OFS as well as DOI were determined using a Wilcoxon rank sum test. Linear regression and a one-way ANOVA were used to compare quantitative variables and OFS or DOI. Associations between presence of qualitative features and type of treatment (medical versus surgical) were established by constructing contingency tables and using a Fisher's exact test whereas associations between quantitative variables and type of treatment were determined using a Wilcoxon rank sum test. $P < 0.05$ significant with adjusted p values calculated for multiple comparisons using Holm's correction calculator.

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research (protocol #'s D11-015-O, 15-004-01).

Results

Thirty-five dogs met inclusion criteria (Supplementary Information 1). There were 13 Dachshunds, 8 mixed breed dogs, 3 Pit Bull Terriers, 2 each of miniature schnauzers, Australian cattle dogs, Shih Tzus and French Bulldogs, and 1 each of Cocker Spaniel, Boston Terrier and Bichon Frise. Mean age was 5.5 years (SD 2.24) and median body weight was 8.8kg (range: 4.6-33kg). Intervertebral disc herniation (IVDH) was the most common diagnosis (n=27) followed by fibrocartilaginous embolism (FCE, n=2), vertebral column fracture (n=5) and traumatic intervertebral disc extrusion (n=1). For dogs with IVDH, 19 were treated with decompressive surgery at the time of injury and 8 were managed conservatively. Median DOI (time of injury to imaging) was 13 months (range: 3-83months)

with 18 dogs having a DOI \leq 13 months and 17 dogs with a DOI $>$ 13 months. Median OFS was 2 (0-6). Two dogs were independently ambulatory (OFS 5 and 6), 4 dogs took some weight bearing steps (OFS 4) and 29 were non-ambulatory (OFS 0-3).

Lesion location ranged from the level of the 7th thoracic vertebra to the 4th lumbar vertebra, with 16 dogs having lesions at T12-T13 or cranial, 16 dogs with lesions at T13-L1 or caudal and 3 with lesions spanning cranial and caudal to T13. The spinal cord was abnormal in the region of prior injury in all dogs. No dogs had extended spinal cord atrophy, while all dogs had areas within the lesion consistent with myelomalacia (Figure 1). A lesion consistent with myelomalacia was the only abnormality noted in 4 dogs (4/35, 11%). Twenty-nine of 35 dogs (83%) had an area of fluid accumulation as part of the lesion with focal cystic change present in 20/35 (57%) and syringomyelia noted less commonly in 9/35 (26%) (Figure 1C,D).

Among dogs with a syrinx, length ranged from 2-5 vertebral bodies. Findings consistent with intramedullary fibrosis within the lesion were uncommon (6/35, 17%), present only in dogs that had undergone surgical decompression for IVDH or had suffered a fracture/luxation and, in the 8 dogs in which gradient echo sequences (T2*) were performed, none had evidence of hemorrhage (Figure 1E,F). A summary of quantitative imaging abnormalities is provided in Table 3. MSCC was 100% (no normal spinal cord tissue discernible at lesion epicenter) in 29 dogs while 6 dogs had a MSCC of less than 100% (median: 77%, range: 38-90%). Lesion length and length of 100% MSCC varied extremely widely between dogs.

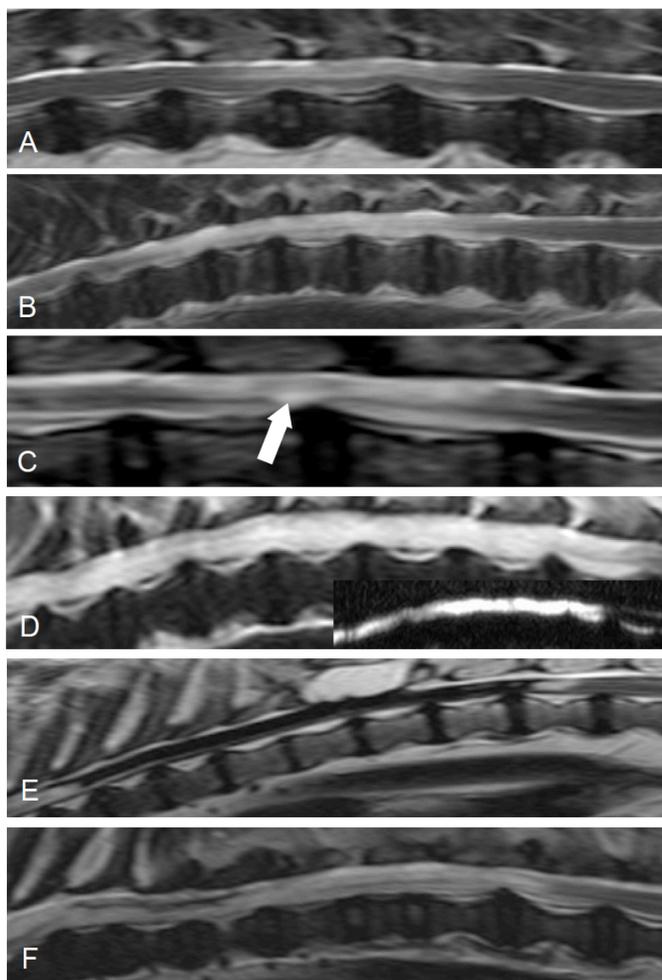


Figure 1. Representative images depicting qualitative imaging abnormalities. A: T2W sagittal image showing ill-defined intramedullary hyperintensity consistent with myelomalacia centered over L1-3. B: T2W sagittal image showing more extensive hyperintensity consistent with myelomalacia. C: T2W sagittal image showing a small, well-defined hyperintense region representative of focal cystic change (arrow). D: T2W sagittal image showing a well-defined hyperintensity extending more than 1 vertebral body in length consistent with syringomyelia with inset showing sagittal HASTE image of the pictured region. E: T2W sagittal image showing intramedullary hypointensity consistent with extensive intraparenchymal fibrosis. F: T2W sagittal image of hypointensity consistent with more focal fibrosis.

There were no relationships identified between qualitative imaging features and OFS or DOI (Figure 2, $p > 0.05$). There were also no significant associations identified between qualitative

imaging features and dogs with IVDH managed medically or surgically ($p>0.05$). The relationship between OFS, DOI or treatment for IVDH and quantitative variables are presented in Figure 3. Having less than 100% MSCC within the lesion ($p=0.219$, $p_a=0.219$) and LL:L2 ($p=0.106$, $p_a=0.212$) were not associated with OFS but the length of 100% MSCC:L2 ($p=0.0041$, $p_a=0.012$) was associated with OFS. No associations were identified between quantitative variables and DOI or treatment type (medical versus surgical) for IVDH dogs ($p>0.05$).

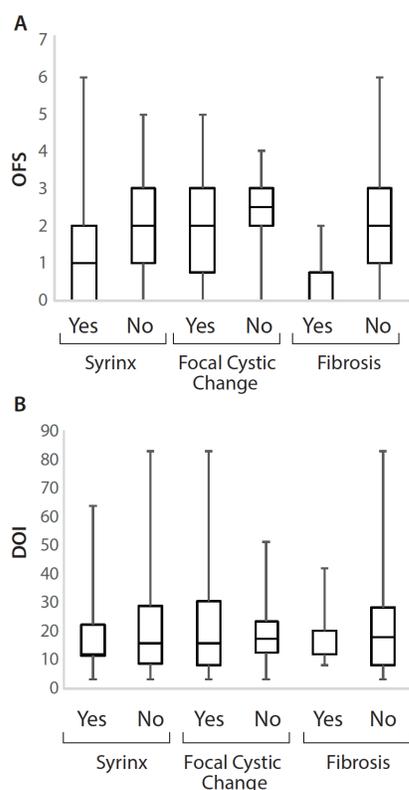


Figure 2. Relationship between qualitative imaging features and (A) OFS or (B) DOI. No significant associations were identified ($p>0.05$).

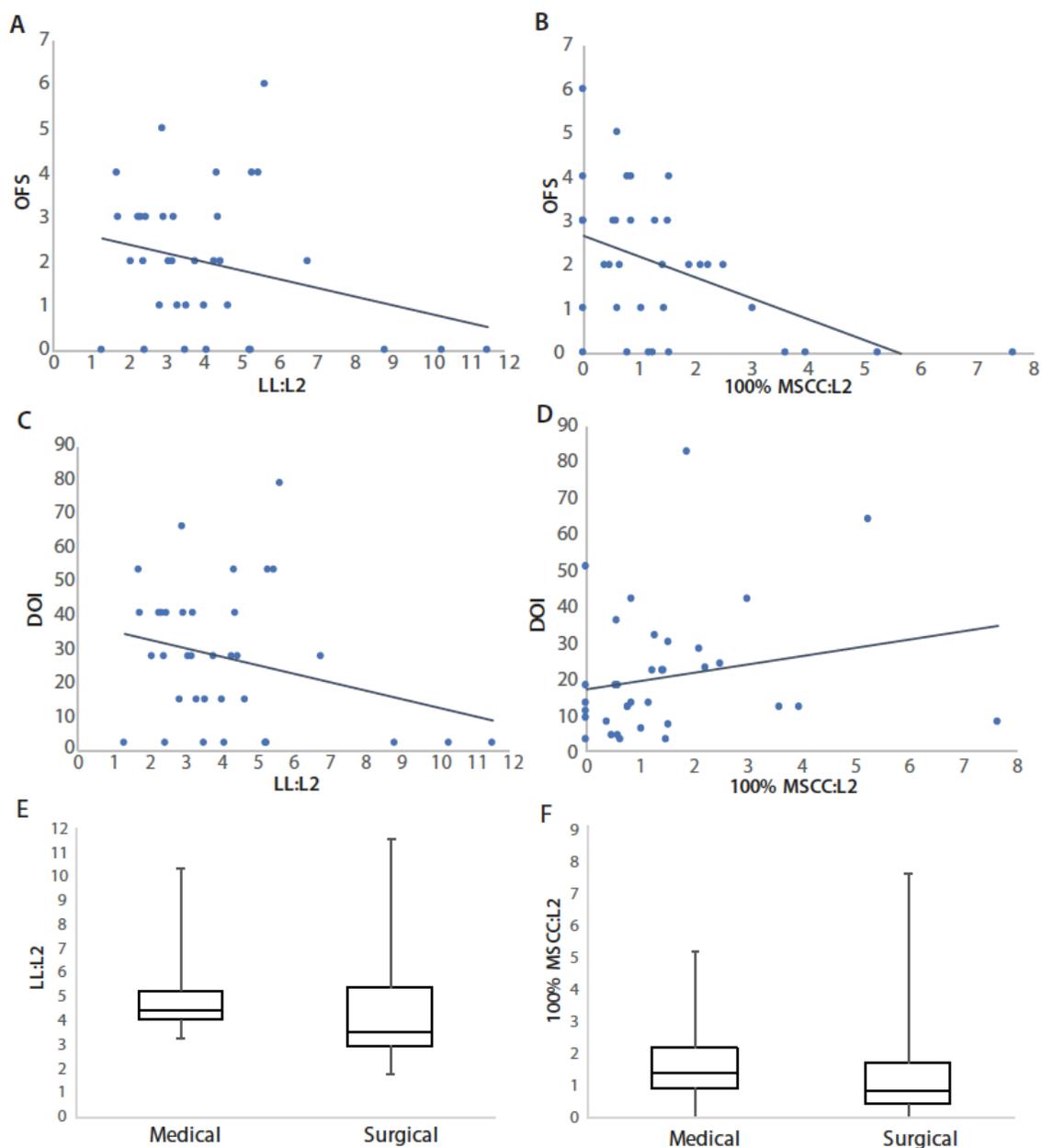


Figure 3. Relationship between quantitative imaging abnormalities and motor function, injury duration or treatment type. A, B: OFS and LL:L2 ($p=0.106$) or 100% MSCC:L2 ($p=0.0041$); C, D: DOI and LL:L2 ($p=0.461$) or 100% MSCC:L2 ($p=0.244$), E, F: treatment type (medical or surgical) for dogs with IVDH and LL:L2 ($p=0.265$) or 100% MSCC ($p=0.455$). OFS: open field score, LL: lesion length (cm), 100% MSCC: length of 100% compromised spinal cord region (cm), DOI: duration of injury, IVDH: intervertebral disc herniation.

Discussion

The results of this study establish MRI lesion characteristics in a population of dogs with incomplete recovery after severe, acute SCI. Lesion appearance and extent were varied although certain features were common. Frequency of cystic change and syringomyelia were comparable to findings in humans. We demonstrated that the length of the region within the spinal cord lesion that had 100% abnormal signal intensity in the transverse plane was associated with hind limb motor function. This suggests that the craniocaudal length of apparently complete transverse myelopathy on MRI impacts the functional status in dogs judged clinically to have a complete SCI.

Specific MRI changes have been described for humans with acute and chronic SCI.²⁰⁻²⁵ Acute qualitative features include hemorrhage, edema, cord swelling, adjacent soft tissue changes, canal stenosis and intervertebral disc herniation while defined quantitative abnormalities include lesion length, maximum canal compromise and maximum spinal cord compression.²¹ MRI features of chronic SCI in humans include intramedullary changes such as myelomalacia, syrinx or cyst formation as well as spinal cord compression, atrophy, disruption and tethering.^{20,22-25} The MRI appearance of acute myelopathies in dogs has also been reported.^{2,3,5,6,8-12,14,15} Qualitative features evaluated have included changes to intervertebral discs including classifications of degeneration and herniation, intramedullary signal changes, extradural compression, presence of spinal cord swelling, gray-white matter lesion distribution, contrast enhancement and presence of fractures, luxations or other evidence of vertebral column instability.^{2,5,6,9-11,14} Variables quantified have included the length of spinal cord compression, the length of intramedullary hyperintensity on T2W sagittal images, the degree of maximum spinal cord compression, and the maximum cross-sectional area of the lesion in the transverse plane.^{3,6,10,12,13,16}

The characteristic MRI features of lesions in dogs with chronic deficits after acute, functionally complete injury have not been previously reported. We describe the frequency of major pathologic features as well as lesion dimensions in a population of chronically paralyzed dogs. While the spinal cord can be normal on MRI in dogs who recover clinically from acute SCI, all dogs in our study had persistent lesions in the chronic setting, supporting permanent damage to the spinal cord after severe injury.¹⁸ All dogs had areas consistent with myelomalacia within the lesion, but the character and extent of the compromised area on MRI was otherwise quite variable. Specifically, LL and length of 100% MSCC varied widely between dogs even after adjusting by the length of L2 to account for dogs of differing body size, illustrating the continuum of injury severity in this clinically similar population. Studies of acute injury in dogs suffering from IVDH and acute, non-compressive nucleus pulposus extrusion have demonstrated that the presence and dimensions of T2W intramedullary hyperintensity were associated with outcome.^{3,6,10,13,17} We found that 83% of dogs in this study had an area of the spinal cord with no normal signal intensity and lack of 100% MSCC was not associated with improved motor function suggesting that the MSCC (percentage) by itself does not appear to be a useful parameter in the chronic setting. Similarly, LL was extremely variable between dogs using either the raw numbers or as a ratio to L2 and was not associated with functional status. In an attempt to capture injury severity by an alternative means, we measured the length of the spinal cord that had 100% altered signal intensity. While also variable, the length of the 100% MSCC (raw and adjusted by L2) was associated with OFS. This suggests that the length of complete disruption of the spinal cord is important functionally. The ability of conventional MRI to detect single or small numbers of intact axons traversing a site of severe injury is limited. Our findings support an increased likelihood of such trans-lesional fibers as the longitudinal extent with no discernible normal tissue decreases.

