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Kinematic analysis of treadmill walking in normal and contused mice using the Treadscan system.

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KINEMATIC ANALYSIS OF TREADMILL WALKING IN NORMAL AND CONTUSED MICE USING THE TREADSCAN® SYSTEM

By

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B.S., Centre College, 2002

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ABSTRACT

KINEMATIC ANALYSIS OF TREADMILL WALKING IN NORMAL AND CONTUSED MICE USING THE TREADSCAN® SYSTEM

Jason Beare

July 18, 2007

Activity-based rehabilitation is important for clinically treating spinal cord injury (SCI). Advances in SCI research are dependent on quality animal models, which rely on our ability to detect functional differences in animals following injury. The purpose of this study is to analyze the gait of normal and contusion-injured mice using the TreadScan® system. TreadScan® utilizes a transparent treadmill belt and a high-speed camera to capture the footprints of animals and automatically analyze gait characteristics. Adult female C57Bl/6 mice were gentled and introduced to the treadmill. Animals received either a standardized mild or moderate contusion injury or a sham injury. TreadScan® gait analyses were performed weekly for ten weeks and compared with scores on the Basso Mouse Scale (BMS). Animals were perfused, and the spinal cords assessed histologically. Results indicate that the TreadScan® system will allow for a more objective, rapid behavioral assessment of locomotor function following SCI than previous measures.

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INTRODUCTION

The successful treatment of spinal cord injury (SCI) in humans is dependent on reliable animal models. Numerous studies have focused on the behavioral deficits following SCI in cats, rats, and monkeys. Rats have long been considered the most advantageous model of SCI due to the relatively low cost of purchasing and caring for the animals, less difficulty in screening procedures for possible treatments of SCI, and well-established protocols for producing reliable, consistent, and graded injuries (Kuhn and Wrathall, 1998). The advent of the transgenic mouse has shifted the focus of SCI research towards a murine model. These mutations, both naturally-occurring and genetically engineered, could offer invaluable insight into the mechanisms of SCI and possible therapeutic agents (Kuhn and Wrathall, 1998; Farooque, 2000; Guertin, 2005; Basso et al., 2006). A number of recent studies using transgenic mice have led to increased understanding of the role of various proteins in the biological response to SCI (Table 1).

As more studies begin to utilize mouse models of SCI, the search for a reliable, consistent, and objective behavioral assessment tool becomes more vital than ever (Table 2). Many assessment measures exist, each with its own advantages and disadvantages. Open-field assessments offer valuable insight into the animal's behavior in a natural setting. Until recently, many of the open-field assessment measures used for the mouse were variations of assessment tools designed for the rat. For example, several studies (Farooque, 2000; Mikami et al., 2002; Hsu et al., 2006; Stieltjes et al., 2006) utilized the Basso, Beattie, & Bresnahan scale (1995) to assess mouse behavior following SCI, despite the fact that this 21 point assessment tool was created specifically for scoring motor deficits in the rat. Other laboratories (Ma et al., 2001; Joshi and Fehlings, 2002a,b; Apostolova et al., 2006; Li et al., 2006) attempted to modify the BBB, taking into account that some measures such as coordination and toe clearance are difficult to evaluate in the mouse (Ma et al., 2001).

While these adaptations of rat assessment tools provided valuable information of locomotor functioning in the mouse following SCI, it was clear that a more specific mouse scale was necessary to provide the most accurate data. Basso et al. (2006) created the Basso Mouse Scale (BMS), a nine point assessment scale designed specifically for studying mouse behavior. The creators of this tool were mindful of the difficulties of studying behavior in very small animals. Toe clearance is not assessed using the BMS, and coordination is scored as None, Some, or Most in order to avoid potential confusion; scoring on the BBB requires the rater to determine whether coordination is Occasional, Frequent, or Consistent. In addition to high inter-rater reliability, the BMS has significant face validity (sensitive to locomotor recovery across lesion severity and time) and predictive validity (correlates significantly with spared white matter). Overall, the BMS has proved reliable for assessing the locomotor activity of several strains of mouse following SCI (Basso et al., 2006).

The BMS is not free of criticism, however. Both the inherent subjectivity of this measure as well as its ordinal nature are recognized by the assessment's creators as intrinsic disadvantages of the tool. The BMS relies on the visual observation of two raters, and while the creators of the scale were careful in their definitions of motor behaviors, the fact remains that the small, rapid movements of the mouse may be difficult to assess. The most significant example of this is coordination. The mouse often moves in quick bursts throughout the open field, making observation of a one-to-one relationship between forelimbs and hindlimbs very difficult to prove (Basso et al., 2006). In addition, the scale provides ordinal data, leading to potentially confusing results following treatment. For example, if a therapeutic agent increases the final BMS score from a 3 to a 4, this seemingly small increase has profound effects on the animal's motor abilities, as the animal has improved from no stepping to stepping. However, a treatment that increases the final BMS score from a 1 to a 2 does not provide as much biological relevance (Basso et al., 2006).

