Dynamic “Range of Motion” Hindlimb Stretching Disrupts Locomotor Function in Rats with Moderate Subacute Spinal Cord Injuries

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Abstract

Joint contractures and spasticity are two common secondary complications of a severe spinal cord injury (SCI), which can significantly reduce quality of life, and stretching is one of the top strategies for rehabilitation of these complications. We have previously shown that a daily static stretching protocol administered to rats at either acute or chronic time points after a moderate or moderate-severe T10 SCI significantly disrupts their hindlimb locomotor function. The objective of the current study was to examine the effects of dynamic range of motion (ROM) stretching on the locomotor function of rats with SCI as an alternative to static stretching. Starting at 6 weeks post-injury (T10 moderate contusion) eight adult Sprague–Dawley rats were subjected to hindlimb stretching for 4 weeks. Our standard stretching protocol (six maneuvers to stretch the major hindlimb muscle groups) was modified from 1 min static stretch-and-hold at the end ROM of each stretch position to a dynamic 2 sec hold, 1 sec release rhythm repeated for a duration of 1 min. Four weeks of daily (5 days/week) dynamic stretching led to significant disruption of locomotor function as assessed by the Basso, Beattie, Bresnahan (BBB) Open Field Locomotor Scale and three-dimensional (3D) kinematic and gait analyses. In addition, we identified and analyzed an apparently novel hindlimb response to dynamic stretch that resembles human clonus. The results of the current study extend the observation of the stretching phenomenon to a new modality of stretching that is also commonly used in SCI rehabilitation. Although mechanisms and clinical relevance still need to be established, our findings continue to raise concerns that stretching as a therapy can potentially hinder aspects of locomotor recovery.

Keywords: dynamic stretching; locomotor function; physical therapy; rehabilitation; SCI

Introduction

SPINAL CORD INJURY (SCI) that causes paralysis below the level of the lesion results in a multitude of secondary complications that commonly include joint contractures and spasticity.¹ Both complications can significantly reduce the quality of life of SCI patients.² Currently, stretching remains the first line therapy to treat and prevent contractures and spasticity.^{3–5} Previously in our laboratory, we showed that a 24 min protocol of “static” stretching of the major hindlimb muscles of rats with mild to moderately acute and chronic SCI resulted in significant declines in locomotor function.^{6,7} The effects of therapeutic stretching on motor function of SCI patients is currently not known; the main outcome measures of clinical studies on stretching are range of motion and/or spasticity.⁸ However, a multitude of studies have investigated static stretching in uninjured athletic populations and have observed

negative effects on some aspects of motor function.⁹ Specifically, static stretching results in decrements in vertical jumping and running performance,^{10–12} muscle strength endurance,¹³ and isometric strength^{14,15} as well as isokinetic torque production.^{16,17} Dynamic stretching is an alternative technique to improve range of motion (ROM) and flexibility that has been recommended as part of the warmup routine for athletic events; it is not thought to cause detriments in performance, and may actually improve it.^{18,19} Dynamic stretching involves multiple repetitions of limb movement through its entire range to the end ROM.²⁰ Such ROM exercises are also commonly performed during the rehabilitation of SCI patients.²¹ Therefore, we wanted to determine if a dynamic stretching protocol involving all major hindlimb muscle groups in rats with SCI disrupted locomotor function similarly to our static stretching protocol. For a variety of reasons including a reduction in the total time at end ROM and the involvement of a rhythm, we

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hypothesized that dynamic stretching would not be detrimental to locomotor function in rats with moderate SCI.

Methods

Eight young adult female Sprague–Dawley rats were used in this experiment. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Louisville. After a week of acclimatization and baseline data collection, the animals received moderate spinal cord contusion injuries (NYU, 12.5 g/cm) at T10 as described previously.²² Locomotor function was assessed at least weekly using the Basso, Beattie, Bresnahan (BBB) Open Field Locomotor Scale,²³ and video-based kinematic and gait analysis was performed biweekly. As described previously,⁷ we used a three segment, two angle model of the hindlimb, and “joint” excursions were measured as the hip–ankle–toe (HAT) and iliac crest–hip–ankle (IHA) angles. Two gait indices were also used, the regularity index (RI) and the plantar stepping index (PSI), based on ventral-view video allowing paw placement order, timing, and location to be determined for every step taken (from initial contact to lift-off) using MaxTraq software (Innovision Systems Inc., Columbiaville, MI) and custom-designed Excel macros. The RI is calculated as the number of correctly patterned plantar steps over the total number of steps (dorsal and plantar).²⁴ The PSI is

calculated as the number of plantar hindlimb steps over the total number of forelimb step.²⁵

At 6 weeks post-injury, the animals entered a 4 week dynamic stretch protocol that was performed 5 days a week for 4 weeks. Dynamic stretching consisted of the same six bilateral stretches of major hindlimb muscle groups as our previous static stretching protocol;^{6,7} however, instead of holding the limb at the end ROM of each stretch for 1 min, each stretch was held for only 2 sec, followed by a 1 sec release, repeated 20 times over a 1 min period. A metronome (at 1 Hz) was set to the 2-1 ratio, using different tones, in order to give an audible clue to the rat physical therapists (PT) allowing them to maintain the rhythm and ensuring that timing was consistent and accurate between therapists for each animal every day. During the 4 weeks of stretching, BBB scores were assessed three times weekly (Monday a.m., Monday p.m., and Friday p.m.).