There are ongoing efforts to identify specific imaging features or combinations of abnormalities in people with SCI to establish non-invasive biomarkers predictive of outcome

or functional status with a focus on changes that occur away from the site of injury. While specialized imaging modalities (such as functional MRI or diffusion imaging) are being increasingly utilized, several studies have identified that spinal cord atrophy distant to the site of injury (typically measured at C2-3 on T2W transverse images) was associated with outcome.²⁶⁻²⁸ This suggests that extensive changes occur to the entire neuroaxis after focal SCI and offers a way to capture injury severity and changes over time such as Wallerian degeneration away from the complexities inherent at the lesion itself. We did not identify extended atrophy associated with the lesion in any dogs but its presence might have been difficult to discern objectively given the variable length and location of lesions and differing spinal cord diameter amongst dogs of different breeds and body sizes. Additionally, we were unable to evaluate for potential atrophy farther from the site of injury in this study based on the fact that only the thoracolumbar spinal cord in the region of injury was scanned. Further investigation using a standard site such as C2-3 might help to determine if similar atrophy occurs distant to the lesion in dogs and if it is associated with motor function. Identification of non-invasive imaging biomarkers could provide a means to monitor changes after injury or serve as an outcome measure in clinical trials.

Qualitative features other than myelomalacia were less common in this population and their relationship to functional status remains unclear. Syringomyelia was identified in approximately one quarter while focal cystic change was present in just over half of cases. This is comparable to humans in which approximately 21-28% of chronic SCI patients develop post-traumatic syringomyelia (PTSM) and 30-50% demonstrate some degree of cystic change.³³⁻³⁴ The precise cause of PTSM in people is not well understood and it remains unclear if focal fluid pocketing secondary to areas of myelomalacia is a necessary precursor to PTSM.³³ In experimental models of SCI, concurrent induction of arachnoiditis (via kaolin injection) potentiates the development of syringomyelia and parenchymal damage suggesting an important role for arachnoid inflammation and scarring in conjunction with direct tissue loss at the site of injury.³⁵⁻³⁷ Rats with induced SCI and arachnoiditis also demonstrated

larger syrinxes and greater locomotor dysfunction at 6-weeks post-injury compared to rats with SCI alone suggesting that development of PTSM contributes to injury severity and influences recovery of motor function.³⁷ While only a small percentage of people develop symptomatic syrinxes, affected patients experience neurologic deterioration after a period of static signs and effective treatment is challenging.^{33,34,38,39} No dogs in our study demonstrated an overt deterioration in neurologic status after reaching a plateau following injury and the importance of identifying a syrinx on MRI in dogs with chronic SCI is unknown. The presence of a syrinx or focal cystic change were not associated with functional status or injury duration. Serial imaging in the chronic setting would be needed to determine if dogs with focal cystic change go on to develop overt syringomyelia over time or if there is an association between progressive syrinx elongation and outcome in dogs. Given the similar frequency of development of this abnormality between dogs and people, dogs with spontaneous SCI might be a useful population in which to continue to investigate causes and interventions for PTSM.

Findings consistent with intramedullary fibrosis were relatively uncommon, occurring in about one third of cases. No dogs had detectable parenchymal hemorrhage, which might be more expected in the acute injury phase. While fibrosis was not associated with functional status or DOI, only dogs who suffered IVDH and underwent surgery or had vertebral column trauma had findings consistent with fibrosis. Some veterinary neurosurgeons perform a durotomy following decompression of the spinal cord in dogs with acute, severe IVDH with the intended goal of relieving intramedullary pressure and improving spinal cord blood flow.^{40,41} The dog illustrated in figure 1E for example had undergone a multilevel laminectomy and durotomy. Spinal fractures and luxations can result in dural lacerations which may also explain why fibrosis was present in these cases.⁴²⁻⁴⁴ It has been shown in experimental SCI in rodents that dural disruption results in invasion of fibroblasts into the spinal cord parenchyma and that repair of such damage (via duroplasty) reduces fibrous scarring.^{45,46} While determination of the status of the dura at the time of injury was not

possible in this study, our findings might reflect a similar response to damage to the dura in dogs with SCI. Imaging in the acute and chronic setting from a larger number of dogs with a range of cause of injury including dogs with varying treatment interventions would be necessary to see if this trend is confirmed.

Limitations of this study include the relatively small sample size with variable cause and treatment of the SCI, the inherent subjectivity of imaging-based definitions and lack of histopathologic confirmation since this is a pet dog population. While myelomalacia was the most likely explanation for ill-defined areas of T2 hyperintensity within the spinal cord under chronic conditions, other causes such as edema or inflammation could not definitively be excluded. While all lesions were easy to identify, it is possible that truncation artifact mimicked the intramedullary hyperintensity obscuring the true cranial and caudal extent of the lesion and impacted LL measurements.⁴⁷ Given the chronicity of these injuries, fibrosis was most likely for areas of T1 and T2 hypointensity identified within the lesion but incidental mineralization or even hemorrhage cannot be excluded. Gradient echo sequences were only performed in a small number of dogs (n=8) hindering the ability to rule out hemorrhage. However, 5 of these were imaged within 9 months of injury and none showed changes consistent with hemorrhage suggesting the likelihood of such a finding would be even less with increasing chronicity. As mentioned above, extended atrophy as a feature of the lesion was not identified but the possibility of its presence was not excluded and atrophy away from the site of injury was not evaluated. While quantifying atrophy, focusing on white matter loss secondary to Wallerian degeneration distant from the site of injury, might prove useful in this population, prospective studies using a standard location and accounting for breed and body size differences are needed.

Overall, these findings described the spectrum of MRI changes in a population of chronically paralyzed dogs and identified that the length of the spinal cord region with 100% abnormal signal intensity in the transverse plane was associated with motor function. Establishing a

continuum of injury severity in the chronic setting will aid in understanding the importance of lesion heterogeneity amongst clinically severely affected dogs and enhance the ability to utilize such dogs as a model of chronic paralysis.

Supplementary information is available at Spinal Cord's website.

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SUPPLEMENTARY INFORMATION

Supplementary information 1. Bwt: Body weight (in kg), DOI: Duration of injury (in months), OFS: Open field score (0-12). MixB: Mixed breed, Dach: Dachshund, Chi: Chihuahua, Eng Bull: English bulldog, IVDH: Intervertebral disc herniation, TD: traumatic intervertebral disc extrusion, Fx/Lux: fracture and/or luxation, FCE: fibrocartilaginous embolism.

Dog	Age (yr)	Breed	BWt (kg)	DOI (mo)	Lesion Location	Diagnosis	Treatment Category	OFS
1	5	Cocker spaniel	16.5	23	T13-L1	IVDH	Surgical	2
2	6	Min Schnauzer	9.3	36	L1-L2	FCE	Medical	3
3	6	Min Schnauzer	8	42	T11-T12	Fx/Lux	Medical	4
4	3	Bichon	9	12	L1-L2	IVDH	Surgical	0
5	4	French Bulldog	18.4	8	T11-13	IVDH	Surgical	0
6	4	MixB	9.4	13	T12-L2	IVDH	Surgical	3
7	3	Dach	6	3	T13	Fx/Lux	Medical	3
8	4	French Bulldog	9.6	12	T12-L1	IVDH	Surgical	0
9	10	Shih Tzu	6	83	T13-L1	IVDH	Surgical	2
10	4	Dach	6.5	6	L3-4	IVDH	Surgical	1
11	3	Cattle dog	22	4	L1-2	TD	Medical	1
12	9	MixB	6.5	18	L1-2	IVDH	Surgical	3
13	6	Dach	7.1	7	L1-3	IVDH	Surgical	0
14	6	Dach	8.8	32	T11-12	IVDH	Medical	3
15	5	Dach	6.5	30	T12-13	IVDH	Medical	4
16	5	Shih Tzu	6.1	18	L1-3	IVDH	Medical	1
17	6	Boston	11	18	L1-3	IVDH	Surgical	5
18	3	Pit Bull	26.5	4	T13-L1	IVDH	Surgical	2
19	9	MixB	8.5	22	L1-2	IVDH	Medical	0
20	6	MixB	10	3	L2-3	IVDH	Surgical	0
21	5	Dach	6.9	9	T11-12	IVDH	Surgical	4
22	5	Dach	7.3	3	T12-13	IVDH	Surgical	2
23	2	Pit Bull	33	22	T7-9	Fx/Lux	Medical	2
24	7	Dach	7.1	22	T13-L1	IVDH	Surgical	1
25	8	Cattle dog	15.5	51	T11-12	IVDH	Medical	3
26	7	Dach	5.7	13	L2-4	IVDH	Surgical	0
27	6	MixB	10.3	11	T11-13	IVDH	Surgical	6
28	9	MixB	4.6	24	T8-11	IVDH	Medical	2
29	5	MixB	19.9	12	T9-12	FCE	Medical	4
30	5	Dach	6.4	8	L1-2	IVDH	Surgical	2
31	11	Dach	10	64	T11-12	IVDH	Medical	0
32	5	MixB	14.6	13	T10-11	IVDH	Surgical	3
33	5	Dach	5	28	T12-13	IVDH	Medical	2
34	4	Dach	4.74	42	T11-12	Fx/Lux	Medical	1
35	1	Pit bull	18	12	T12-13	Fx/Lux	Medical	0

CHAPTER 6

The relationship between lesion severity characterized by diffusion tensor imaging and motor function in chronic canine spinal cord injury

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Abstract:

Lesion heterogeneity amongst chronically paralyzed dogs after acute, complete thoracolumbar spinal cord injury (TLSCI) is poorly described. We hypothesized that lesion severity quantified by Diffusion Tensor Imaging (DTI) is associated with hind limb motor function. Our objectives were to quantify lesion severity with fractional anisotropy (FA), mean diffusivity (MD) and tractography and investigate associations with motor function.

Twenty-two dogs with complete TLSCI in the chronic stage were enrolled and compared to 6 control dogs. All underwent thoracolumbar MRI with DTI and gait analysis. FA and MD were calculated on regions of interest (ROI) at the lesion epicenter and cranial and caudal to the visible lesion on conventional MRI and in corresponding ROI in controls. Tractography was performed to detect trans-lesional fibers. Gait was quantified using an ordinal scale (OFS). FA and MD were compared between cases and controls, and relationships between FA, MD, presence of trans-lesional fibers and OFS were investigated.

FA at the epicenter (median:0.228, 0.107-0.320), cranial (median:0.420, 0.391-0.561), and caudal to the lesion (median:0.369, 0.265-0.513) were lower than combined ROI in controls (median:0.602, 0.342-0.826, $p < 0.0001$). MD at the epicenter (median: 2.06×10^{-3} , $1.33 - 2.96 \times 10^{-3}$) and cranially (median: 1.52×10^{-3} , $1.03 - 1.87 \times 10^{-3}$) were higher than combined ROI in controls (median: 1.28×10^{-3} , $0.81 - 1.44 \times 10^{-3}$, $p \leq 0.001$). Four dogs had no trans-lesional fibers. Median OFS was 2 (0-6). FA at the lesion epicenter and presence of trans-lesional fibers were associated with OFS ($p \leq 0.0299$).

DTI can detect degeneration and physical transection after severe TLSCI. Findings suggest DTI quantifies injury severity and suggests motor recovery in apparently complete dogs is due to supraspinal input.

Key words: Magnetic resonance imaging, fractional anisotropy, mean diffusivity, tractography, chronic paralysis

Introduction

Spinal cord injury (SCI) is common in dogs and frequently leaves severely affected dogs with permanent deficits.¹⁻³ Despite this, the variability and complexity of lesions amongst chronically paralyzed dogs after acute, functionally complete thoracolumbar SCI is poorly understood, especially how structural lesion characteristics relate to functional status.

The use of magnetic resonance imaging (MRI) has become widespread in the evaluation of spinal cord disease in dogs. While MRI provides excellent macroscopic anatomic detail and specific imaging features have been reported to be prognostic in various acute, canine myelopathies, there are limitations to the sensitivity of conventional MRI.⁴⁻⁸ Diffusion tensor imaging (DTI) is an imaging technique related to diffusion weighted imaging (DWI) that relies on the diffusion of water molecules in tissues to create images.⁹⁻¹¹ Since pathologic changes in tissue microstructure alter water diffusion, DTI allows assessment of spinal cord white matter tracts at the microstructural level following injury. Quantitative analysis involves calculations of various parameters with fractional anisotropy (FA) and mean diffusivity (MD) or apparent diffusion coefficient (ADC) being most common.¹¹ Quantitative data can then be leveraged to generate tensor maps and ultimately, create a visual representation of white matter tracts of the spinal cord.^{12,13}

In a rodent model of SCI, FA values identified abnormal regions of spinal cord that appeared normal on standard T2-weighted (T2W) images suggesting that DTI improves sensitivity of detection of the extent of the region affected by injury.¹⁴ In addition, FA values were associated with motor scores showing that FA can provide information on lesion evolution and functional outcome.¹⁴ Similarly, DTI has been shown to increase sensitivity of detection of spinal cord pathology in people with various myelopathies compared to conventional imaging.^{13,15-17} In people with chronic SCI, decreased FA values in areas remote from the

visible lesion on T2W images has been interpreted as identifying regions in which Wallerian degeneration was occurring secondary to the injury.^{17,18} While both FA and ADC/MD values can typically differentiate SCI and control populations, FA has been shown to correlate with motor and sensory function suggesting it is the more useful quantitative imaging parameter.¹⁶⁻¹⁹ Tractography has been used to visualize the continuity or disruption of fibers across an area of injury in experimental SCI and in people with SCI, but the relationship between lesion continuity and functional status has not been extensively evaluated.^{16,20}

DTI has been reported in normal dogs with values established for quantitative parameters for the cervical and thoracolumbar spinal cord.²¹⁻²³ There are limited reports of DTI in dogs with SCI and primarily include dogs with acute SCI.^{22,24,25} There are no studies specifically examining DTI in chronically paralyzed dogs secondary to prior acute SCI. Further exploration of the utility of DTI in this population is warranted with the goals of improving delineation of injury and characterization of injury severity. We hypothesized that SCI severity quantified by DTI would be associated with hind limb motor function. Our aims in this study were to perform DTI in a population of chronically paralyzed dogs, to generate FA and MD values cranial, caudal and within the lesion, to use tractography to determine integrity through the lesion epicenter, and to investigate associations between DTI indices and motor function. Establishing the relationship between DTI characteristics and motor recovery will enhance the utility of using dogs as a model of chronic paralysis by providing a means by which to stratify cases based on lesion severity and to track the effects of interventional clinical trials.

Materials & Methods

Control dogs: Clinically normal dogs were prospectively recruited to establish normal values for quantitative DTI analysis performed using the same protocol on the same magnet as SCI dogs. Informed consent was obtained and examinations were conducted in accordance with

the North Carolina State University Institutional Animal Care and Use Committee (protocol #15-150-O). All dogs had to have a normal neurologic examination and no history of neurologic disease.

Case Selection: Dogs were recruited prospectively from the patient pool of the Canine Spinal Cord Injury Program at the North Carolina State University College of Veterinary Medicine and via trial advertisement online (<https://cvm.ncsu.edu/research/labs/clinical-sciences/canine-spinal-cord-injury/>, www.dodgerslist.com). Previous open field gait score (OFS) data from a comparable population (based on inclusion criteria) were used to determine that 20 dogs would allow detection of a 3-point difference in mean OFS between dogs with and without intact trans-lesional fibers with a power of 90%. Given the lack of preliminary DTI data, we aimed to enroll a minimum of 20 dogs. To be included, dogs must have suffered an acute, clinically complete (hind limb paralysis with loss of pain perception) thoracolumbar SCI and demonstrated an incomplete recovery at least three months following injury characterized by chronic motor deficits and severely reduced to absent hind limb and tail pain perception (with or without urinary and fecal incontinence). Prior advanced imaging (computed tomography or MRI) at the time of injury, or definitive diagnosis were not required for inclusion. Exclusion criteria included implants that could generate MRI artifact, co-morbidities precluding general anesthesia, and aggression or anxiety preventing gait analysis and handling. Data collection on each dog included signalment, diagnosis, lesion location, duration of injury (interval from acute injury to imaging) and prior treatment of the SCI. Prior therapy including participation in interventional clinical trials were noted but not utilized as exclusion criteria for the purposes of this study. Informed consent was obtained for all animals and examinations were conducted in accordance with the NCSU Institutional Animal Care and Use Committee (protocol #15-004-01).