With limitations of open-field assessment tools in mind, other behavioral tools have been created and implemented over the years. Many of these tools involve the observation and rating of the animal's performance on natural behavioral tasks. For example, the righting reflex provides insight into the animal's ability to right itself when dropped from an upside-down position onto a cushion; scores range from 0 (no righting reflex) to 3 (animal rights itself immediately after it is dropped) (Farooque, 2000). The inclined plane task focuses on the maximum degree of incline that animal can hold for 5 seconds when facing downward; the maximum angle is the final score (Kuhn and Wrathall, 1998; Li et al., 2006). The ladder rung task has several variations: the animal is forced to walk across either a horizontal or an inclined ladder with rungs of either equal or uneven spacing. In the ladder rung task, the observer counts the number of mistakes, misses, or slips by each animal (Apostolova, 2006; Farr et al., 2006; Hsu et al., 2006). A variation of the ladder rung task is the grid walk, in which the animal walks on a wire mesh grid and the observer counts the number of foot misses (Ma et al., 2001). Beam walking involves an assessment of fine locomotor function; the animal is scored based on the narrowest beam it is able to traverse without slipping or falling (Farooque et al., 2006). The hindlimb Motor Function Score (MFS) is a ten-point scale. The animal is rated 0 to 5 based on its performance in the open-field. Animals that demonstrate normal movement (score = 5) then undergo a beam walking task, with additional points awarded based on the narrowest beam the animal is able to traverse (Farooque et al., 2006). Finally, footprint analysis involves inking the paws of the animal and manually calculating gait characteristics such as forelimb-hindlimb coordination, paw rotation, and hindlimb base of support (Ma et al., 2001; Faulkner et al., 2004). While all of these assessment tools provide insight into the animal's performance on the specific task, it is debatable whether any of this information is clinically relevant to human patients. For instance, many of these behavioral measures look specifically at fine motor control, whereas human SCI patients often have limited or no gross motor control. It is difficult to understand how an improvement in a mouse's ability to traverse a 1-cm steel bar reflects a treatment's efficacy in improving locomotor function in a human patient.

Several recent advancements in computer-assisted locomotor scoring tools have begun to address the need for objectivity in assessment of behavior following SCI. For example, electromyographic (EMG) recordings measure the onset and burst duration of muscle activation during locomotion (Fortier et al., 1987; Leblond et al., 2003). In addition, the automated animal movement analysis system SCANET utilizes infrared beam sensors to scan small horizontal movements, large horizontal movements, and vertical rearing across an open-field. Rearing seems to be the best reflection of SCI severity, as animals that are more severely injured lack the hindlimb stability to support weight for rearing activity (Mikami et al., 2002). Robotic step training utilizes a motor driven treadmill belt and robotic arms to train the spinal mouse to step. The number of steps and quality of stepping are assessed following training via manual placement of limbs by a robotic training algorithm (Cai et al., 2006). The Rotorod measures a variety of behaviors including posture, head position, limb lift, limb carry, limb advance, limb placement, and stride length as the animal moves on a rotating drum. These measures are calculated manually by the experimenter (Faulkner et al., 2004; Farr et al., 2006; Hsu et al., 2006). A computerized locomotor activity tool utilizes force transducers to provide objective data collection as the animal moves in the open-field. This system measures overall locomotor activity (i.e. distance traveled) and whole-body tremors in the open-field (Fowler et al., 2001; Farooque et al., 2006). Finally, kinematic analysis provides a wealth of information regarding hindlimb position, joint movement, joint angle displacement, and step cycle analyses. This information is gathered by placing markers on anatomical features such as toe, ankle, knee, hip, and iliac crest, then manually creating stick diagram representations of the hindlimb during either locomotor or swimming tasks (Leblond et al., 2003; Guertin, 2004; Guertin and Steuer, 2005). This is a highly useful, albeit highly time-consuming, method for analyzing behavior following SCI.