Tonic stretching triggered several observable responses in both the stretched and unstretched limbs that rat PTs took note of.⁶ Dynamic stretching resulted in a new and highly consistent response that was observed as paw “vibrations”: high frequency and low amplitude fluctuations in one or two joints. Because of its resemblance to human clonus, which can be triggered by sudden stretching or upon the release of stretch,^{26,27} we sought to better understand this phenomenon. To quantify this response, single stretching sessions for four animals were recorded (digital video at

FIG. 1. Locomotor function. (A) Dynamic stretching protocol began 6 weeks after spinal cord injury (SCI) when locomotor function had reached a stable plateau and continued on for 4 weeks. At the end of the first week of stretching, the animals had significantly lower Basso, Beattie, Bresnahan (BBB) scores as compared with their pre-stretch values. Over the weekend animals achieve significant recovery; however, the first session of the 2nd week of stretching induced deficits in the locomotor function that persisted to the end of the week. Similar pattern of recovery/disruption followed for the remaining 2 weeks of the stretching protocol. Four of the eight animals were euthanized after 2 h of stretching, for histological muscle assessment and the other four were allowed to recover for 4 weeks before being euthanized. Their locomotor function was assessed weekly (dotted line). (B,C) Analysis of biweekly kinematic and gait recordings showed that the animals had significantly reduced joint excursions (B) and gait indices (C) indicative of lack of stepping or even sweeping in some animals during the weeks of the stretching therapy (weeks 6 and 8), as compared with the pre-stretch stepping ability and hindlimb joints excursions (week 4). By week 12, the locomotor function of the remaining four animals had achieved significant recovery back to pre-stretch levels.

100Hz frame rate with the same PT stretching all animals). Most often vibrations occurred in both limbs simultaneously; however, kinematic analysis was performed on the contralateral limb, as more joint markers were easily viewable and could be tracked with the software for analysis. The toe, ankle, knee, hip, and iliac crest were marked with a black marker for kinematic analysis of the movement using MaxTraQ software. Three vibration responses per stretch per animal were analyzed for ROM (excursion of movement) and frequency (peaks per second). Averages of these outcome measures from the four animals for each stretch are reported in the Results.

After the last week of stretching (week 4), four of the eight animals were euthanized 2 h after the last stretching session, and the other four animals were maintained for an additional 5 weeks. The spinal cords and major hindlimb muscles (tibialis anterior [TA], medial gastrocnemius [MG] and biceps femoris [BF]) were dissected out, post-fixed in 4% paraformaldehyde (PFA) and cryoprotected in 30% sucrose. Muscles (mid-belly) were sectioned at 10 μ m and stained with Hematoxylin and Eosin. Muscle fibers (MF) were analyzed for the presence of centralized nuclei, a marker of regeneration.²⁸ Spinal cords were sectioned at 50 μ m and stained with eriochrome cyanine to determine the percentage of white matter sparing at the injury epicenter using ImageJ software as described previously.²²

Statistical Analysis

Each animal served as its own control for the locomotor outcome measures, and the data were analyzed for changes over time using a mixed model analysis of variance (ANOVA) (BBB scores and joint excursions) followed by Tukey *post-hoc*. Nonparametric Wilcoxon signed ranks test was used to make comparisons between time points in the gait indices RI and PSI. One way ANOVA followed by independent *t* tests were used to compare the percentages of MF containing centralized nuclei between the animals euthanized 2 h after the last stretching session (group 1, *n*=4) and the animals euthanized 5 weeks later (group 2, *n*=4). Significant differences were established at *p*<0.05.