General neurologic and gait evaluation: All cases underwent a neurologic examination including standard evaluation of gait, proprioception, spinal reflexes and pain perception.

More extensive gait analysis consisted of walking each dog on a non-slip surface for approximately 3-5 minutes. All examinations were videotaped. Gait was categorized as ambulatory (able to take at least 10 consecutive weight bearing steps unassisted), or not and quantified using an ordinal gait scale (open field score, OFS, ranging from 0-12).^{26,27} OFS \geq 4 corresponds to taking at least some weight-bearing steps.

Imaging acquisition and processing: All dogs (cases and controls) were anesthetized and scanned in dorsal recumbency. Pre-medication consisted of 1-4 mcg/kg IV dexmedetomidine (Dexdomitor, Orion Pharma, Espoo, Finland) and 0.1-0.2 mg/kg IV butorphanol (Torbugesic, Zoetis, Kalamazoo, MI, USA) followed by propofol induction (Propoflo 10 mg/ml, Abbott Laboratories, North Chicago, IL, USA) and inhaled isoflurane for maintenance (VET ONE Fluriso, MWI, Boise, ID, USA). All MRIs were performed using a 1.5T scanner (Symphony; Siemens Medical Solutions USA Inc., Malvern, PA) using circularly polarized spine array and body array flex coils with acquisition of standard transverse and sagittal sequences (T1W pre- and post-contrast, T2W, STIR, half-Fourier acquisition single-shot turbo spin-echo (HASTE)) of the thoracolumbar spinal cord.²⁸ Additional sequences utilized in some, but not all, dogs included proton density and GRE/T2*. DTI was obtained for the same region in the transverse plane using a protocol adapted from Jones et al 2002 with 35 diffusion directions with a scan time of approximately 5 minutes (Table 1).²⁹ Five B0 non-diffusion weighted images were acquired with diffusion weighted scans. Post processing of the diffusion data consisted of conversion from DICOMs to 4D image volumes using MRI Convert and stored in NIfTI format. A diffusion tensor model was fitted to these images using Diffusion Toolkit (<http://trackvis.org/dtk/>), providing a matrix-valued tensor for each voxel that was used to compute standard indices.

Table 1. DTI Protocol parameters

Imaging plane	Transverse
Slice thickness (mm)	3.5
Voxel dimensions	2.5x2.5x3.5
Number of diffusion directions	35
Number of slices	50
Field of view	240x240
Averages	1
TR (ms)	7700
TE (ms)	100
b-value (s/mm ²)	1000
Scan time (min)	5

Imaging analysis: Standard MR images were used to identify relevant anatomy and identify the lesion in cases. Post-processed DTI images were imported into Mango (<http://ric.uthscsa.edu/mango/>) in order to outline regions of interest (ROI) on which quantitative analyses were then performed. In cases, ROI were hand drawn using B0 non-diffusion weighted transverse images at the lesion epicenter as well as 1-2 vertebral segments cranial and caudal to the visible extent of the lesion in normal-appearing areas on conventional MRI (T2W images). Each ROI was composed of the 2-dimensional cross-sectional area of spinal cord on 3 consecutive slices to create a volume of tissue for analysis. Care was taken when drawing ROI to exclude extramedullary structures including cerebrospinal fluid and epidural fat. Quantitative analysis consisted of calculation of FA and MD for each ROI constructed (cranial, lesion epicenter, caudal). FA and MD were calculated on corresponding ROI in controls to generate normal canine values along the thoracolumbar spinal cord using this protocol and to allow comparison to cases. Qualitative analysis consisted of generating maps of the orientation of tensors across all voxels. These tensor maps were used to perform tractography using TrackVis (<http://trackvis.org>) in which adjacent tensors aligned in the same direction were linked with blue color indicating axons

traveling in a cranial-caudal direction. Tractography provided a visual representation of the spinal cord and was assessed visually for continuity of white matter tracts across the lesion. Tractography was also performed in controls.

Statistical analysis: Analyses were performed using Jmp 12 Pro (SAS Institute, Cary, NC, USA). Summary statistics for continuous data (FA and MD values, OFS) were reported as mean and standard deviation if normally distributed or median and range if not using the Wilk-Shapiro test for normality. Trans-lesional fibers were noted to be present or absent based on tractography. FA and MD values were compared between cases and controls using Wilcoxon rank sum test. Amongst cases, a model was constructed that incorporated age (categorical, \leq or $>$ 5 years old) and duration of injury (continuous, in months) as covariates with OFS. Using this model, relationships between FA or MD and OFS were investigated by performing an ANCOVA. The association between the presence of trans-lesional fibers and OFS was evaluated by Wilcoxon rank sum. $P < 0.05$ was considered significant with adjusted p values calculated to account for multiple comparisons using Holm's correction calculator.

Results

Clinical information for controls: Six neurologically normal adult dogs were enrolled (Supplementary data 1). Median body weight was 12.5 kg (6.3-41). Mean age was 6.5 years (SD 2.7).

Clinical information and gait scoring in cases: Twenty-two dogs with SCI were enrolled. There were 9 Dachshunds, 6 mixed breed dogs, 3 Pit bull terriers, 2 Australian cattle dogs and 1 each of Shih Tzu and Boston Terrier. Median body weight was 9.25 kg (range 4.6-33). The mean age was 5.5 years (SD 2.3) and median duration of injury was 15.5 months (range 3-64). Intervertebral disc herniation was the most common diagnosis (17 dogs) followed by vertebral column fracture (3 dogs), fibrocartilaginous embolism (1 dog), and traumatic disc

extrusion (1 dog). In all dogs, neurolocalization was between the third thoracic and third lumbar spinal cord segments based on neurologic exam findings. Twenty-one dogs had no pelvic limb or tail pain perception while 1 dog had a severely blunted response in the toes of the left hind limb. The median OFS for all dogs was 2 (range 0-6). Two dogs were independently ambulatory (OFS 5 and 6), 3 dogs took some weight bearing steps (OFS 4) and 17 were non-ambulatory (OFS 0-3). Treatments at the time of acute injury were variable and depended on underlying cause. Amongst dogs with a diagnosis of intervertebral disc herniation or fracture/luxation, surgery (decompression +/- stabilization) was performed in 10 dogs and medical management with or without formal rehabilitation therapy was performed in 10 dogs.

Imaging results in controls: Conventional MRI sequences revealed no apparent lesions of the thoracolumbar spinal cord. Diffusion imaging captured a region ranging from T8-T10 vertebrae cranially through L3-L6 caudally, with variation in length imaged depending on the size of the dog. Quantitative calculations and tractography were performed in all control dogs. The cranial ROI ranged from T11-T12, middle ROI from T13-L2 and caudal ROI from L2-4, with each individual ROI drawn within the spinal cord at the level of the mid-vertebral body. Median ROI size across all spinal cord locations was 26 pixels and ranged from 18-46. FA and MD values for controls are outlined in Table 2. FA and MD values were not different between cranial, middle and caudal ROI in controls ($p=0.85$, $p=0.36$, respectively). Therefore, combined overall FA and MD for controls were used for comparison to cases. Tractography revealed uniform fiber distribution in control dogs (Figure 1A-B).

Imaging results in cases: Conventional MR images identified the lesion in all cases. Lesion location ranged from the level of thoracic vertebra T7 to lumbar vertebra L4, with 13 dogs having lesions at T12-T13 or cranial and 9 dogs with lesions at T13-L1 or caudal. DTI was centered over the lesion epicenter for each dog and extended from T3-L7 across all cases. Diffusion imaging was of adequate quality to perform quantitative analysis and tractography

in all cases. One dog had a lesion that extended to the cranial aspect of the window imaged and prevented drawing a ROI cranial to the visible extent of the lesion. Cranial ROI FA and MD calculations were not performed in this case. Median ROI size across the three spinal cord locations was 26 pixels and ranged from 15-55. FA and MD values for cases are outlined in Table 2. FA and MD values for cranial and caudal ROI were significantly different from the lesion ROI ($p_a < 0.0001$). FA but not MD values for cranial and caudal ROI were different from each other ($p_a = 0.008$, $p_a = 0.202$). Tractography revealed absence of trans-lesional fibers in 4 cases and partial disruption in 18 (Figure 1C-F).

Table 2. Summary of median (range) FA and MD values in controls and cases. FA: fractional anisotropy, MD: mean diffusivity, ROI: region of interest

		Cranial ROI	Middle/Lesion ROI	Caudal ROI	Overall (controls only)
FA	Controls	0.601 (0.578-0.774)	0.611 (0.554-0.826)	0.589 (0.342-0.817)	0.602 (0.342-0.826)
	Cases	0.420 (0.391-0.561)	0.228 (0.107-0.32)	0.369 (0.265-0.513)	
MD	Controls	1.31×10^{-3} (.98-1.41)	1.29×10^{-3} (0.82-1.35)	1.13×10^{-3} (0.81-1.44)	1.28×10^{-3} (0.81-1.44)
	Cases	1.52×10^{-3} (1.02-1.87)	2.06×10^{-3} (1.33-2.96)	1.31×10^{-3} (0.82-2.08)	

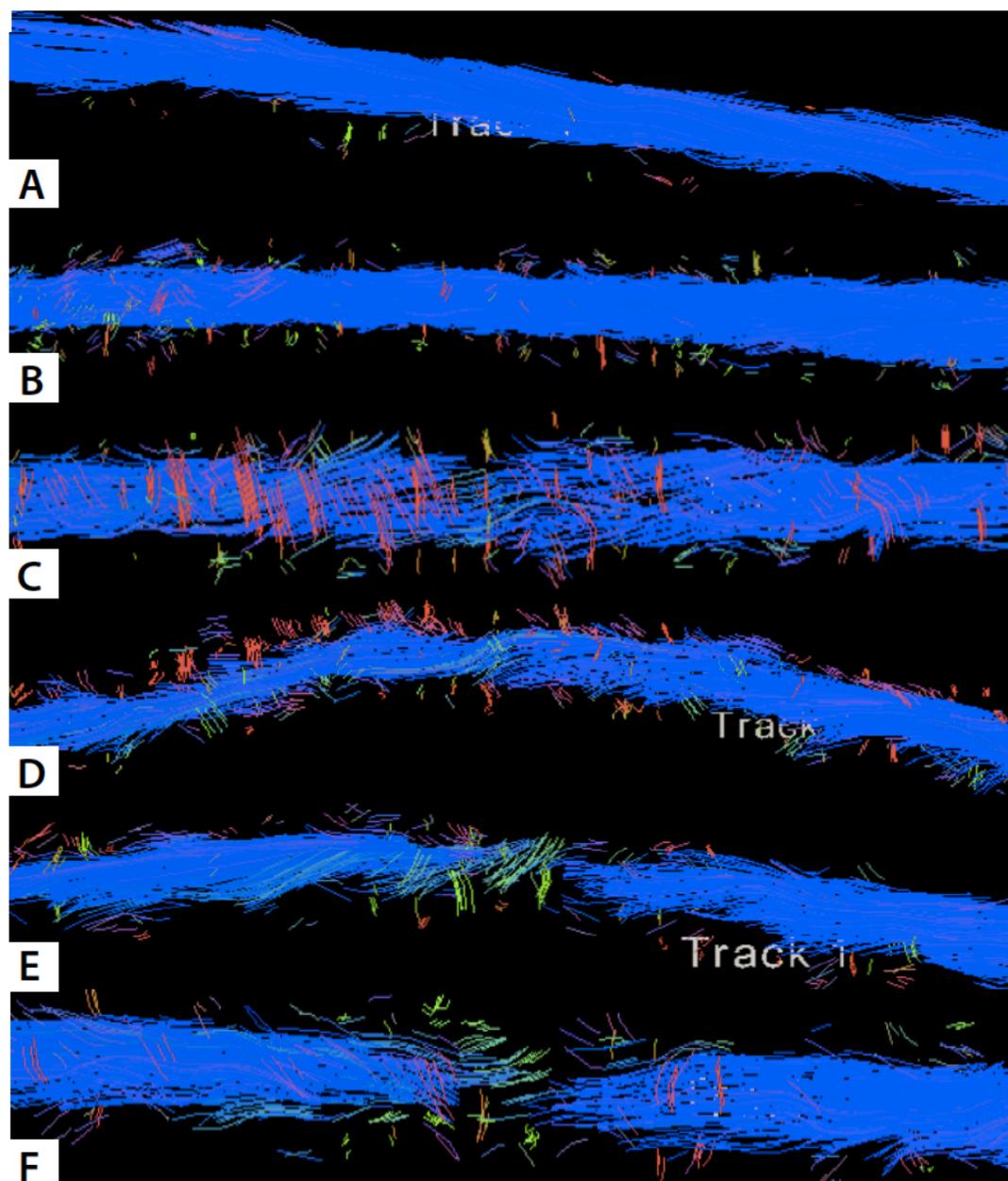


Figure 1. Tractography in controls and cases. Sagittal (A) and dorsal (B) views in normal dogs showing normal fiber density and distribution. C-F. Tractography in dogs with SCI showing varying degrees of fiber thinning and disruption. Sagittal (C) and dorsal (D) views showing moderate fiber thinning. Sagittal (E) and dorsal (F) views showing absence of translesional fibers in a dog secondary to intervertebral disc herniation. Blue color corresponds to cranial-caudal fiber direction; Cranial is to the left and caudal to the right.

Using overall ROI for controls (combined cranial, middle and caudal ROI), FA was significantly higher in controls compared to each ROI in cases ($p_a < 0.0001$) (Figure 2A). MD was significantly lower in controls compared to cranial and lesion epicenter ROI in cases ($p_a = 0.001$, $p_a < 0.0001$, respectively) (Figure 2B). MD in controls was also lower than MD for caudal ROI in cases but the difference was not significant ($p_a = 0.237$).

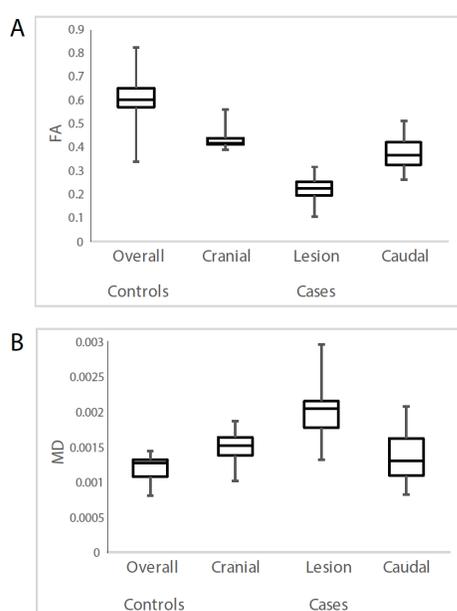


Figure 2. FA and MD values compared between controls and cases. A: Overall FA in controls was significantly different from FA for each ROI in cases ($p_a < 0.0001$). In cases, FA for cranial ROI and FA for caudal ROI were each significantly different from FA for lesion ROI ($p_a < 0.0001$). FA for cranial ROI was significantly different from FA for caudal ROI ($p_a = 0.008$). B: Overall MD in controls was significantly different from cranial ROI and lesion ROI in cases ($p_a = 0.001$, $p_a < 0.0001$). Overall MD in controls was not significantly different from MD for caudal ROI in cases ($p_a = 0.237$). In cases, MD for cranial ROI and MD for caudal ROI were each significantly different from MD for lesion ROI ($p_a < 0.0001$). MD for cranial ROI was not significantly different from MD for caudal ROI ($p_a = 0.202$). FA: fractional anisotropy, MD: mean diffusivity, ROI: region of interest.