Gait analysis assessment measures are especially useful in quantifying locomotor behavior following SCI. Computer-assisted footprint analysis – sometimes referred to as the CatWalk system – allows the experimenter to manually identify step cycles and calculate gait characteristics such as velocity, stance time, swing time, and stride length as the animal traverses a clear stationary walkway (Clarke and Still, 1999; Hamers et al., 2001; Apostolova, 2006; Hamers et al., 2006). The major drawback of this system is that the animal is allowed to traverse the walkway at its own pace; there is no ability to achieve a constant speed for comparing individual animals. A clear belt-driven treadmill device offers the same ability to manually identify step cycles and calculate gait characteristics, but with the added advantage of speed control (Leblond et al., 2003; Guertin et al., 2004; Herbin et al., 2004; Kale et al., 2004; Amende et al., 2005). Until recently, the drawback of the treadmill system was the time-consuming nature of the video analysis. Individual footprints were identified manually, and many of the gait characteristics such as stance time, swing time, stride length, paw rotation, hindlimb base of support, etc, were measured and/or calculated by the experimenter. Table 2 summarizes all aforementioned mouse behavioral measures, including sample references that have utilized each assessment tool.

The purpose of the present study is to characterize a new motor driven treadmill device in conjunction with the TreadScan® software system (CleverSys, Inc, Reston, VA) for gait analysis. Similar to the computer-assisted footprint analysis method, animals are recorded from underneath by a high-speed digital camera as they walk across a clear surface. The treadmill allows the experimenter to control for speed, and with minimal training the TreadScan® software automatically detects the individual footprints of the animal. To assess the system's utility in differentiating normal from SCI animals, adult female C57BL/6 mice were recorded on the treadmill both before and following SCI. In addition, we wanted to determine if the TreadScan® system is capable of detecting differences between a mild and a moderate contusion SCI. Finally, Kuhn and Wrathall (1998) indicate that mice have the ability to recover from the initial injury, often reaching a plateau on behavioral measures around two to three weeks post-injury. Other studies confirm the initial recovery phase that peaks around two weeks post-injury (Ma et al., 2001; Basso et al., 2006). We used the sensitivity of the TreadScan® system to ascertain if functional recovery continues beyond this initial recovery phase, examining the progress of injured animals for 10 weeks.

METHODS

Treadmill Training

Adult female C57BL/6 mice (20 g) were used to obtain normal baseline levels for mouse treadmill locomotion. Prior to treadmill introduction, mice were gentled in the BMS field for four sessions over a period of one week. Initial BMS scores were obtained before treadmill training to assure that all animals performed at the highest behavioral level (BMS = 9). An initial group of mice (n = 15) was trained to walk on a motor driven treadmill belt at a constant speed of 15 cm/sec for 20-second periods. This group received 34 training sessions over a period of seven weeks. A second (n = 20) and third group (n = 20) were trained on the treadmill at 15 cm/sec for 20-second periods for 12 training sessions over a three week period or five training sessions over a ten day period, respectively. The final three training sessions were recorded for each group to serve as the baseline for normal locomotion. The response rates of each animal were recorded throughout the three recording sessions, in order to assess which training group performed most frequently on the treadmill. The treadmill speed of 15 cm/sec was chosen based on the work of Heglund and Taylor (1988), which suggests that the mouse changes its locomotor pattern from a walk to a trot at 19 cm/sec. Since our interest was in the walking pattern of the mouse, we chose a baseline treadmill speed of 15 cm/sec, a speed also favored by Leblond et al. (2003).

Surgical procedures and postoperative care

All animal care and surgical interventions were undertaken in strict accordance with the Public Health Service Policy on Humane Care and Use of

Laboratory Animals, Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996), and with the approval of the University of Louisville Institutional Animal Care and Use Committee.

Fifty-five adult female C57BL/6 mice (20g) were purchased from Jackson Laboratory (Bar Harbor, ME). Of these, 37 mice were consistently able to walk on the treadmill, and were divided randomly into three injury groups. A first group (n = 12) received mild contusion injuries. A second group (n = 11) received moderate contusion injuries. The final group (n = 9) were sham controls. The remaining five mice were excluded from the study due to overly severe injuries. The mice were anesthetized using 0.1 ml ketamine/xylazine administered by intraperitoneal injection. A dorsal laminectomy was performed at the ninth thoracic vertebral (T9) level to expose the spinal cord. Mice were placed in a custom stabilizer device which holds the spinal cord level and steady for impaction using the Louisville Injury System Apparatus (LISA)(Zhang et al., 2007b). Briefly, the LISA utilizes a laser sensor to measure the velocity and displacement of an injury obtained via a pneumatically-driven impactor. Mice in the mild injury group received a 0.25 mm displacement contusion at a velocity of 1.0 m/s. Mice in the moderate injury group received a 0.40 mm displacement contusion at a velocity of 1.0 m/s. Sham control mice remained uncontused and were sutured after receiving a T9 laminectomy. After surgery, animals were given 1 cc of sterile saline subcutaneously; 0.1 cc of Gentamycin intramuscularly on the day of surgery, day 3, and day 5 post-surgery; and 0.1 ml Bupronorphine subcutaneously on the day of surgery, day 1, and day 2 post-surgery. Animals were placed on a heating pad until full recovery from anesthesia. Postoperative care included the manual expression of bladders twice a day for seven to ten days or until spontaneous voiding returned. The animals were sacrificed 10 weeks post-injury.