Results

Based on a dense, compact appearance on eriochrome cyanin stained sections, moderate SCI at T10 resulted in an average spared

white matter (SWM) at the injury epicenter of $7.35\% \pm 3.36$. The average SWM for group 1 was $6.08 \pm 2.54\%$ and $8.61 \pm 3.96\%$ for group 2. There was no significant difference in SWM between the groups euthanized at two different time points (*p*=0.322). Despite the low percentage of spared white matter, the animals achieved substantial locomotor recovery: BBBs plateaued by week 6 with an average score of 11, which is indicative of consistent weight-supported stepping without forelimb–hindlimb coordination (Fig. 1). One week of dynamic stretching starting at 6 weeks post-SCI resulted in a significant decrease in locomotor function as average BBB scores dropped to 3.7 ± 2.3 (*p*=0.004). Thereafter, at the end of each week of stretching, BBB scores hovered near 2.5 (indicative of slight movement of one or two joints only). As in previous studies, these animals achieved some locomotor recovery over the weekends when they were not stretched.^{6,7} However, one stretching session (Monday morning) was enough to again reduce the BBB scores to below 4 (Fig. 1). Lack of hindlimb movement was further confirmed with the more objective kinematic and gait analysis. The animals had significantly reduced excursions (ROM) of both IHA and HAT hindlimb angles during the weeks of stretching (weeks 6 and 8). RI and PSI were reduced to 0 at week 6 (measured on the 3rd day of the 1st week of stretching) indicating a complete lack of plantar stepping (Fig. 2B). These gait indices remained at 0 until stretching ceased. Figure 2 depicts some characteristics of the vibration response. In B, a stick figure is shown representing a single episode of the vibration response upon the release of stretch (in this case of the hamstring stretch): black line showing a starting position and gray lines showing a full ROM that the limb goes through during the response. As the foot “vibrates,” (multiple flexion/extension phases) the positions of the toe, ankle, and knee change, and Figure 2A shows the velocity profile of these points. Table 1 shows the average frequencies (calculated as number of peaks in knee–ankle–toe [KAT] per second), and averaged ROM (degrees) in the KAT angle during the vibration response for each stretch. The percentages of muscle fibers containing centralized nuclei for each muscle analyzed are listed in Table 2. Animals euthanized 2 h after stretching had a significantly higher percentage of muscle fibers with centralized nuclei in the MG muscle than the group euthanized 5 weeks later (*p*<0.05). There were no significant differences for the BF and TA muscles.

FIG. 2. Characteristics of vibration response. Kinematic analysis was performed on the contralateral limb vibration response during a release of 2 sec hamstring stretch of the opposite limb. Vibrations occurred in both limbs simultaneously; however, kinematic analysis was performed on the contralateral limb, as more points were visually available and could be tracked with the software for analysis. **(A)** During a vibration response, the limb goes through multiple clonic-like contractions, as evidenced by the multiple peaks in velocity of the toe–ankle–knee angle in 1 sec. **(B)** Stick figure of the contralateral limb during a vibration response. The black line shows a steady position of the limb during the 2 sec stretch hold period of the opposite limb. Upon release of stretch, the clonic-like contractions (gray stick figures) are initiated as the limb is pulled in toward the body (knee and hip are flexed). Most of the movement occurs in the ankle joint, with an average range of motion of 14 degrees, and contractions occurring at high frequency (~ 12 peaks per second).

TABLE 1. CHARACTERISTICS OF CLONIC-LIKE MUSCLE RESPONSES TO STRETCH

<i>Muscle/Stretch</i>	<i>Clonic-like stretch response characteristic</i>	
	<i>Frequency (peaks/sec)</i>	<i>Range of Motion (degrees)</i>
Tibialis anterior	12.86 ± 3.44	16.24 ± 12.29
Gastrocnemius	13.29 ± 5.55	11.74 ± 7.81
Quadriceps	16.35 ± 1.41	7.04 ± 2.29
Hamstring	15.38 ± 1.72	8.56 ± 3.55
Hip abductors	15.48 ± 2.65	8.33 ± 8.28
Hip adductors	13.14 ± 2.51	2.96 ± 0.93

Data reported as means ± SD.

Discussion

In this study, we sought to determine if an alternative dynamic hindlimb stretching protocol would result in disrupted locomotor function in rats with SCI. Based on the simple concept that phasic or rhythmic afferent input would be less detrimental to spinal cord circuitry function as it relates to locomotor function than tonic afferent input,²⁹ we hypothesized that dynamic stretching would not induce locomotor deficits. Contrary to our hypothesis, dynamic stretching dramatically disrupted locomotor function, reducing BBB scores to levels similar to those of static stretching, even though the total amount of stretch that the animals received on a daily basis was less (20 sec less/stretch) than in our previous studies.^{6,7,29} Histological analysis of the muscles revealed that for the ankle extensor MG, the group euthanized 2 h after stretching had elevated numbers of centralized nuclei (8%) as compared with the rats euthanized 4 weeks later (~4%), suggesting that more muscle fiber regeneration was occurring caused by the strain resulting from multiple stretching sessions. It is important to note however, that the additional muscle fiber regeneration was apparently complete by 4 weeks, suggesting that the stretching did not cause damage beyond what the muscle repair system is equipped to handle, and is, therefore, unable to explain the locomotor deficits. Nevertheless, this is the first evidence suggesting some degree of muscle damage caused by stretching, potentially because our previous studies had animals surviving for several weeks after the last stretching session.