Age and duration of injury were variably associated with the FA and MD calculations and, thus, were included in the model when investigating associations between DTI variables and

motor function. OFS was higher for dogs with higher FA values at the lesion epicenter but this association was no longer significant once corrected for multiple comparisons ($p=0.0387$, $p_a=0.116$) (Figure 3A). The presence of trans-lesional fibers, as demonstrated by tractography, was associated with a higher OFS ($p=0.0299$) (Figure 3B).

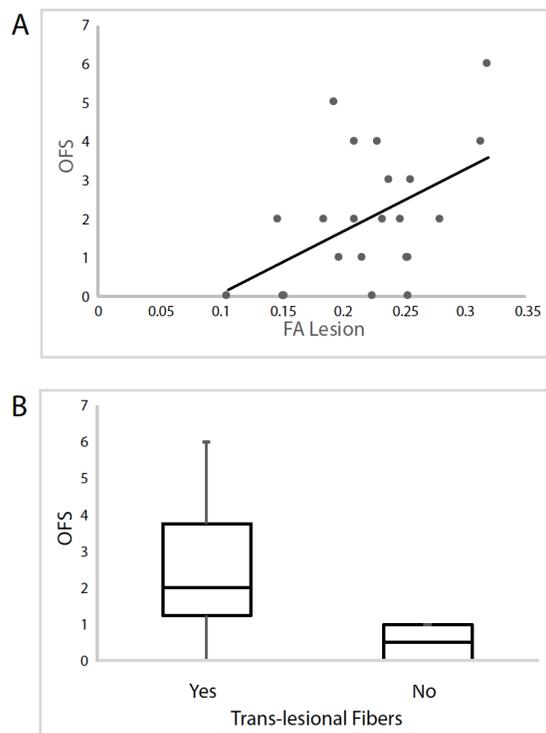


Figure 3. Relationship between imaging findings and gait scores. A: FA for lesion ROI was associated with OFS ($p=0.0212$, $p_a=0.127$). B: Presence of trans-lesional fibers was associated with OFS ($p=0.0299$). FA: fractional anisotropy, ROI: region of interest, OFS: open field score.

Discussion

We established FA and MD values at the lesion epicenter in dogs with chronic SCI and demonstrated significant differences compared to neurologically normal dogs. Furthermore, values calculated on areas cranial and caudal to the lesion that appeared normal on T2W images were also abnormal relative to the control population. Tractography depicted varying degrees of fiber disruption at the lesion epicenter including apparent physical transection in a subset. These findings suggest that DTI can capture microstructural changes such as Wallerian degeneration distant from the injury site not evident on conventional MRI and identify complete structural compromise at the epicenter in dogs with severe SCI. FA at the lesion and the presence of trans-lesional fibers were associated with hind limb motor function. This suggests that DTI can not only establish a continuum of lesion severity and quantify lesion characteristics but also improve our understanding of motor recovery in dogs following severe thoracolumbar SCI, enhancing their utility as a model of chronic paralysis.

MRI has become standard in the assessment of the injured spinal cord and is invaluable to achieving an accurate diagnosis. However, conventional MRI has limited sensitivity to detect more subtle changes that occur within the spinal cord after injury. DTI provides microstructural information and has been shown to enhance detection of pathology in the spinal cord in regions that appear normal using standard sequences in people with cervical spondylotic myelopathy and experimental rodent SCI models.^{15,16,30-33} Our results are consistent with this showing abnormal DTI values relative to control dogs in normal-appearing areas of the spinal cord above and below the apparent lesion on T2W images. While our findings showed decreased FA and increased MD within and beyond the visible lesion, prior studies in dogs with SCI have found variable increases, decreases or indistinguishable changes in these indices relative to control values.^{22,24,25} Direct comparisons are challenging, however, as the majority of dogs previously reported were evaluated at the time of an acute SCI in which the pathologic changes (i.e. edema,

hemorrhage, inflammation, etc) are distinct from those that characterize the chronic injury setting. Since these were pet dogs who survived their SCI, obtaining tissue samples from the regions of spinal cord that looked normal on T2W images was not possible, and determining the precise histologic correlate for specific DTI changes is challenging.¹¹ However, quantitative indices have been previously confirmed in experimental models to reflect a variety of histopathologic changes in the chronic injury setting including Wallerian degeneration and demyelination supporting a similar explanation for the FA and MD changes noted cranial and caudal to the visible lesion in these dogs.^{11,33-37} The ability of DTI to capture the degenerative changes and axonal loss that occurs in areas distant from the lesion epicenter, offers a sensitive way to evaluate the effects of severe injury and avoids the complex pathologic changes at the lesion epicenter commonly noted on conventional MR images that can obscure the ability to determine axonal integrity.

Tractography, which leverages the microstructural information provided by quantitative analysis to produce a visual depiction of spinal cord white matter tracts, showed varying degrees of fiber thinning and deviation from the normal cranial to caudal orientation through the area of injury in all dogs.^{12,13,16,31} Four dogs had an absence of any trans-lesional fibers, consistent with prior reports that physical spinal cord transections are rare.³⁸⁻⁴⁰ Since all 22 dogs were judged to be clinically complete based on loss of pain perception and had severe lesions evident on conventional MRI, tractography might be useful to identify cases with spinal cord transection. While 2 of the 4 dogs suffered severe luxation, the other 2 had intervertebral disc herniation suggesting that transection is possible secondary to etiologies other than trauma. Although this was not confirmed histologically, identification of physical transection using DTI along with quantification of degenerative changes improves the characterization of the spectrum of injury severity that occurs amongst dogs with naturally occurring, severe SCI and underscores the overlap between canine and human SCI.

FA at the lesion epicenter and the presence of trans-lesional connections depicted via tractography were each positively associated with OFS although the relationship between FA and gait was no longer significant after correction for multiple comparisons. Similar reports in people have shown that FA and other DTI parameters correlated with function, suggesting that quantitative analysis, particularly FA, might provide clinically relevant information on motor function in dogs with SCI.^{11,14,17,18} While we did not establish cutoff values for FA or MD (or explore the utility of other DTI indices) that could predict motor function, DTI along with other newer MRI applications (e.g. functional MRI) may produce useful non-invasive injury biomarkers capable of relating structural severity to function.^{11,14,17} Furthermore, for dogs with no trans-lesional fibers, taken to mean there was a spinal cord transection, the association with worse gait scores implies that at least some supraspinal influence might play a role in functional motor recovery in dogs after severe, spontaneous SCI. This is supported by reports in people and animal models on the role of residual supraspinal input on regaining locomotion after injury.^{41,42}

Historically, dogs who regain ambulation but not pain perception after clinically complete SCI are labeled as ‘spinal walkers’ exhibiting purely reflexive movement generated by intraspinal circuitry contained below the level of injury. Our findings suggest that interplay between residual supraspinal influence and the intraspinal circuitry that produces reflexive stepping might be important.⁴³⁻⁴⁶ However, the identification of trans-lesional connections on imaging does not differentiate between functional and non-functional axons. It is also unclear how many intact axons are needed to interact with intraspinal motor circuitry to result in meaningful function below the level of injury. We have previously shown that the presence of trans-lesional motor conduction in a similar population of dogs was associated with hind limb motor function.⁴⁷ This offers additional support that functional connections delivering supraspinal input are present in some dogs with severe lesions and likely contribute to regaining independent ambulation after injury. DTI complements other means of evaluation such as electrodiagnostic testing in dogs with incomplete recovery after severe SCI. Further

investigation of the relationship between residual structure and function focused on development of DTI indices as non-invasive biomarkers of motor recovery has important applications for both canine and human SCI.

Overall, we demonstrated the utility of quantitative and qualitative DTI analyses to better characterize the spectrum of lesion severity, specifically the extent of spinal cord degeneration distant from the lesion and the structural continuity at the epicenter, in dogs with chronic impairment after severe SCI. These findings emphasize the lesion complexity within this population and highlight the similarities between dogs with naturally occurring SCI and humans with SCI. Our results also provide clues regarding the association between motor recovery and structural spinal cord integrity and might allow identification of subsets amongst severely injured individuals with differing potential for recovery or differing ability to respond to certain interventions. This has implications for clinical trial design and supports the use of the chronically paralyzed dogs to investigate potential therapies for chronically paralyzed humans.

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ADDENDUM TO DTI CHAPTER
Investigating associations between spasticity, electrodiagnostic and imaging variables

In addition to the data presented in the preceding chapters, the following addendum outlines relationships between spasticity, electrodiagnostic and imaging variables. Continuous variables (CSS Overall score, H:M ratio, H-reflex threshold, MEP latency, MEP conduction velocity, FA and MD values) were compared using logistic regression. Presence or absence of MEPs and presence or absence of trans-lesional fibers were compared to continuous spasticity and imaging variables with a Wilcoxon rank sum test. Presence or absence of MEPs was compared to presence or absence of trans-lesional fibers by constructing a contingency table and performing a Fisher's exact test. $P < 0.05$ was considered significant.

Associations between spasticity and electrodiagnostic variables: No relationship was identified between H:M ratio and CSS Overall score ($p=0.7584$) but H-reflex threshold intensity was inversely associated with CSS Overall score ($p=0.0337$). The presence of hind limb MEPs was also associated with CSS Overall score ($p=0.0038$).

Associations between spasticity and imaging variables: CSS Overall score was inversely associated with FA cranial to the lesion ($p=0.006$) and FA caudal to the lesion ($p=0.0006$) but not FA at the lesion epicenter ($p=0.6594$). CSS Overall score was also positively associated with MD cranial to the lesion ($p=0.0173$) but not MD at the lesion epicenter or caudally ($p=0.1172, 0.7573$, respectively). No relationship was identified between CSS Overall score and the presence of trans-lesional fibers ($p=0.6668$).

Associations between electrodiagnostic and imaging variables: No relationships were identified between H-reflex threshold intensity and FA, MD or the presence of trans-lesional fibers ($p > 0.05$). The presence of MEPs were also not associated with FA, MD or the presence of trans-lesional fibers in this population, and no relationships were identified between MEP

latency or conduction velocity and FA or MD ($p>0.05$). Only 3 dogs with intact hind limb MEPs underwent DTI but all 3 had trans-lesional connections on tractography.

Conclusions: Improved understanding of the relationships between spasticity, electrodiagnostic and imaging parameters in dogs with chronic impairment after severe, acute SCI offers insight into lesion complexity and injury severity.

Our findings suggest that H-reflex threshold intensity might provide a more reliable measure of spasticity severity than H:M ratio, which was quite variable in our canine population. While an increased H:M ratio has been reported in spastic humans with SCI compared to healthy controls, there can be overlap between the groups, as was noted in our testing.¹⁻³ Additionally, electrodiagnostic and clinical measures for evaluating spasticity in humans with SCI have shown limited correlations with each other.^{2,3} This likely reflects the complexity of the syndrome of spasticity and the inability of one test to comprehensively evaluate it. Continued evaluation of the H-reflex and CSS in dogs with SCI is warranted to determine if the combination of clinical and electrodiagnostic evaluation can provide useful, reliable information on motor neuron pool excitability and the clinical manifestation of spasticity in this population.

The positive association between trans-lesional motor conduction and spasticity severity might appear contradictory since disruption of supraspinal influence is considered an important component in the development of spasticity in humans after SCI.⁴ However, disrupted influence is not synonymous with absent input and spasticity is not limited to patients with complete injuries. In fact, problematic spasticity was found to be significantly associated with incomplete SCI.⁵ The lack of association between CSS overall score (or H-reflex threshold intensity) and the presence or absence of trans-lesional fibers offers additional support that spasticity can develop in dogs with physically complete or incomplete SCI. In either case, the relationship between the structural severity of the injury and

development of spasticity is complex. Based on our finding of lower FA and higher MD distant from the lesion, it is possible that more extensive axonal loss and degeneration after injury results in more severe spasticity in this population. However, H-reflex threshold intensity, which we contend is a measure of motor neuron pool excitability and was associated with spasticity severity, was not associated with FA or MD. These results do highlight the potential ability of DTI to quantify functionally relevant microstructural changes distant from the lesion epicenter and characterize changes to the neuraxis more broadly after severe injury. Further evaluation of the inter-relationship between residual supraspinal connections and reorganized intraspinal circuitry after injury (including motor neuron pool excitability) and how this impacts functional status and the manifestation of spasticity are warranted.

DTI indices have been shown to relate to functional status and outcome in experimental SCI and humans with chronic SCI.^{6,7} We also speculated that intact motor conduction across the site of injury (demonstrated electrophysiologically) would be associated with evidence of structural connections traversing the site of injury (demonstrated by DTI tractography). While we were unable to confirm this structural-functional correlate, we evaluated a relatively small number of dogs including only 3 with recordable hind limb MEPs and DTI. Continued evaluation of both structural and functional modalities in dogs will be important for enhancing our understanding of injury severity and for ongoing development as robust outcome measures in dogs with chronic impairment after severe, acute SCI.

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CHAPTER 7

Predictors of response to 4-aminopyridine in a population of chronically paralyzed dogs

Lewis MJ, Laber E, Williams K, Olby NJ

Abstract

4-aminopyridine (4AP), a potassium channel antagonist, can improve hind limb motor function in dogs with chronic thoracolumbar spinal cord injury (SCI) but benefits are variable between individuals. We hypothesized that injury characteristics would be able to differentiate between dogs that do and do not respond to 4AP. Our objective was to compare clinical, electrodiagnostic, gait and imaging variables between dogs that do and do not respond to 4AP to identify predictors of response.

Thirty-four dogs with permanent deficits after acute thoracolumbar SCI were enrolled. Spasticity, motor and sensory evoked potentials (MEPs, SSEPs), H-reflex, F-waves, gait scores and lesion characteristics on magnetic resonance imaging were evaluated at baseline and after 4AP administration. Baseline variables were assessed as predictors of response; response was defined as ≥ 1 change in open field gait score. Variables were also compared between pre- and post-4AP to evaluate 4AP effects.

Fifteen of 33 (45%) dogs were responders, 18/33 (55%) were non-responders and 1 was eliminated due to adverse event. Pre-H-reflex threshold $< 1.2\text{mA}$ was predictive of non-response (11 non-responders, 2 responders); pre-H-reflex threshold $> 1.2\text{mA}$ and pre-F-wave latency $< 13\text{ms}$ were predictive of response (0 non-responders, 7 responders). All responders had trans-lesional connections. MEPs were more common post-4AP versus pre-4AP (10 versus 6 dogs), but no significant differences were identified amongst any variables evaluated.

4AP improved central conduction and suggested electrodiagnostic evaluation might predict a favorable response. Further exploration in more dogs might elucidate additional factors that contribute to response and improve understanding of injury complexity in dogs with chronic SCI.

Introduction

Severe spinal cord injury (SCI) in humans and dogs commonly results in permanent functional impairment. While injuries classified functionally as complete (no pain perception or motor below the level of injury) presume disconnection from all supraspinal influence, physical transection of the spinal cord is uncommon.¹⁻³ Preserved rims of tissue, typically subpial in location, have been demonstrated traversing the site of severe injury on histopathology in humans and animals.¹⁻⁶ Evidence of spinal cord continuity on MRI amongst humans with complete injuries has also been noted.⁷ We and others have demonstrated motor conduction across the injury and presented evidence of structural continuity using diffusion tensor imaging (DTI) in some chronically paralyzed dogs (Chapter 4).^{8,9} While motor evoked potentials (MEPs) were recordable in a subset of these SCI dogs, amplitude was small and latencies notably delayed compared to normal values. These data suggest that a percentage of axons can survive severe injury but might be rendered dysfunctional due to demyelination preventing conduction of the action potential.