Recording and analysis procedures

The treadmill device was purchased from Columbus Instruments (Columbus, OH). Briefly, the tread consisted of a motor driven transparent treadmill belt. A high-speed digital video camera was mounted below the treadmill; digital video images of the underside of the mouse were recorded at 100 frames/second. An adjustable compartment measuring 17 cm by 5 cm was mounted over the treadmill belt, ensuring that the mouse would remain in the view of the camera at all times. TreadScan® software (CleverSys, Inc, Reston, VA) identified each individual paw of the mouse in each frame as it walked on the treadmill. With minimal training, this software was used to correctly identify stance duration, stride duration, swing duration, stride length, track width, and toe spread data for each foot. Software training was performed by the experimenter with assistance provided from a CleverSys, Inc. representative. Briefly, a training session video was chosen for software training based on its representation of normal mouse locomotion. The outline of each paw was drawn on the computer screen using the software's built-in tracing system. Care was taken to ensure that only the paws were selected; the software uses color differentiation to identify paw placement. A sampling of 10-12 outlines for each paw was sufficient to train the software to correctly identify paw placement and liftoff. Mice were walked on the treadmill for 20-second sessions, resulting in 2000 captured frames. For each 20-second session, the video was previewed to determine the best four- to ten-second window of consistent walking for video analysis. Shorter windows were utilized when animals walked at higher speeds, due to the increased number of strides per second. Similarly, longer windows were necessary for slower speeds. These windows were chosen subjectively to include representative step cycles of the animal's best performance on four to six consecutive step cycles for each foot; fewer strides have been shown to increase variability, while additional strides do not tend to reduce variability (Wooley et al., 2005).

Beginning one week post-injury and continuing once weekly for 10 weeks, BMS scores were obtained for all mice by observers who had completed BMS training at Ohio State University under the direction of Dr. Basso and other faculty who designed the scale (Basso, 2006). BMS observers were blinded to the treatment group. On the same day as BMS testing, mice from the mild, moderate, and sham groups were run on the motor-driven treadmill at constant speeds. Animals in the moderate injury group were walked at speeds of 5, 7, & 10 cm/s once weekly for 10 weeks. Animals in the mild and sham injury groups were walked at speeds of 7, 10, & 15 cm/s once weekly for 10 weeks.

Electrophysiology

All transcranial magnetic motor-evoked potential (tcMMEP) recordings were obtained from stimulation of awake, nonanesthetized, restrained mice, as described previously for rats (Magnuson et al., 1999; Loy et al., 2002; Cao et al., 2005) and mice (Hill et al., 2007). Briefly, mice were placed in a prone position on a wooden board and wrapped in a cloth stocking tacked to the board surface. The hindlimbs and tail were left exposed to enable the insertion of needle electrodes into the gastrocnemius muscle of each hindlimb, with the active electrode placed into the gastrocnemius muscle belly and the reference electrode placed near the distal tendon. The ground electrode was placed in the base of the tail. tcMMEP responses were elicited using a magnetic stimulator with a 5.0 cm coil placed over the cranium. A single magnetic pulse with 100% intensity was performed twice with each animal to ensure accuracy. The onset latency to the initiation of response and amplitude were recorded at eight weeks post injury. One mildly injured animal showed normal tcMMEP responses and was therefore removed from the study.

Histochemistry and Analysis

Following final behavioral and physiological assessments, the mice were sacrificed at ten weeks post-injury. They were anesthetized with a solution of 60% ketamine 40% xylazine (0.25 ml/20 g) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA), consecutively. The spinal cords were dissected, submerged in PFA solution for one hour, and stored in a 30% sucrose solution overnight at 4° C. The spinal cords were cut into one cm segments centered on the epicenter, embedded in tissue freezing medium (Triangle Biomedical Sciences, Durham, NC), flash frozen on dry ice, and stored at -80° C. Serial 30 μm thick sections spanning the injury site were cut coronally and stored at -80° C. After thawing, iron eriochrome cyanine (EC) staining was done to delineate spared myelin. Slides were cover-slipped with Cytoseal® (Fisher, Atlanta, GA) and dried in the ventilated hood overnight. The total cross-sectional area of the spinal cord and the lesion boundary were captured with an Olympus BX60 microscope and measured and analyzed using Neurolucida® (Microbrightfield Inc., Colchester, VT). The epicenter of each injury was determined based on the section with the least amount of spared white matter. Randomization of the epicenter sections allowed an unbiased quantification. The data were normalized to find an estimated area at the epicenter of the injury. This was confirmed by evaluation of uninjured animals. The spared white matter area at the epicenter was compared to normal average white matter area at the same location. A percent of spared tissue score was calculated for each animal. At this point, the code was broken and the subjects were divided into mild, moderate, severe, and sham groups. Mean values of percent spared white matter area were compared statistically using one-way ANOVA followed by Tukey's HSD post hoc testing.