During stretching, our rat PTs kept a thorough record of all observable hindlimb responses, among which are contralateral limb kicking, air stepping, and spasms (contralateral and ipsilateral

limbs). A new response was uncovered with dynamic stretching, which we refer to as “vibrations.” These were induced by the onset and the release of stretch, and were seen in the both the stretched and the contralateral unstretched limb. Although we did not record electromyography (EMG) during these responses, we did determine the kinematics of the vibrations. We found that the vibrations had a frequency range of 10–19 Hz, with a mean of 14.42 for all stretches combined, and a very low amplitude, ~10 degrees (in the KAT angle). Onset of muscle contractions when the limb was being stretched and the muscles lengthened, or in other words, eccentric muscle contractions, were being generated during our stretching protocol. Eccentric muscle contractions that occur at the ground contact phases of normal locomotion are a part of stretch-shortening cycle, one of the fundamental functions of the skeletal muscle.³⁰ This type of muscular activation serves to decelerate the limb for smooth and controlled contact with a surface,³¹ and is often utilized in athletic training because it provides an excellent stimulus for muscle hypertrophy and strength gains.^{32–34} However, eccentric contractions have been also recognized to result in a greater amount of disruption in myofibrillar structure,³⁵ which could potentially explain why we see an increased number of regenerating fibers in the muscles of the animals that were euthanized within hours of the last stretching session. Delayed onset muscle soreness (DOMS), which is a type of mechanical hyperalgesia, occurs 1–2 days after eccentric contractions as a result of nociceptive afferents sensitization in response to release of bradykinin and nerve growth factor.³⁶ Consistent with the DOMS phenomenon is the following observation: we have never observed any decrements in locomotor function following the initial stretching session in this (Fig. 1) or any of our earlier studies.⁷ However, in the 2nd week of stretching, one session did cause the deficits, presumably because of the sensitization of afferents, which now were functioning at lower thresholds.

Nociceptive afferents have complex modulatory effects on locomotor circuitry. Kniffki and coworkers have observed that stimulation of group III and IV afferents can either accentuate an ongoing fictive locomotor rhythm or block the rhythmic output by inducing a period of tonic hyperactivity.³⁷ In healthy humans, activation of nociceptive afferents may only have mild inhibitory effects on locomotor function, as supraspinal modulation may limit their activity. Electrical stimulation of mesencephalic locomotor region in the cat inhibits group III and IV transmission in the dorsal horn.³⁸ After SCI, however, central pattern generators in the lumbar cord become much more reliant on peripheral inputs for their activation,³⁹ and, therefore, locomotor circuitry is also likely to be more vulnerable to inhibitory effects of group III and IV afferents. Moreover, nociceptive afferents undergo substantial plasticity after SCI, which has been implicated in the development of neuropathic pain^{40,41} and autonomic dysreflexia.⁴² Activation of nociceptive afferents impairs locomotor recovery after SCI in rodents, and spinal learning in rats^{43–45} and humans.⁴⁶ Therefore, it would not be surprising that nociceptive afferents are also involved in mediation of the negative effects of stretching on locomotor function.

Conclusion

In conclusion, muscle stretching is a commonly used technique aimed at increasing/maintaining joint ROM, and is used across multiple populations from the athletic to the clinical. We have now shown that both static and dynamic stretching applied to the hindlimbs of spinal cord injured animals result in the dramatic but temporary disruption of their locomotor function. The physiological

TABLE 2. PERCENT OF MUSCLE FIBERS WITH CENTRALIZED NUCLEI

<i>Muscle</i>	<i>Percent of muscle fibers with centralized nuclei</i>	
	<i>Group 1 (n = 4)</i>	<i>Group 2 (n = 4)</i>
Tibialis anterior	3.77% ± 1.62	4.7% ± 1.81
Medial gastrocnemius	8.21% ± 2.06 ^a	4.18% ± 1.12
Biceps femoris	4.49% ± 2.45	4.65% ± 1.53

Data reported as means ± SD.

Group 1, animals euthanized 2 h after the last stretching session; Group 2, animals euthanized 5 weeks after the last stretching session.

^aGroup 1 had a significantly greater percentage of muscle fibers with centralized nuclei in the medial gastrocnemius muscle than Group 2 ($p < 0.05$).

mechanism of this phenomenon is not yet known, although based on our observations and histological findings it is unlikely to be caused by frank muscle damage, but rather may involve activation of nociceptive afferents. The clinical relevance of our findings needs to be established, but given that static stretching has been shown to have detrimental effects on performance in healthy humans,^{9,14,47} it is conceivable that after SCI, the motor circuitry is even more vulnerable to the negative effects of stretch.

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Author Disclosure Statement

No competing financial interests exist.

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