4-aminopyridine (4AP), a potassium channel antagonist, has been evaluated as a treatment strategy for SCI specifically aimed at restoring function to anatomically intact but physiologically dysfunctional axons across the injury site. 4AP primarily exerts its effect by blocking exposed fast-gated potassium channels in demyelinated axons although it also has other mechanisms of action including increasing synaptic transmission at the pre-synaptic level.¹⁰⁻¹³ Prior studies in humans with SCI have shown 4AP can improve electrodiagnostic measures of central conduction including higher amplitude, lower stimulation threshold and shorter latency MEPs.¹⁴⁻¹⁶ However, functional benefits at clinically safe doses of 4AP or its derivatives have generally been modest and appear more prominent in people with incomplete injuries (Hansebout, Qiao, Cardenas 2014, Hayes 1993, Hayes 1994, Potter).^{14,15,17-20} Targeting patients with complete injuries in whom there is MRI evidence of cord continuity with higher doses resulted in some functional improvements, but the benefits

must be balanced with the greater likelihood of side effects as the dose increases.⁷ Blight et al., reported similar effects in chronically weak or paralyzed dogs after naturally occurring SCI in which approximately two thirds showed varying degrees of functional improvement after a single dose.⁵ However, all but one dog with no pain perception at the time of evaluation showed no overt change in neurologic function after administration of 4AP.⁵ In a placebo controlled cross over clinical trial 19 chronically non-ambulatory dogs, administration of 4AP and a derivative, T-Butyl Carbamate, resulted in an improvement in stepping function compared to placebo, but the benefit was very variable between individuals ranging from no response to restoration of independent ambulation.²¹ Wide variability in 4AP concentration in urinary excretion profiles between individuals has also been demonstrated in humans with SCI.¹⁹

Despite promise as a potential therapy for human SCI, disappointing large-scale clinical trial results, narrow therapeutic window and variable response between individuals have limited the widespread use of 4AP.^{18,21} It has been assumed that injuries of comparable severity using clinical measures of function have the same spinal cord pathology whereas a range of lesion characteristics and subtler functional distinctions are more likely. We hypothesized that there would be significant differences in one or more of clinical, electrodiagnostic, gait and imaging variables between dogs that respond to 4AP and dogs that show no response. Our objective was to compare spasticity severity, trans-lesional motor and sensory conduction, motor neuron pool excitability, gait scores and lesion characteristics on conventional MRI and DTI between dogs that do and do not respond to 4AP amongst a population with chronic impairment after acute, functionally complete SCI. The ability to predict responders using baseline characteristics will improve our understanding of injury complexity and its relationship to functional status in naturally occurring SCI in dogs. This will not only allow tailored therapy regimens but will facilitate targeted clinical trial recruitment amongst this heterogeneous population with direct application to humans with SCI.

Materials & Methods

Case Selection: Dogs were recruited prospectively from the patient pool of the Canine Spinal Cord Injury Program at the North Carolina State University College of Veterinary Medicine and via trial advertisement online (<https://cvm.ncsu.edu/research/labs/clinical-sciences/canine-spinal-cord-injury/>, www.dodgerslist.com). To be included, dogs must have suffered an acute, clinically complete (hind limb paralysis with loss of pain perception) thoracolumbar SCI and demonstrated an incomplete recovery at least three months following injury characterized by chronic motor deficits and severely reduced to absent hind limb and tail pain perception (with or without urinary and fecal incontinence). Dogs were excluded who had concurrent health issues precluding general anesthesia or sedation for procedures as well as dogs with seizure disorders because 4AP can exacerbate seizures. For this study, dogs underwent baseline (pre-4AP) and post-4AP evaluation of spasticity, gait, long tract and local circuitry function using electrodiagnostics and structural lesion severity using magnetic resonance imaging (MRI). Please refer to prior chapters on spasticity, advanced gait, electrodiagnostic, imaging analyses in this population for additional, more detailed inclusion and exclusion criteria and description of specific procedures. Brief explanation of the procedures performed are outlined below. Informed consent was obtained for all animals and examinations were conducted in accordance with the NCSU Institutional Animal Care and Use Committee (protocol #15-004-01).

4AP protocol: Dogs were admitted to the hospital at the North Carolina State University College of Veterinary Medicine for baseline (pre-4AP) procedures, drug titration and then repeat testing of the same procedures post-administration of 4AP. The targeted dose for this study was determined based on prior evaluation of 4AP in chronically non-ambulatory dogs.²¹ That work showed that a dose range of 0.5-0.75mg/kg was likely to be safe for the majority of dogs. Compounded 4AP oral capsules were specifically prepared for this study and included 1mg, 2.5mg, 5mg and 10mg sizes. Using each dog's current body weight, the

appropriate combination of capsules was used to achieve a dose of approximately 0.5mg/kg given orally. If that dose was well-tolerated with no apparent side effects, each dog received a second dose of approximately 0.75mg/kg orally at least 8 hours after the initial dose. If a dog displayed mild drug-related anxiety at the higher dose, the lower dose was used for the remainder of the study. If more severe side effects were noted, the drug was withdrawn and the dog did not participate in post-4AP testing. The maximum tolerated dose (up to approximately 0.75mg/kg) was then utilized to determine if a dog was a responder to 4AP and for repeat testing of baseline procedures after administration of 4AP (at least 8-12 hours after prior drug administration). A responder was defined as a dog showing an improvement in motor function quantified as an increase in open field scale (OFS) of ≥ 1 point between baseline and repeat testing. On the day of repeat testing, each dog was administered the maximum tolerated dose of 4AP and an hour later all baseline procedures were repeated using the same protocols except MRI and advanced gait analysis. MRI was only performed at baseline. Due to the location and logistics of testing, instrumented treadmill analysis was performed on a separate day from the rest of the procedures. Baseline (pre-4AP) instrumented treadmill data was acquired followed by administration of the same maximum tolerated dose previously established for each dog. One hour post-4AP administration, treadmill data was acquired using the same recording protocol. Dogs were under the supervision of one of the investigators or licensed veterinary technicians throughout hospitalization for the study and monitored for adverse drug reactions or other adverse events. Seizure activity, if it occurred, was treated with 0.25mg/kg midazolam IV, repeated if necessary, and the 4AP was withdrawn. Excessive anxiety was treated with drug withdrawal and 0.25mg/kg midazolam or other sedative (such as butorphanol) at the discretion of study investigators. Any other adverse events were recorded and treated accordingly at the discretion of investigators.

Standard neurologic and gait evaluation: All dogs underwent a neurologic examination including standard evaluation of gait, proprioception, spinal reflexes and pain perception.

Dogs were also walked on a non-slip surface and on a treadmill for approximately 3 minutes with the speed adjusted to a comfortable pace for each individual. Sling support was provided for the hind limbs as needed. All gait examinations were videotaped including footage with and without sling support. Gait was categorized as ambulatory (able to take at least 10 consecutive weight bearing steps unassisted), or not and quantified using an ordinal, open field scale (OFS) that ranges from 0-12.^{22,23} Treadmill footage was scored using previously described measures of pelvic limb stepping (stepping score, SS) and coordination (regularity index, RI).^{24,25} Gait scores (OFS, SS, and RI) generated without sling support were utilized for the purposes of this project unless otherwise specified.

Spasticity evaluation: Spasticity testing was performed in all dogs using the Canine Spasticity Scale (CSS) adapted from human clinical scales (Chapter 2).²⁶⁻²⁹ The CSS is composed of the assessment of patellar clonus duration and flexor spasm duration on each hind limb with a standard scoring system developed for each component. Tests were repeated 3 times (ie, 3 trials) on each hind limb, with at least 30 seconds separating trials. Ordinal scores (0 to 3) were assigned for each hind limb for each scale component (patellar clonus duration, flexor spasm duration, flexor spasm degree) which were then summed to give an overall CSS score representing all components and both hind limbs (0 to 18).

Instrumented treadmill gait evaluation: A subset of dogs underwent novel gait analysis using instrumented, force-plate treadmill (Fully Instrumented Treadmill (FIT), Bertec Corporation, Columbus, OH). To participate in this portion, dogs had to be between 3-30kg and amenable to walking on the treadmill with only verbal encouragement to ensure accurate data capture. Dogs were outfitted with reflective markers including an interscapular marker utilized for center of pressure (COP) measurements assessed in the x (lateral) and y (craniocaudal) directions. For non-ambulatory dogs, a standard sling (Walk-a-bout) provided hind quarter support and was attached to a load cell to capture the percentage body weight support provided during testing (support percentage). Dogs walked at a steady, comfortable pace

until at least 5 trials of adequate quality were recorded. Data for each trial in each dog were collected as .c3d files, which were converted to .txt files (Visual 3D Software, C-Motion) and imported into MATLAB (MATLAB Software, Mathworks) for analysis. Mean COP as well as the root mean square of the COP in both the x and y directions (COPx, COPy, RMS_COPx, RMS_COPy) and support percentage were calculated for each trial in each dog.

Electrodiagnostic evaluation: Long tract function was evaluated via transcranial magnetic stimulation (TMS) and recording of motor evoked potentials (MEPs) and cortical somatosensory evoked potentials (SSEPs) and local reflex circuitry was assessed by H-reflex, F-waves and M-waves (Chapter 4).⁹ All procedures were performed under sedation (dexmedetomidine and butorphanol) in lateral recumbency (left side tested) in a darkened, quiet, shielded room. TMS was performed using a Magstim 200² magnetic stimulator (The Magstim Company Limited, Version 1.9, Spring Gardens, United Kingdom) with MEPs recorded from the left extensor carpi radialis muscle in the thoracic limb (positive control) and the left cranial tibial muscle in the pelvic limb according to standard protocol.³⁰ Stimulation was repeated 4 times at supramaximal stimulus for each limb and the presence (yes/no) and minimum latency of MEPs were recorded with the conduction velocity calculated from the latency. SSEPs were recorded following electrical stimulation of the tibial nerve for the pelvic limb and ulnar nerve for the thoracic limb (positive control) as well as recording of cord dorsum potentials (positive control) according to standard protocol.^{31,32} The presence (yes/no) and minimum latency of SSEPs and cord dorsum potentials were recorded. M-waves, F-waves and H-reflexes were generated for sciatic/tibial nerve according to standard protocol.³³⁻³⁸ The minimum latency of the F-waves, F-wave persistence (percentage of F-waves present in 10 stimulations) and the F-ratio $[(\text{latency F} - \text{latency M} - 1) / 2 \times \text{latency M}]$ were recorded. The H-reflex threshold (stimulus intensity at which H-reflex first appeared), the minimum H-reflex latency and the maximum H-reflex amplitude and maximum M-wave amplitude (defined as the largest negative to the largest positive peak

for each waveform) were each recorded during H-reflex testing and used to calculate the H:M ratio (maximum H amplitude/maximum M amplitude).

Imaging acquisition and analysis: A subset of dogs underwent thoracolumbar MRI with acquisition of standard transverse and sagittal sequences (T1W pre- and post-contrast, T2W, STIR, half-Fourier acquisition single-shot turbo spin-echo (HASTE), +/- proton density and GRE/T2*). DTI was obtained for the same region acquired in the transverse plane using a protocol adapted from Jones et al 2002 with 35 diffusion directions with a scan time of approximately 5 minutes.³⁹ T2-weighted images were used to identify the lesion and to measure lesion length defined as length of abnormal signal intensity. Post-processed DTI images were imported into Mango (<http://ric.uthscsa.edu/mango/>) in order to manually outline regions of interest (ROI) within the spinal cord above, below and within the lesion epicenter. FA and MD were calculated for each ROI constructed (cranial, lesion epicenter, caudal). Tensor maps displaying the orientation of tensors across all voxels were used to perform tractography using TrackVis (<http://trackvis.org>) Tractography was assessed visually for continuity of the represented white matter tracts across the lesion.

Statistical analysis: All analyses were performed using R (Version 3.3.0, <http://cran.r-project.org/>) and Jmp 12 Pro (SAS Institute, Cary, NC, USA). Summary statistics for baseline variables and relationships between baseline variables are presented in prior chapters. Being a responder to 4AP was defined as having a 1+ improvement in OFS between pre- and post-4AP administration whereas non-responders were defined as showing no positive change. The following variables at baseline were assessed as predictors of response to 4AP: age, duration of injury, SS, RI, overall CSS score, F latency, F ratio, H-reflex latency, H-reflex threshold, H:M ratio, lesion length on T2W images, FA for each ROI, MD for each ROI, and the presence of trans-lesional fibers. We fit a classification tree for the responder status given baseline patient characteristics and models were fit using R.⁴⁰ There were too few dogs with hind limb MEPs present at baseline to include in the model.

Pairwise analysis (ANOVA or Wilcoxon rank sum test, as indicated, in Jmp or R) was also performed to directly compare responders and non-responders for each continuous variable (those outlined above as well as body weight, limb length, hind limb MEP latency, HL MEP conduction velocity, RMS_COPx, RMS_COPy and support percentage). The presence of HL MEPs and presence of trans-lesional fibers were each investigated between responders and non-responders by constructing a contingency table and using Fisher's exact test.

Additionally, in order to determine the effect of 4AP, the following continuous variables were compared between baseline and post-4AP administration using a Wilcoxon rank sum test or one-way ANOVA as indicated: SS, RI, support percentage, mean COPx, mean COPy, RMS of COPx, RMS of COPy, overall CSS score, F ratio, H-reflex latency, H-reflex threshold and H:M ratio. Presence of MEPs were compared between baseline and post-4AP administration by constructing a contingency table and using Fisher's exact test. $P < 0.05$ was considered significant and the Holm-Bonferroni method was used to calculate adjusted p-values (p_a) for multiple comparisons.

Results

Thirty-four dogs with SCI were enrolled. There were 14 Dachshunds, 10 mixed breed dogs, 3 Pit bull terriers, 2 Australian cattle dogs and 1 each of Shih Tzu, Boston Terrier, Miniature Poodle, English Bulldog and Miniature Schnauzer. Median body weight was 7.65 kg (range 3.1-33). The mean age was 5.97 years (SD 2.55) and median duration of injury was 17 months (range 3-84). Intervertebral disc herniation was the most common diagnosis (26 dogs) followed by vertebral column fracture (4 dogs), fibrocartilaginous embolism (2 dogs), and traumatic intervertebral disc extrusion (2 dogs). In all dogs, neurolocalization was between the third thoracic and third lumbar spinal cord segments based on neurologic exam findings. Thirty-two dogs had no pelvic limb or tail pain perception while 1 dog had a severely blunted response in the medial and lateral toes of the left hind limb and one dog had

a subtle response in the medial toe of the left hind limb. The median OFS for all dogs at enrollment was 2 (range 0-9), the median SS was 0 (0-89) and median RI was 0 (0-46.56). Seven of 34 (21%) dogs were independently ambulatory and 27/34 (79%) were non-ambulatory including 4 dogs who took some weight bearing steps (OFS = 4). Treatments at the time of acute injury were variable and depended on underlying cause. Amongst dogs with a diagnosis of intervertebral disc herniation or fracture/luxation, surgery (decompression +/- stabilization) was performed in 16 dogs and medical management with or without formal rehabilitation therapy was performed in 14 dogs.

All dogs participated in spasticity and electrodiagnostic evaluation. One dog was removed from pre- and post-4AP electrodiagnostic analysis of local circuitry (H-reflex, F-waves) due to prior distal limb self-mutilation precluding reliable data capture. One additional dog did not undergo any repeat testing due to an adverse event and was eliminated from responder/non-responder analysis. Thirteen of the 34 dogs participated in instrumented treadmill analysis (pre- and post-4AP) and 22 dogs underwent MRI (at baseline). Fifteen of 33 (45%) dogs were responders and 18/33 (55%) were non-responders.