Statistical Analyses

To assess the optimal number of training sessions for non-injured animals, Chi-square analyses were run on baseline response rates for each training group. Repeated Measures Analyses of Variance (ANOVA) were run on baseline data to determine any gait parameter differences between recording sessions. If no significant differences were found, baseline data from the three recording sessions were averaged for each group, resulting in an average baseline performance for each training group. Repeated Measures ANOVA with Tukey's post hoc analyses were run on the new averages to assess whether any significant differences existed in the gait characteristics of the training groups.

Following injury, Repeated Measures ANOVA with Tukey's HSD post hoc t-tests were run on hindlimb gait characteristics across speeds at an early time point (Week 2) and at a late time point (Week 10) to reveal how the animals responded to changes in treadmill speed, and whether responses changed as animals recovered over time. To assess whether hindlimb gait characteristics changed over time, as well as to determine whether any differences existed between injury groups, Repeated Measures ANOVA with Tukey's HSD post hoc t-tests were run on gait parameter data over the entire ten week testing period. For Sham and Mild animals, weeks 1, 2, 3, 4, 5, 6, and 10 were analyzed at 7 cm/sec. At 10 cm/sec, weeks 2, 3, 4, 5, 6, 8, and 10 were examined for the Sham and Mild groups. For 15 cm/sec data, baseline data as well as data from weeks 2, 4, 5, and 10 were investigated for the Sham and Mild groups. Due to the lower number of animals performing the treadmill task in the Moderate injury group, fewer weeks were used in these ANOVA. At 5 cm/sec, weeks 1, 2, 3, 5, 6, and 8 were analyzed. At 7 cm/sec, weeks 2, 4, 6, and 8 were evaluated. At 10 cm/sec, only weeks 2, 5, and 10 were examined. The data points for these analyses were chosen in order to obtain a representative sampling of early, middle, and late time points.

To reveal whether any differences existed in spared white matter between injury groups, a One-Way ANOVA was run on the spared white matter percentage for each injury group. In addition, Pearson 2-tailed correlations were run on several of the hindlimb gait parameters and spared white matter percentage, BMS and spared white matter percentage, and hindlimb gait parameters and BMS. In addition, a correlation was run on hindlimb swing time and hindlimb stride length. In order to minimize the number of correlations being run, the data was split into early weeks (1, 2, 3, and 4) and late weeks (5, 6, 8, and 10) for these correlations.

RESULTS

Baseline

For baseline recordings, all animals were recorded for a total of three training sessions. Repeated measures ANOVA were run to determine whether any significant differences existed between the training sessions. A between sessions difference was found in right hindlimb stride length (F=5.605, df=2, 54, p=.006), left hindlimb stride length (F=6.589, df=2, 54, p=.003), and left hindlimb swing time (F=5.429, df=2, 54, p=.007). Tukey's HSD post hoc t-tests revealed only one significant difference. The group with the least amount of training had a significantly longer right hindlimb stride length during its third baseline session than its first baseline session (t=7.1, df=3, 54, p<.05). No other significant differences existed between sessions. Due to the very low incidence of significant differences between training sessions, we were comfortable averaging the three baseline sessions for each gait parameter to give an overall baseline level for each animal.

Animals that received the least amount of training prior to recording sessions performed more consistently than animals that received higher levels of training. For the three baseline recording sessions (treadmill speed = 15 cm/sec), the response rate for mice receiving only five training sessions (Low Training) was 93.33%. In contrast, animals that received 12 training sessions (Medium Training) had a response rate of 65%, while animals with 34 training sessions (High Training) exhibited a response rate of only 57.78% (Figure 1). A Chi Square Analysis of this data indicated that the Low Training group had a significantly higher response rate than both the Medium Training and the High Training groups (p<.05). In addition, animals in the

Low group showed significant differences in gait characteristics compared to more extensively trained animals.

Due to the location of the contusion injury at the T9 level, we were only interested in differences in hindlimb gait parameters. Thus, forelimb data were ignored in all statistical analyses. Left hindlimb stance was longer in the Medium group than in the Low group ($p<.05$), whereas right hindlimb stance times were longer in the High group than in the Low group ($p<.05$). The Medium training group approached a significantly longer right hindlimb stance time than the Low group ($p=.051$). Figure 2A shows hindlimb stance differences between training groups.

Left hindlimb stride length was longer in the Medium ($p<.01$) and High ($p<.01$) groups than in the Low group. In addition, Medium ($p<.01$) and High ($p<.01$) groups had longer right hindlimb stride lengths than the Low training group. Figure 2B shows hindlimb stride length differences between training groups.

Due to the higher response rates and lower standard deviations of the Low training group, we believe this group's data to be the most representative of baseline locomotion in the intact mouse. At 15 cm/sec, the C57BL/6 mouse exhibited left forelimb stance and swing times of 195.93 ± 17.75 ms and 124.75 ± 12.81 ms, respectively. The right forelimb stance and swing times were 204.51 ± 12.60 ms and 114.31 ± 12.76 ms. For the hindlimbs, the left stance and swing times were 219.38 ± 17.94 ms and 103.50 ± 11.94 ms, while the right stance and swing times were 221.26 ± 16.40 ms and 100.07 ± 11.57 ms. Left forelimb stride length at 15 cm/sec was 47.48 ± 3.39 mm, right forelimb stride length was 47.54 ± 3.59 mm, left hindlimb stride length was 47.67 ± 3.61, and right hindlimb stride length was 47.86 ± 3.61 mm. Rear track width, which measures an animal's hindlimb base of support, was measured to be 23.85 ± 1.69 mm in the intact mouse. Finally, hindlimb toe spread, or the distance between the first and fifth digits on the rear paws, was 7.86 ± 0.58 mm for the left paw and 7.48 ± 0.36 mm for the right paw. Table 3 shows baseline data for all three training groups.

BMS Differences

Animals from all three training groups (High, Medium, and Low) were divided randomly into Sham, Mild, or Moderate injury groups. Figure 3A shows weekly BMS scores of each injury group for ten weeks post-injury (wpi). A BMS difference was found between injury groups. Sham animals exhibited significantly higher BMS scores than the Moderate injury animals at Weeks 1, 2, 3, 4, 6, 8, and 10. Mild injury animals showed higher BMS scores than Moderate injury animals at Weeks 1, 2, and 8 (Figure 3A). In addition, Moderate animals exhibited a change in BMS over time; in these animals, Week 1 BMS scores were significantly lower than Weeks 3, 4, 5, 6, 8, and 10 (Figure 3B). Terminal BMS scores were 8.78 ± 0.44 for Shams, 7.68 ± 1.60 for the Mild group, and 5.41 ± 1.45 for the Moderate group.

Speed Differences

Hindlimb gait characteristics across speeds at an early time point (Week 2) and a late time point (Week 10) were analyzed specifically to reveal how the animals respond to changes in treadmill speed, and whether responses changed as animals recovered over time. At 2 wpi, as speed increased from 7 cm/sec to 10 cm/sec, Sham animals shortened their right hindlimb stance time by 22% ($p<.01$), and left hindlimb stance times were shortened by 23% ($p<.01$). Similarly, as speed increased from 10 cm/sec to 15 cm/sec, Sham animals shortened their right hindlimb stance times by 27% ($p<.01$), and their left hindlimb stance times by 27% ($p<.01$). Figure 4A represents right hindlimb speed-dependent stance differences in all injury groups at two weeks post-injury. In Sham animals, right hindlimb stride length increased by 13% (p<.01) and left hindlimb stride length increased by 22% (p<.01) as speed increased from 7 cm/sec to 10 cm/sec. A similar stride length speed dependence was found as speed increased from 10 cm/sec to 15 cm/sec, as right hindlimb stride length increased by 16% (p<.01) and left hindlimb stride length increased by 15% (p<.01). Figure 5A represents right hindlimb speed-dependent stride length differences in all injury groups at two weeks post-injury. Repeated measures ANOVA also indicated that rear track width, a measure of the animal's hindlimb base of support, was dependent on speed in Sham animals (F=9.682, df=2, 14, p=.002). Tukey's HSD post hoc t-tests revealed that rear track width was 8% wider in the 7 cm/sec condition than in the 10 cm/sec condition (t=2.4, df=3, 14, p<.05), and 12% wider in the 7 cm/sec condition than the 15 cm/sec condition (t=3.7, df=3, 14, p<.01).