Mean drug dosage used for post-4AP testing was 0.78mg/kg (SD 0.05). All dogs received at least 3 doses of 4AP (1 at ~0.5mg/kg and 2 at ~0.75mg/kg) during the study, each separated by at least 8-12 hours. Dogs who participated in instrumented treadmill analysis also received an additional dose at ~0.75mg/kg separated by at least 24 hours from the previous dose. Adverse events were noted in 7 dogs consisting of seizure activity (1 dog), anxiety (5 dogs) and suspected burns (1 dog). The mean 4AP dose amongst the 6 dogs with possible drug-related adverse effects was 0.79mg/kg. One dog had a generalized seizure after receiving the second of the higher doses of 4AP (0.77mg/kg). This resolved with 0.25mg/kg IV midazolam and drug withdrawal and the patient had no further seizure activity or drug-related effects. This dog did not participate in any post-4AP testing. Five dogs demonstrated anxiety potentially attributable to the study drug. In 3 dogs, this was mild and did not require

treatment or adjustments to the dosing regimen. One dog exhibited a mild episode of anxiety 1-2 hours after receiving the higher dose (0.8mg/kg). No treatment was initiated but the next dose was lowered to 0.7mg/kg and was well-tolerated for repeat testing. The dog remained on that dose after completion of the study with no anxiety or other side effects reported at home. One additional dog demonstrated increasing anxiety after receiving the higher doses of the study drug (0.77mg/kg). This patient was able to complete post-4AP testing but was treated with 0.25mg/kg IV midazolam and 0.2mg/kg IV butorphanol upon study completion due to progressive anxiety. While 4AP was considered a factor, this dog also demonstrated moderate behavioral anxiety at baseline likely attributable to separation from her owner and hospitalization. Excessive anxiety resolved with treatment and drug withdrawal and no further issues were reported by her owner. The medication was otherwise well tolerated in remaining dogs. One dog suffered suspected thermal burns along both flanks secondary to heat support provided during MRI acquisition. These resolved with supportive therapy and pain medications.

A summary of baseline spasticity, electrodiagnostic, instrumented treadmill and imaging variables are presented in prior chapters. No dogs demonstrated changes in their hind limb or tail pain perception after drug administration. Comparison of variables between responders (n=15) and non-responders (n=18) is outlined in Table 1. The classification tree model found that a baseline H-reflex threshold <1.2mA was predictive of being a non-responder (11 non-responders, 2 responders), and that H-reflex threshold >1.2mA combined with a baseline minimum F-wave latency <13ms were together predictive of being a responder (0 non-responders, 7 responders). No other baseline variables (or combinations of variables) evaluated were found to be predictive of response to 4AP (presence or absence of MEPs excluded due to small numbers). Two of 15 (13%) responders had detectable hind limb MEPs compared to 4/18 (22%) non-responders (p=0.8672). All responders (15/15, 100%) had trans-lesional fibers whereas all 4 dogs with absent connections were in the non-responder group, resulting in 14/18 (78%) non-responders with intact trans-lesional fibers

($p=0.035$). Pairwise comparison of variables between baseline and post-4AP across all dogs is outlined in Table 2. Detectable MEPs were more common post-4AP versus pre-4AP, present in 10 dogs versus 6 at baseline. No significant differences were identified amongst any of the variables evaluated.

Table 1. Comparison of clinical, spasticity, electrodiagnostic, gait and imaging variables between responders and non-responders to 4AP. DOI: duration of injury, CSS: Canine Spasticity Scale, HL: hind limb, MEP: motor evoked potential, CV: conduction velocity, SS: stepping score, RI: regularity index, RMS_COP: Root mean square of the Center of Pressure in 'x' (right to left) or 'y' (craniocaudal) directions, FA: fractional anisotropy, MD: mean diffusivity. $P<0.05$ significant.

Baseline Variable	Mean (SD) or Median (Range)		p-value
	Responders	Non-responders	
Age (years)	5.5 (2.6)	6.26 (2.49)	0.49
DOI (months)	17 (3-69)	18 (3-84)	0.46
Body weight (kg)	7.1 (3.7-33)	8 (3.1-29)	0.81
Limb length (cm)	25.2 (18.5-48)	26 (20.5-53)	0.9
CSS Overall Score	6.88 (2.6)	8.26 (2.4)	0.09
HL MEP Latency	51.8 (18.38)	54.12 (10.93)	0.85
HL MEP CV	15.05 (2.33)	13.41 (3.89)	0.58
F-wave latency	13.12 (3.8)	13.56 (3.37)	0.24
F ratio	1.79 (0.57)	1.97 (0.89)	0.44
H-reflex latency	13.02 (3.57)	13.35 (3.32)	0.62
H:M ratio	0.28 (0.2)	0.29 (0.17)	0.32
H-reflex threshold	2.1 (0.3-9.4)	1.1 (0.1-7.8)	0.29
OFS	2 (0-6)	2 (0-9)	0.77
SS Unsupported	0 (0-75)	0 (0-89)	0.41
RI Unsupported	0 (0-34.29)	0 (0-46.56)	0.21
RMS_COPx	0.022 (0.005)	0.028 (0.009)	0.24
RMS_COPy	0.026 (0.004)	0.028 (0.009)	0.58
Support percentage	24.32 (20.4-27.7)	13.08 (0-30.17)	0.25
Lesion length (cm)	7.7 (2.73-15.02)	4.6 (2.65-9.22)	0.074
FA Cranial	0.44 (0.039)	0.44 (0.055)	1
FA Lesion	0.23 (0.056)	0.22 (0.047)	0.46
FA Caudal	0.39 (0.073)	0.37 (0.06)	0.34
MD Cranial	0.0015 (0.0002)	0.0015 (0.0003)	0.79
MD Lesion	0.0021 (0.0003)	0.0020 (0.0004)	0.85
MD Caudal	0.0014 (0.0003)	0.0013 (0.0004)	0.7

Table 2. Comparison of spasticity, electrodiagnostic and gait analysis variables between baseline and post-4AP administration. 4AP: 4-aminopyridine, CSS: Canine Spasticity Scale, HL: hind limb, MEP: motor evoked potential, CV: conduction velocity, SS: stepping score, RI: regularity index, RMS_COP: Root mean square of the Center of Pressure in 'x' (right to left) or 'y' (front to back) directions. P<0.05 significant.

Variable	Mean (SD) or Median (Range)		P-value
	Pre-4AP	Post-4AP	
CSS Overall Score	7.56 (2.56)	8.3 (2.84)	0.2783
HL MEP Latency	53.35 (11.86)	57.16 (16.21)	0.4474
HL MEP CV	13.96 (2.95)	13.72 (4.26)	0.6642
F ratio	1.87 (0.765)	1.97 (0.85)	0.6203
H-reflex latency	13.3 (3.39)	13.04 (3.42)	0.8224
H:M ratio	0.273 (0.17)	0.228 (0.18)	0.2181
H-reflex threshold	1.9 (0.1-9.4)	1.3 (0.5-8)	0.4996
SS Unsupported	0 (0-89)	0 (0-90)	0.3678
RI Unsupported	0 (0-46.56)	0 (0-58.95)	0.9616
RMS_COPx	0.026 (0.008)	0.026 (0.011)	0.8576
RMS_COPy	0.027 (0.007)	0.031 (0.012)	0.5903
Support percentage	18.36 (11.19)	16.49 (10.63)	0.3805

Discussion

The results of this study suggest that a combination of electrodiagnostic variables might be helpful in identifying which dogs are more likely to respond to 4AP amongst a population that exhibit chronic impairment after suffering a prior acute, complete SCI. Specifically, a lower H-reflex threshold intensity was associated with non-response while the combination of a higher H-reflex threshold and shorter F-wave latency were predictive of being a responder. Hind limb MEPs were more common after 4AP administration and no dogs with absent trans-lesional fibers on tractography were responders suggesting that at least some degree of long tract integrity is integral to potential response. The functional benefit, even for responders, was modest and only 2 dogs regained the ability to ambulate independently. On the basis of these results, electrodiagnostic evaluation of local circuitry and long tract

integrity in a larger cohort may enhance our understanding of the mechanisms underlying the variable response and help to identify dogs in whom 4AP may be a useful therapy.

Electrodiagnostic evaluation at baseline, focusing on tests of motor neuron pool excitability, might be helpful in differentiating potential responders from non-responders. A lower H-reflex threshold was predictive of non-response while a higher threshold and shorter F-wave latency were together predictive of response. A lower H-reflex threshold in this population indicates increased motor neuron pool excitability so it is possible that these results demonstrate that drug-related effects of increasing central conduction and synaptic transmission have limited influence when the alpha motor neuron population is already in a state of greater excitability. A higher H-reflex threshold might imply the opposite response to the drug for a more inhibited motor neuron pool population. However, the explanation is likely more complex and might be confounded by our ability to detect a change in motor function amongst dogs with differing degrees of hind limb motor impairment using a non-linear ordinal scale. We have previously shown that a lower H-reflex threshold (i.e. increased motor neuron excitability) was associated with higher gait scores and our study population included 7 dogs who were independently ambulatory (but lacked sensation) at baseline (Chapter 4).⁹ Responders to 4AP generally had lower gait scores at baseline (median OFS 2 (0-6) vs 2 (0-9)) although this difference was not significant and was not predictive of response in our model. These findings might suggest that dogs with greater motor function were less likely to respond to 4AP and mirrors the findings of the previously reported clinical trial in which all participants had an OFS of 5 or less at entry into the trial.²¹ It is also possible that OFS changes between 0-3 were easier to detect (i.e. going from paraplegic to any movement of the limbs) than changes in scores of 4 and above (i.e. more nuanced changes in ambulation), implying that the association between higher H-reflex threshold and response to 4AP was due to difficulty in detecting an improvement in motor in higher functioning dogs rather than decreased motor neuron pool excitability. Further exploration

into this relationship might focus on a larger number of exclusively non-ambulatory dogs and utilize a combination of the ordinal OFS and treadmill-based SS and RI to define response.

The association between minimum F-wave latency and response to 4AP is also unclear and warrants further investigation, especially since the latency measurement is affected by limb length and is primarily determined by the peripheral nerve function with only a minor contribution at the level of the motor neurons in the spinal cord. One possible explanation is that dogs with shorter limbs were more likely to respond to 4AP since F latency but not F ratio, which corrects for limb length, was found to be predictive. While smaller dogs do tend to demonstrate improved motor recovery compared to larger dogs with severe SCI, their spontaneous recovery potential does not provide an explanation for a more favorable response to this drug. Limb length and body weight were not associated with response to 4AP when examined individually by univariate analysis suggesting size alone does not predict response. An alternate explanation is that the mechanism of action of 4AP is multifactorial including having effects on peripheral nerve function and neuromuscular transmission.¹⁰⁻¹³ However, M-waves in our dogs were all within the normal range supporting normal peripheral nerve function and H-reflex and F-waves have also previously been reported to remain unchanged after administration of 4AP in humans with SCI.^{15,16,19} While there has been no specific investigation of H-reflex or F-wave variables at baseline as predictors of response, our results suggest that the drug effect due to peripheral mechanisms is likely limited in this population. If anything, an indication of nerve dysfunction at baseline (i.e. a longer F-wave latency), might be the more expected scenario to predict a favorable effect of 4AP.

Using non-invasive electrodiagnostic testing methods of long tract function, the presence of hind limb MEPs was not associated with being a responder and no dogs demonstrated cortical SSEPs after hind limb stimulation, at baseline or after 4AP. There were also no consistent changes in MEP latency, conduction velocity or amplitude amongst dogs with

MEPs both pre- and post-4AP, although improvement in these variables has been previously reported with 4AP administration in humans with chronic SCI.¹⁴⁻¹⁶ However, the number of dogs with recordable hind limb MEPs increased from 6 at baseline to 10 after 4AP administration and no dogs with complete disruption of fibers on tractography were responders. These findings support enhanced central conduction as the main mechanism of action of the drug and imply that connections across the injury are necessary for the drug to exert a beneficial effect on function. It is possible that the presence of MEPs or SSEPs could be a predictor of response but determination of this was not possible given the small number of dogs which demonstrated trans-lesional conduction. However, only 1 of 4 dogs who had MEPs after 4AP not present at baseline was classified as a responder. This suggests that the drug might enhance central conduction without a notable change in hind limb motor function in chronically paralyzed dogs or be only one of multiple factors integral to a positive response in a given individual. Utilizing more invasive stimulation and recording methods from the level of the dura, which has been shown previously to increase sensitivity to detect sensory conduction in dogs, might identify additional dogs in whom trans-lesional conduction is present and help to answer this question.⁴¹

Similarly, tractography appeared useful for detecting the uncommon cases of physical spinal cord transection and suggested that imaging measures of structural lesion severity might provide useful information on likelihood of response. Cord continuity on T1W images in people with functionally complete lesions has previously been associated with a favorable response to 4AP.⁷ However, the ability of tractography or other DTI indices to accurately differentiate varying levels of incomplete fiber disruption remains unknown. Ongoing investigation in this population is warranted to determine if microstructural abnormalities on DTI provide useful information regarding response to 4AP.

The average dose received for the post-4AP testing was 0.79mg/kg. It is possible that further dose escalation might have resulted in classification of additional dogs as responders,

potentially uncovering predictors of response. However, doses exceeding 1mg/kg might have been associated with increased drug-related side effects and do not necessarily produce a predictable, progressive increase in function.^{5,21} The prior blinded trial in dogs also administered 4AP for two weeks while we only investigated the effects of a single dose limiting direct comparisons between these studies regarding the relationship between dose and response.²¹ While the drug was generally well-tolerated, 6/34 (20%) dogs exhibited side effects that could be attributed to the study medication. The mean 4AP dose amongst these dogs (0.79mg/kg) was comparable to the average dose for the entire population (0.79mg/kg). Specific treatment beyond drug withdrawal was needed in 2 dogs and 1 additional dog responded to dose reduction. This highlights the importance of choosing the appropriate dose with careful balance between the potential for side effects and the potential benefits, and that dose alone does not predict response in this population.

Consistent with prior reports in people with SCI, the functional benefit among responders was generally modest.^{14,15,17-20} In a blinded, placebo-controlled trial in chronically non-ambulatory dogs, the majority of whom (17/19) lacked hind limb and tail sensation, 4AP improved the primary outcome measures, OFS and treadmill-based gait scores.²¹ However, only 3/19 (16%) dogs regained the ability to walk independently reflecting a limited benefit in daily functioning for most dogs.²¹ While direct comparison is not possible due to differences in dosing regimen and the fact that 7 dogs in our study were independently ambulatory prior to 4AP, only 2/34 (6%) dogs improved from being non-ambulatory to ambulatory (an OFS increase of 1 in both dogs) amongst a similarly severely affected population. Additionally, the primary goal with this study was to investigate potential mechanistic reasons for a hind limb motor response or lack thereof to 4AP, rather than to confirm its variable functional benefits.

Spasticity has previously been suggested to improve with 4AP in humans with SCI although this trend could not be confirmed in a Phase 3 clinical trial of fampridine-SR (a sustained

release derivative of 4AP) in patients with chronic SCI.^{14,17,18,20} We did not find a similar reduction in spasticity between pre- and post-4AP administration. In fact, the overall CSS score went up slightly and the number of dogs categorized as having moderate to severe spasticity was unchanged. This might reflect differences in spasticity between the two species (e.g. the lower prevalence of extensor spasms in the dogs we tested compared to spastic people), differences in the manner of evaluating spasticity as well as differences in response to the drug between the two species.²⁷

While we investigated a variety of clinical, electrophysiological and imaging variables, it remains possible that there are other, as yet unidentified, predictors of response to 4AP in dogs with SCI. Limitations of this study included the small number of dogs and using a conservative definition for a responder (1+ increase in OFS). This may have decreased our ability to detect any potential differences between groups. Only 4 dogs demonstrated a more pronounced change in OFS (including OFS increase of 2 points in 2 dogs and 3 points in 1 dog) precluding the ability to analyze the data using OFS change ≥ 2 as the definition of being a responder. We also purposely chose a group of dogs with severe SCI but a range of impairment in order to explore potential predictors of response. As such, this study was not designed as a clinical trial for efficacy, but rather as an extension of prior work in dogs with the goal of identifying factors underlying the variable response to 4AP in this population. Future studies in a larger number of dogs with incomplete recovery focusing on electrodiagnostic evaluation at baseline might confirm these preliminary results and provide more insight into the mechanism of response to 4AP. Additionally, we only evaluated dogs at one time point after a single oral dose (3 total if titration is included) and, thus it is possible that benefits might have become apparent if dogs were re-evaluated on multiple occasions or after receiving a larger number of doses. Prior pharmacokinetic evaluation and studies in dogs and cats, however, have shown detectable effects within minutes after IV injection and within an hour after single oral dose with peak plasma levels in dogs occurring at 2-3 hours after administration.^{5,42,43}

Overall, this study demonstrated that 4AP improves central conduction in a population of dogs with chronic SCI and that electrodiagnostic evaluation might be useful to predict a favorable response. A simple relationship between patient or lesion characteristics and improvement in motor skills secondary to 4AP was not identified. It is possible that multiple factors contribute to response or non-response that will only become apparent through additional studies in a larger number of dogs. Ongoing work to better our understanding of injury features in dogs with chronic SCI might allow improved differentiation of responders from non-responders and provide insight into targets for manipulation with novel or combinations of therapeutic interventions with application for both canine and human SCI.

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CHAPTER 8

Conclusions, Limitations and Future directions

Conclusions

This work enhanced understanding of both structural and functional aspects of severe SCI in a naturally occurring canine model of chronic paralysis. Outcome measures were developed and refined to improve the ability to evaluate this population. Furthermore, it was demonstrated that injury severity is a continuum even amongst severely affected individuals who lack pain perception chronically after SCI. Lesion features ranged from findings consistent with physical transection to dogs with evidence of structural and functional connections through the lesion epicenter, highlighting injury complexity and heterogeneity. Careful analysis of injury characteristics suggested that at least some degree of spared supraspinal influence on reorganized motor circuitry below the level of injury is likely integral to the delayed motor recovery exhibited by some dogs with absent pain perception after severe SCI. This finding triggered a recalibration of how motor and ambulation are interpreted in this population and provided important insight into mechanisms of recovery of function. Exploration into reasons for the variability in response to the potassium channel antagonist, 4-aminopyridine (4AP) showed that electrodiagnostic evaluation might be useful to predict positive responders. This further highlighted injury complexity and underscored that there is unlikely to be a simple relationship between one aspect of injury and functional outcome or response to a specific therapy.

In order to expand the ability to broadly assess dogs with SCI, additional outcome measures were developed. While spasticity has been referred to in the veterinary literature in the context of SCI, it had never been formally evaluated in dogs with spontaneous injury.^{1,2} We confirmed that spasticity is common in dogs with chronic, severe SCI and that its presence and severity could be quantified via the Canine Spasticity Scale (CSS), which was adapted from human spasticity scales and composed of assessment of patellar clonus and flexor

spasms.³⁻⁵ Additionally, overall CSS scores were associated with gait scores and H-reflex threshold. While spasticity is a complex phenomenon, these results led to speculation that spasticity severity and hind limb stepping ability might both be manifestations of differences in motor neuron pool excitability between dogs. Therefore, the CSS might be helpful for investigating plasticity in the motor circuitry below the level of lesion and how that reorganization relates to function for dogs with severe thoracolumbar SCI. While differences were noted between dogs and humans with regard to the relative prominence of the different aspects of spasticity and the impact of spasticity in dogs (positive or negative) remains unclear, the CSS is a simple clinical tool that might be a useful adjunct to the standard neurologic examination in the chronic setting. Upon further validation, the CSS could be incorporated as an outcome measure in clinical trials involving dogs with chronic thoracolumbar SCI.

A gait analysis tool was also established for use in dogs with chronic gait deficits after severe, acute thoracolumbar SCI. Results demonstrated that center of pressure (COP) measurements could be reliably obtained from this population and offered information on global locomotion changes post-injury. Measurement of COP variability during locomotion showed increased variability across all levels of dysfunction (paraplegia to ambulatory with weakness and ataxia) in dogs with SCI compared to neurologically normal dogs.⁶ Additionally, the percentage of hind limb support provided during ambulation was associated with validated gait scales. Standardization and refinement of the sling support mechanism are needed, however, this method provides a means to quantify weight support for non-ambulatory dogs and to investigate the impact of weight support on other gait parameters. Evaluating dogs on multiple occasions might produce useful data on changes in weight support over time due to compensation, recovery or in response to a particular intervention. Further testing in a larger number of dogs with SCI is also necessary to determine if COP and COP variability measurements can discern different locomotor patterns between dogs of differing functional injury severity or detect changes in function over time. There were

notable technical considerations that would need to be addressed and COP does not offer information on whether a dog is ambulatory or not, a crucial benchmark in evaluating gait post-SCI. However, given the dynamic information on locomotion and postural control, COP and COP variability might be able to complement currently available means of gait analysis as outcome measures in clinical trials.

In addition to broadening the repertoire for evaluating this population, this work also sought to better describe the range of injury severity within a severely affected population. It is well known that a proportion of severely affected dogs show spontaneous motor recovery despite no return of pain perception below the level of injury.^{7,8} Imaging and electrodiagnostic techniques were utilized to more fully explore the extent of lesion complexity and severity both structurally and functionally and to investigate how severity relates to the level of motor function manifested in this population. Conventional magnetic resonance imaging (MRI) was somewhat limited in determining structural integrity through the lesion as the vast majority of dogs had regions with completely abnormal signal intensity (suggesting an apparent complete transverse myelopathy on MRI). However, diffusion tensor imaging (DTI) tractography showed only a minority had a complete absence of connections through the lesion epicenter consistent with physical transection while the rest displayed varying degrees of fiber disruption. Fractional anisotropy (FA) and mean diffusivity (MD) were also able to identify regions with microstructural change consistent with Wallerian degeneration and demyelination that appeared normal on T2W images. These findings provide broad information on lesion characteristics and suggest DTI is a sensitive means to evaluate structural change and quantify lesion severity in the chronic setting after severe, acute SCI. The presence of hind limb motor evoked potentials (MEPs) also confirmed functional integrity through the lesion epicenter in some dogs. As expected, no dogs with absent trans-lesional connections on tractography had recordable hind limb MEPs. No associations were identified between FA or MD values and the presence of MEPs, but MEPs were only present

in a small number of dogs limiting the ability to demonstrate correlation between structural and functional lesion severity.

In assessing the connection between structural and functional lesion characteristics and hind limb motor function, it was found that the craniocaudal length of the region with 100% abnormal signal intensity on T2W images, FA at the lesion epicenter, the presence of trans-lesional fibers and the presence of trans-lesional motor conduction were each associated hind limb motor function. This suggests that a less severe injury with evidence of structural and functional integrity through the lesion is integral to regaining the ability to walk in dogs with absent pain perception. These results support a role for residual supraspinal influence on motor recovery after severe injury and argue against reflexive spinal walking as a sufficient explanation for dogs with spontaneous SCI that regain independent ambulation despite absent pain perception. However, there was also evidence suggestive of post-injury plasticity in the neural circuitry contained below the level of the lesion. The inverse association between H-reflex threshold intensity and gait scores implied that motor neuron pool excitability might be an additional contributing factor to the level of motor function displayed after injury. This association was not upheld when a larger number of dogs were examined perhaps suggesting a complex role for changes to the central pattern generator and motor neuron excitability post-injury that warrants further investigation. Additionally, the precise relationship between trans-lesional connections and the local intraspinal motor circuitry still needs to be elucidated including the degree of supraspinal input needed to impact hind limb function.

Potential predictors of response to 4AP were also explored amongst dogs with a broad range of hind limb function due to severe SCI. While a simple relationship between patient or lesion characteristics and improvement in motor skills secondary to 4AP was not identified, results did suggest that a combination of electrodiagnostic variables might be useful in predicting response. It was also noted that 4AP improved central conduction, positive

response was more common in dogs with lower gait scores and evidence of transection on tractography was only seen in non-responders. These results support further examination of 4AP in a population of non-ambulatory dogs with evidence of structural spinal cord integrity on MRI. The ability to identify one, or more likely, multiple factors that contribute to response or non-response will not only allow targeted, personalized therapy with this medication but will continue to broaden the concept of injury complexity and uncover mechanisms involved in post-injury functional status.

Overall, this work expanded the body of knowledge regarding the heterogeneous, multifaceted nature of severe chronic SCI in dogs. This population displayed a range of injury characteristics, despite all having severe lesions, and specific injury features related to functional status in the chronic setting. These results offer clues regarding mechanisms involved in recovery and possible therapeutic targets which will facilitate expanded use of dogs with SCI as a model of chronic paralysis. Well-designed canine clinical trials have the potential to complement research in experimental models and humans with SCI with the ultimate goal to improve outcomes for both dogs and humans with SCI.

Limitations and future directions

Limitations of this work include the small number of dogs included in conjunction with the relatively subjective nature and limited sensitivity of some of the evaluation methods. For example, transcranial magnetic stimulation demonstrated long tract conduction across the site of injury in a small proportion of dogs. However, this likely underestimated the number of dogs with functional trans-lesional connections, perhaps skewing the results. Samples for histopathology were also not obtained but, as pet dogs who were living with their SCI, this was not a feasible option. The use of MRI including DTI provided a reasonable alternative for tissue samples to assess structural integrity of the spinal cord. Additionally, the novel

evaluation methods introduced for dogs with SCI require further development and validation before more widespread implementation can be considered.

Evaluation also focused heavily on motor function as recovery of ambulation is an important functional benchmark for SCI patients (humans and dogs). However, integrating structural, functional (electrodiagnostic) and clinical approaches to analyze the entire neuraxis and to describe a more comprehensive model of functional status after injury is warranted. It is suspected that other aspects of neurologic dysfunction including sensory and autonomic impairment (e.g. urinary and fecal incontinence) share overlapping characteristics with human SCI patients and are worthy of further in-depth investigation in this population.

While a number of interesting associations were uncovered, causality was not established and more work is needed in a larger number of dogs to determine if these relationships are maintained. For example, the initial 20 dogs showed the H-reflex threshold was inversely associated with treadmill-based stepping and coordination scores but that association was no longer present when the same variable was re-examined in more dogs (n=33). This does not discount the potential relationship between motor neuron pool excitability and recovery of motor function but rather highlights the need for further investigation to understand how reorganized circuitry facilitates or impedes recovery of function in individual animals. Importantly, this work can be seen as an exploratory investigation into severe SCI in dogs in the chronic setting and, as such, offers targets for further, in-depth study and potential interventional studies. The value in this expanded characterization of canine SCI lies in designing subsequent clinical trials testing therapeutic interventions aimed at manipulating one or more aspects of injury.

Future directions include confirming these findings in a larger number of dogs and ongoing development of outcome measures. Of particular interest is to establish the sensitivity of DTI not only to measure integrity at the lesion epicenter but also to quantify degenerative changes

distant from the site of injury. DTI indices are being investigated as potential biomarkers in human and experimental SCI and could prove useful for canine SCI as well.^{9,10} Building on this work as a foundation, interventional studies can also be designed to target specific aspects of injury with apparent functional significance. For example, the effect of epidural stimulation on the motor circuitry below the level of injury can be studied via monitoring changes in H-reflex and F-waves as well as long tract function compared to these baseline results. While this work purposely focused on dogs with a range of motor function in order to more fully characterize the spectrum of injury severity possible amongst severely injured dogs, an interesting group to focus on moving forward are dogs that display evidence of continuity through the lesion epicenter and some recovery of hind limb function but remain chronically non-ambulatory (e.g. OFS 3-5). These dogs are sufficiently impaired such that meaningful improvement can be targeted (i.e. ambulation) but lack evidence of physical transection that might limit response to various therapeutic strategies. Additionally, these dogs might mirror people with incomplete injury but severe neurologic impairment and, as such, offer a useful subset in which to evaluate novel or combinations of treatment interventions for translational application to human SCI.

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APPENDICES

APPENDIX A

MATLAB code for center of pressure analysis

```

% Code by: Leighanne Davis with help from William Pfitzner
% Updated: 4/22/2015
% Inputs: COP data, marker position data, load cell data
% Outputs: Root mean squared: COPx, COPy, RFPz (marker data)

clear all
close all
clc

Trial = dlmread('CP008_New Session 109.txt','\t',5,0);    % reading in trial data
frequency = Trial(1:length(Trial),1);
Trial2 = dlmread('CP008_New Session 109.txt','\t',5,0);
frequency2 = Trial2(1:length(Trial2),1);

% Specify data

Right_front_x = Trial(1:length(Trial),27); % Trial 1
Right_front_y = Trial(1:length(Trial),28); % Marker data (right front paw)
Right_front_z = Trial(1:length(Trial),29);
COP_x_FP1 = Trial(1:length(Trial),12); % Center of Pressure data
COP_y_FP1 = Trial(1:length(Trial),13);
COP_x_FP2 = Trial(1:length(Trial),15);
COP_y_FP2 = Trial(1:length(Trial),16);
Upper_back_x = Trial(1:length(Trial),30); % Marker data (Upper back)
Upper_back_y = Trial(1:length(Trial),31);
Upper_back_z = Trial(1:length(Trial),32);
%Load_Cell_L = Trial(1:length(Trial),33);
Load_Cell_R = Trial(1:length(Trial),33);
total_time = max(frequency)/960; % 120 is the sampling rate; time is in seconds
t_time = linspace(0,total_time,length(Right_front_x));

COP_x = COP_x_FP1+COP_x_FP2;
COP_y = COP_y_FP1+COP_y_FP2;

Right_front_x2 = Trial2(1:length(Trial2),27); % Trial 2
Right_front_y2 = Trial2(1:length(Trial2),28);
Right_front_z2 = Trial2(1:length(Trial2),29);
COP_x2_FP1 = Trial2(1:length(Trial2),12);
COP_y2_FP1 = Trial2(1:length(Trial2),13);
COP_x2_FP2 = Trial2(1:length(Trial2),15);
COP_y2_FP2 = Trial2(1:length(Trial2),16);
Upper_back_x2 = Trial2(1:length(Trial2),30);
Upper_back_y2 = Trial2(1:length(Trial2),31);
Upper_back_z2 = Trial2(1:length(Trial2),32);
%Load_Cell_L2 = Trial2(1:length(Trial2),33);
Load_Cell_R2 = Trial2(1:length(Trial2),33);
total_time2 = max(frequency2)/960; % 120 is the sampling rate; time is in seconds
t_time2 = linspace(0,total_time2,length(Right_front_x2));

COP_x2 = COP_x2_FP1+COP_x2_FP2;
COP_y2 = COP_y2_FP1+COP_y2_FP2;

% Interpolate marker data to length of force data;

finder = find(Right_front_x > 0); % Trial 1
realvalues_RFPx = Right_front_x(finder);
finder2 = find(Right_front_y > 0);
realvalues_RFPy = Right_front_y(finder2);
finder6 = find(Right_front_z > 0);