Repeated measures ANOVA indicated a speed difference in both right (F=7.660, df=2, 14, p=.006) and left (F=6.013, df=2, 14, p=.013) toe spread in the Sham group. Tukey's HSD post hoc t-tests indicated that right hindlimb toe spread was 5% wider at 7 cm/sec than at both 10 cm/sec (t=0.4, df=2, 14, p=.01) and 15 cm/sec (t=0.4, df=2, 14, p=.01); left hindlimb toe spread was 6% wider at 7 cm/sec than at 15 cm/sec (t=0.5, df=3, 14, p=.01) and 5% wider at 10 cm/sec than at 15 cm/sec (t= 0.4, df=3, 14, p=.05).

In Mild animals 2 wpi, left hindlimb stance time was only significantly shorter for 15 cm/sec compared to 7 cm/sec (p<.05). No significant speed differences were found for the right hindlimb stance time. With regard to stride length, Mild animals showed 12% longer right hindlimb stride length as speed increased from 7 cm/sec to 10 cm/sec (p<.05), and 18% longer right hindlimb stride length as speed increased from 10 cm/sec to 15 cm/sec ($p<.01$). The left hindlimb stride length was only longer for 15 cm/sec compared to 7 cm/sec ($p<.05$). Rear track width and right hindlimb toe spread did not exhibit significant speed differences in the Mild group. However, repeated measures ANOVA indicated a speed difference in left toe spread ($F=15.951$, $df=2, 6$, $p=.004$). Tukey's HSD post hoc t-tests revealed that left hindlimb toe spread was 6% wider in the 7 cm/sec condition than in the 15 cm/sec condition ($t=0.5$, $df=3, 6$, $p<.01$) and 4% wider at 10 cm/sec than at 15 cm/sec ($t=0.3$, $df=3, 6$, $p=.05$).

At 2 wpi, Moderate animals exhibited a shorter right hindlimb stance time for 10 cm/sec than for 5 cm/sec ($p<.01$) at 2 wpi. Similarly, Moderate animals had 10% shorter left hindlimb stance time as speed increased from 5 cm/sec to 7 cm/sec ($p<.05$), and 29% shorter left hindlimb stance time as speed increased from 7 cm/sec to 10 cm/sec ($p<.01$). Right hindlimb stride length was 17% longer for Moderate animals in the 10 cm/sec condition than in the 7 cm/sec condition ($p<.05$), and 18% longer in the 10 cm/sec than in the 5 cm/sec condition ($p<.05$); left hindlimb stride length was 23% longer for 10 cm/sec than for 5 cm/sec ($p<.05$). Rear track width and rear toe spreads showed no significant differences across speeds in the Moderate group at 2 wpi.

By 10 wpi, Sham animals showed less gait characteristic differences in response to speed changes. Right hindlimb stance times were 33% shorter at 15 cm/sec than at 7 cm/sec ($p<.01$); similarly, these times were 28% shorter at 15 cm/sec than at 10 cm/sec ($p<.01$). Left hindlimb stance times showed similar speed dependence; 15 cm/sec stance times were 33% shorter than 7 cm/sec stance times ($p<.01$), and 28% shorter than 10 cm/sec stance times ($p<.01$). Figure 4B indicates right hindlimb speed-dependent stance time differences in all injury groups at 10 weeks post-injury. Right hindlimb stride length was 11% longer at 15 cm/sec than at

10 cm/sec (p<.05), and 15% longer at 10 cm/sec than at 7 cm/sec (p<.05). Similarly, left hindlimb stride length was 10% longer at 15 cm/sec than at 10 cm/sec (p<.05), and 15% longer at 10 cm/sec than at 7 cm/sec (p<.01). Figure 5B represents right hindlimb speed-dependent stride length differences in all injury groups at 10 weeks post-injury. The significant differences seen at 2 wpi in rear track width and rear hindlimb stride lengths all but disappeared by 10 wpi; only left hindlimb toe spread showed a speed difference on a repeated measures ANOVA (F=4.175, df=2, 16, p<.05). Tukey's HSD post hoc t-tests revealed that left hindlimb toe spread was 5% wider at 7 cm/sec than at both 10 cm/sec (t=0.4, df=2, 16, p=.01) and 15 cm/sec (t=0.4, df=2, 16, p=.01).

In the Mild group, right hindlimb stance times were 23% shorter at 10 cm/sec than at 7 cm/sec (p<.05), and 28% shorter at 15 cm/sec than at 10 cm/sec (p<.05). Left hindlimb stance times were 31% shorter at 10 cm/sec than at 7 cm/sec (p<.05), and 15 cm/sec was 39% shorter than 7 cm/sec (p<.01). Right hindlimb stride lengths were 23% longer at 15 cm/sec than at 7 cm/sec (p<.01), while left hindlimb stride lengths were 26% longer at 15 cm/sec than at 7 cm/sec (p<.01). Rear track width and rear toe spreads showed no differences across speeds by 10 wpi.