```

```

realvalues_RFPz = Right_front_z(finder6);

finder3 = find(Right_front_x2 > 0);           % Trial 2
realvalues_RFPx2 = Right_front_x2(finder3);
finder4 = find(Right_front_y2 > 0);
realvalues_RFPy2 = Right_front_y2(finder4);
finder5 = find(Right_front_z2 > 0);
realvalues_RFPz2 = Right_front_z2(finder5);

% Define a sample time;

sample_time = 1:length(t_time); % Session 1
sample_time2 = 1:length(t_time2); % Session 2

% Create an array that is the length of sample_time and is the values
% of realvalues_RFPx

sample_x = linspace(1,sample_time(end),length(realvalues_RFPx)); % Trial 1
sample_y = linspace(1,sample_time(end),length(realvalues_RFPy));
sample_z = linspace(1,sample_time(end),length(realvalues_RFPz));

sample_x2 = linspace(1,sample_time2(end),length(realvalues_RFPx2)); % Trial 2
sample_y2 = linspace(1,sample_time2(end),length(realvalues_RFPy2));
sample_z2 = linspace(1,sample_time2(end),length(realvalues_RFPz2));

% Now we do the actual interpolation

sample_RFPx_values = interp1(sample_x,realvalues_RFPx,sample_time,'spline'); % Trial 1
sample_RFPy_values = interp1(sample_y,realvalues_RFPy,sample_time,'spline');
sample_RFPz_values = interp1(sample_z,realvalues_RFPz,sample_time,'spline');

sample_RFPx2_values = interp1(sample_x2,realvalues_RFPx2,sample_time2,'spline'); % Trial 2
sample_RFPy2_values = interp1(sample_y2,realvalues_RFPy2,sample_time2,'spline');
sample_RFPz2_values = interp1(sample_z2,realvalues_RFPz2,sample_time2,'spline');

% Set the reference frame to that of "inside" the dog instead of from the
% frame of reference of the lab

% We will do this by utilizing a marker that stays relatively constant
% on the dog; for these purposes that would be the Upper Back marker

% First we need to interpolate the Upper Back marker data to the
% length of the force data (following the steps above)

finder_UB = find(Upper_back_x > 0);           % Trial 1
realvalues_UBx = Upper_back_x(finder_UB);
finder_UB2 = find(Upper_back_y > 0);
realvalues_UBy = Upper_back_y(finder_UB2);
finder10_UB = find(Upper_back_z > 0);
realvalues_UBz = Upper_back_z(finder10_UB);

finder3_UB = find(Upper_back_x2 > 0);          % Trial 2
realvalues_UBx2 = Upper_back_x2(finder3_UB);
finder4_UB = find(Upper_back_y2 > 0);
realvalues_UBy2 = Upper_back_y2(finder4_UB);
finder6_UB = find(Upper_back_z2 > 0);
realvalues_UBz2 = Upper_back_z2(finder6_UB);

sample_x_UB = linspace(1,sample_time(end),length(realvalues_UBx)); % Trial 1
sample_y_UB = linspace(1,sample_time(end),length(realvalues_UBy));
sample_z_UB = linspace(1,sample_time(end),length(realvalues_UBz));

sample_x2_UB = linspace(1,sample_time2(end),length(realvalues_UBx2)); % Trial 2
sample_y2_UB = linspace(1,sample_time2(end),length(realvalues_UBy2));
sample_z2_UB = linspace(1,sample_time2(end),length(realvalues_UBz2));

```

```

sample_UBx_values = interp1(sample_x_UB,realvalues_UBx,sample_time,'spline');    % Trial 1
sample_UBy_values = interp1(sample_y_UB,realvalues_UBy,sample_time,'spline');
sample_UBz_values = interp1(sample_z_UB,realvalues_UBz,sample_time,'spline');

sample_UBx2_values = interp1(sample_x2_UB,realvalues_UBx2,sample_time2,'spline');    % Trial
2
sample_UBy2_values = interp1(sample_y2_UB,realvalues_UBy2,sample_time2,'spline');
sample_UBz2_values = interp1(sample_z2_UB,realvalues_UBz2,sample_time2,'spline');

% Filter the Data to get rid of unwanted noise

filter_order = 8;
pass_freq = 18;
Fs = 960;
Nyquist = Fs/2;
[a,b] = butter(filter_order,(pass_freq/Nyquist), 'low');
COP_x = filtfilt(a,b,COP_x);
COP_y = filtfilt(a,b,COP_y);
COP_x2 = filtfilt(a,b,COP_x2);
COP_y2 = filtfilt(a,b,COP_y2);

% Putting the data into the reference frame of the dog for COP

ref_COPx = COP_x-sample_UBx_values';    % Trial 1
ref_COPy = COP_y-sample_UBy_values';

ref_COPx2 = COP_x2-sample_UBx2_values';    % Trial 2
ref_COPy2 = COP_y2-sample_UBy2_values';

% Putting the data into the reference frame of the dog for marker
% data

ref_RFPx = sample_RFPx_values-sample_UBx_values;    % Trial 1
ref_RFPy = sample_RFPy_values-sample_UBy_values;
ref_RFPz = sample_RFPz_values-sample_UBz_values;

ref_RFPx2 = sample_RFPx2_values-sample_UBx2_values;    % Trial 2
ref_RFPy2 = sample_RFPy2_values-sample_UBy2_values;
ref_RFPz2 = sample_RFPz2_values-sample_UBz2_values;

% This portion of the code is working towards the outputs

% Find the mean COPx and COPY of the sample

mean_COPx = mean(ref_COPx);    % Trial 1
mean_COPY = mean(ref_COPY);

mean_COPx2 = mean(ref_COPx2);    % Trial 2
mean_COPY2 = mean(ref_COPY2);

% Find change in COP for x and y

delta_COPY = ref_COPY-mean_COPY;    % Trial 1
delta_COPx = ref_COPx-mean_COPx;

delta_COPY2 = ref_COPY2-mean_COPY2;    % Trial 2
delta_COPx2 = ref_COPx2-mean_COPx2;

% Mean of the change in COP x and y

mean_delta_COPY = mean(delta_COPY);    % Trial 1
mean_delta_COPx = mean(delta_COPx);

mean_delta_COPY2 = mean(delta_COPY2);    % Trial 2
mean_delta_COPx2 = mean(delta_COPx2);

```

```

% Find the mean RFPx and RFPy of the interpolated data

mean_RFPx = mean(ref_RFPx);    % Trial 1
mean_RFPy = mean(ref_RFPy);
mean_RFPz = mean(ref_RFPz);

mean_RFPx2 = mean(ref_RFPx2); % Trial 2
mean_RFPy2 = mean(ref_RFPy2);
mean_RFPz2 = mean(ref_RFPz2);

% Make an array of change in RFP in the x and y directions

delta_right_frontx = ref_RFPx-mean_RFPx;    % Trial 1
delta_right_fronty = ref_RFPy-mean_RFPy;
delta_right_frontz = ref_RFPz-mean_RFPz;

delta_right_frontx2 = ref_RFPx2-mean_RFPx2;    % Trial 2
delta_right_fronty2 = ref_RFPy2-mean_RFPy2;
delta_right_frontz2 = ref_RFPz2-mean_RFPz2;

% Find the mean of the change in RFP marker data

mean_delta_RFPx = mean(delta_right_frontx);    % Trial 1
mean_delta_RFPy = mean(delta_right_fronty);
mean_delta_RFPz = mean(delta_right_frontz);

mean_delta_RFPx2 = mean(delta_right_frontx2);    % Trial 2
mean_delta_RFPy2 = mean(delta_right_fronty2);
mean_delta_RFPz2 = mean(delta_right_frontz2);

% Calculate the root mean square for the delta_COP and marker data

% COP data

root_mean_delta_COPx = rms(delta_COPx);        % Trial 1
root_mean_delta_COPy = rms(delta_COPy);

root_mean_delta_COPx2 = rms(delta_COPx2);      % Trial 2
root_mean_delta_COPy2 = rms(delta_COPy2);

COP_x_RMS = [root_mean_delta_COPx,root_mean_delta_COPx2]
COP_y_RMS = [root_mean_delta_COPy,root_mean_delta_COPy2]

% Marker data

root_mean_delta_RFPx = rms(delta_right_frontx);    % Trial 1
root_mean_delta_RFPy = rms(delta_right_fronty);
root_mean_delta_RFPz = rms(delta_right_frontz);

% rms(sqrt(x^2 + y^2))

root_mean_delta_RFPx2 = rms(delta_right_frontx2);    % Trial 2
root_mean_delta_RFPy2 = rms(delta_right_fronty2);
root_mean_delta_RFPz2 = rms(delta_right_frontz2);

RFP_x = [root_mean_delta_RFPx,root_mean_delta_RFPx2];
RFP_y = [root_mean_delta_RFPy,root_mean_delta_RFPy2];
RFP_z_RMS_T1 = root_mean_delta_RFPz
RFP_z_RMS_T2 = root_mean_delta_RFPz2

RFP_1 = [root_mean_delta_RFPx,root_mean_delta_RFPy];
RFP_2 = [root_mean_delta_RFPx2,root_mean_delta_RFPy2];

% Find percent body weight
sling = input('mass of sling in g ')
dog_mass = input('mass of dog from Mass_script.m file in kg ')
mass_sling = sling/1000;    % in kg

```

```

%mean_LC_L = mean(Load_Cell_L);
mean_LC_R = mean(Load_Cell_R);
%mean_LC_L2 = mean(Load_Cell_L2);
mean_LC_R2 = mean(Load_Cell_R2);

%Force_LoadCell_L = mean_LC_L*116;
Force_LoadCell_R = mean_LC_R*116;
%Force_LoadCell_L2 = mean_LC_L2*116;
Force_LoadCell_R2 = mean_LC_R2*116;

%Support_Mass_L = (Force_LoadCell_L/9.81)-mass_sling; % in kg
Support_Mass_R = (Force_LoadCell_R/9.81)-mass_sling;
%Support_Mass_L2 = (Force_LoadCell_L2/9.81)-mass_sling;
Support_Mass_R2 = (Force_LoadCell_R2/9.81)-mass_sling;

%percent_support_L = (Support_Mass_L/dog_mass)*100
percent_support_R = (Support_Mass_R/dog_mass)*100
%percent_support_L2 = (Support_Mass_L2/dog_mass)*100
percent_support_R2 = (Support_Mass_R2/dog_mass)*100

% Filtering Practice
% figure(1)
% subplot(2,1,1)
% plot(t_time,delta_COPy)
% title('Treadmill On Un-Filtered vs Filtered');
% hold on
% plot(t_time,filtered_signal,'r')
% legend('Un-filtered','Filtered');
% subplot(2,1,2)
% plot(t_time,filtered_signal,'r')
% title('Filtered Treadmill On');
%
% figure(2)
% subplot(2,1,1)
% plot(t_time2,delta_COPy2)
% title('Treadmill Off Un-Filtered vs Filtered');
% hold on
% plot(t_time2,filtered_signal2,'r')
% legend('Un-filtered','Filtered');
% subplot(2,1,2)
% plot(t_time2,filtered_signal2,'r')
% title('Filtered Treadmill Off');
%
% figure(3)
% subplot(2,1,1)
% plotyy(t_time,delta_COPy,t_time2,delta_COPy2)
% legend('Tread On','Tread Off');
% title('Un-Filtered Tread On vs Off');
% subplot(2,1,2)
% plotyy(t_time,filtered_signal,t_time2,filtered_signal2)
% legend('Tread On','Tread Off');
% title('Filtered Tread On vs Off');

%% Graph the data

figure(1)
subplot(2,1,1)
plot(t_time,delta_COPy)
hold on

```

```

plot(t_time2,delta_COPy2,'r')
xlabel('time (sec)'); ylabel('Change in COPy (m)'); title('Change in COPy over time');
legend('Session 1', 'Session 2');
subplot(2,1,2)
plot(t_time,delta_COPx)
hold ON
plot(t_time2,delta_COPx2,'r')
xlabel('time (sec)'); ylabel('Change in COPx (m)'); title('Change in COPx over time');
legend('Session 1', 'Session 2');

figure(2)
plot(delta_COPx,delta_COPy,'*')
hold ON
plot(delta_COPx2,delta_COPy2,'*r')
xlabel('Change in COP (x-direction)'); ylabel('Change in COP (y-direction)');
title('Points of COPx vs. COPy')
legend('Session 1', 'Session 2');
axis([-0.1 0.12 -0.1 0.12]);

figure(3)
plot(mean_delta_COPx,mean_delta_COPy,'*');
hold ON
plot(mean_delta_COPx2,mean_delta_COPy2,'*r');
xlabel('Mean of change in COPx (x-direction)');
ylabel('Mean of change in COPy (y-direction)');
title('Average change in COP over two sessions')
legend('Session 1','Session 2');
%axis([(-3*10^-16) (-.6*10^-16) (-3.5*10^-16) (4.5*10^-16)]);

figure(4)
bar(COP_x_RMS); set(gca,'XTickLabel', {'Session 1', 'Session 2'});
ylabel('RMS of the mean change in COPx');
title('Average RMS change over two sessions in the x direction');
%axis([0 0 0 0.04]);

figure(5)
bar(COP_y_RMS); set(gca,'XTickLabel', {'Session 1', 'Session 2'});
ylabel('RMS of the mean change in COPy');
title('Average RMS change over two sessions in the y direction');

figure(6)
plot(delta_right_frontx,delta_right_fronty,'*')
hold ON
plot(mean_delta_RFPx,mean_delta_RFPy,'*k')
hold ON
plot(delta_right_frontx2,delta_right_fronty2,'*r')
hold ON
plot(mean_delta_RFPx2,mean_delta_RFPy2,'*g')
xlabel('RFP (x-direction)'); ylabel('RFP (y-direction)');
title('Points of the Right Front Paw in the x direction vs. the y direction')
legend('Session 1', 'Session1 mean', 'Session 2', 'Session 2 mean');
%axis([-0.12 0.06 -1.5 0.5]);

figure(7)
plot(delta_right_frontx,delta_right_frontz,'*')
hold ON
plot(mean_delta_RFPx,mean_delta_RFPz,'*k')
hold ON
plot(delta_right_frontx2,delta_right_frontz2,'*r')
hold ON

```

```

plot(mean_delta_RFPx2,mean_delta_RFPz2,'*g')
xlabel('RFP (x-direction)'); ylabel('RFP (z-direction)');
title('Points of the Right Front Paw in the x direction vs. the z direction')
legend('Session 1', 'Session1 mean', 'Session 2', 'Session 2 mean');
%axis([-12 .06 -1.5 .5]);

figure(8)
plot(delta_right_fronty,delta_right_frontz,'*')
hold ON
plot(mean_delta_RFPy,mean_delta_RFPz,'*k')
hold ON
plot(delta_right_fronty2,delta_right_frontz2,'*r')
hold ON
plot(mean_delta_RFPy2,mean_delta_RFPz2,'*g')
xlabel('RFP (y-direction)'); ylabel('RFP (z-direction)');
title('Points of the Right Front Paw in the y direction vs. the z direction')
legend('Session 1', 'Session1 mean', 'Session 2', 'Session 2 mean');
%axis([-12 .06 -1.5 .5]);

figure(9)
plot(mean_delta_RFPx,mean_delta_RFPy,'*');
hold ON
plot(mean_delta_RFPx2,mean_delta_RFPy2,'*r');
xlabel('Mean of change in RFPx (x-direction)');
ylabel('Mean of change in RFPy (y-direction)');
title('Average change in RFP over two sessions')
legend('Session 1', 'Session 2');

figure(10)
bar(RFP_x); set(gca,'XTickLabel', {'Session 1', 'Session 2'});
figure(11)
bar(RFP_y); set(gca,'XTickLabel', {'Session 1', 'Session 2'});
bar(RFP_1,diag(RFP_2),'stacked');
xlabel('RMS of the mean change in RFPx');
ylabel('RMS of the mean change in RFPy');
title('Average RMS change over two sessions');
legend('Session 1', 'Session 2');

```