In the Moderate group, right hindlimb stance times were 22% shorter at 7 cm/sec (p<.01) than at 5 cm/sec, and 37% shorter at 10 cm/sec than at 5 cm/sec (p<.01). Left hindlimb stance time was 19% shorter at 7 cm/sec than at 5 cm/sec (p<.05), and 22% shorter at 10 cm/sec than at 7 cm/sec (p<.05). Right hindlimb stride length showed no differences across speeds at 10 wpi. Left hindlimb stride length was 13% longer at 10 cm/sec than at 5 cm/sec (p<.05). Rear track width and hindlimb toe spreads did not show any significant differences across speeds at 10 wpi in the Moderate group.

Differences between weeks

Repeated measures ANOVA with Tukey's HSD post hoc t-tests were run on hindlimb gait parameter data across weeks. A difference in left hindlimb stride length across time was found in the Sham group at 7 cm/sec (F=12.590, df=6, 48, p<.001). The left hindlimb stride length was 42% longer at Week 5 than at Week 1 (t=15.9, df=7, 48, p<.01), 32% longer at Week 5 than at Week 2 (t=13.1, df=7, 48, p<.01), 27% longer at Week 5 than at Week 3 (t=11.6, df=7, 48, p<.01), and 18% longer at Week 5 than at Week 10 (t=8.1, df=7, 48, p<.05). In addition, left hindlimb stride length was also 34% longer at Week 6 than at Week 1 (t=12.8, df=7, 48, p<.01), 25% longer at Week 6 than at Week 2 (t=10.0, df=7, 48, p<.01), and 20% longer at Week 6 than at Week 3 (t=8.5, df=7, 48, p<.01). Left hindlimb stride length was also 25% longer at Week 4 than Week 1 (t=9.5, df=7, 48, p<.01), and 21% longer at Week 10 than Week 1 (t=7.8, df=7, 48, p<.05). No significant differences were found on the right side in Sham animals at 7 cm/sec.

Repeated measures ANOVA indicated a difference in hindlimb base of support over time in the Sham group in the 7 cm/sec condition (F=2.782, df=6, 48, p=.021). Tukey's HSD post hoc t-tests revealed that hindlimb base of support was 11% wider at Week 2 than at Week 5 (t=3.0, df=7, 48, p<.05), 12% wider at Week 2 than at Week 6 (t=3.1, df=7, 48, p<.05), and 11% wider at Week 2 than at Week 10 (t=2.9, df=7, 48, p=.05). Repeated measures ANOVA indicated a difference over time in left hindlimb stance percentage and swing percentage in Sham animals in the 7 cm/sec condition (F=3.668, df=6, 48, p=.004). Tukey's HSD post hoc t-tests revealed left hindlimb stance percentage was 5% higher at Week 4 than at Week 1 (t=0.04, df=6, 48, p=.05), 6% higher at Week 2 than at Week 1 (t=0.05, df=6, 48, p=.01), and 5% higher at Week 2 than at Week 5 (t=0.04, df=6, 48, p=.05). Stance and swing percentages are by definition related with one another; thus, differences seen in left hindlimb stance percentages are exactly the opposite of differences seen in left hindlimb swing percentages. Table 4 provides a summary of all differences over time in the Sham group at 7 cm/sec.

In the 10 cm/sec speed condition, a repeated measures ANOVA indicated a difference across time in right hindlimb toe spread in the Sham group (F=4.382, df=6, 42, p=.002). Tukey's HSD post hoc t-tests revealed that animals in the Sham group exhibited an 8% wider rear right toe spread at Week 5 than at Week 1 (t=0.6, df=5, 42, p=.01), 6% wider at Week 5 than at Week 8 (t=0.5, df=5, 42, p=.05), and 6% wider at Week 6 than at Week 1 (t=0.5, df=5, 42, p=.05). At 15 cm/sec, no significant differences were uncovered between weeks in the Sham group.

In the Mild group, a repeated measures ANOVA indicated a difference over time in the rear right toe spread in the Mild group at 7 cm/sec (F=2.552, df=6, 48, p=.032). Tukey's HSD post hoc t-tests revealed that right toe spread was 12% wider at Week 4 than at Week 1(t=0.8, df=6, 48, p<.05), and 10% wider at Week 10 than at Week 1 (t=0.7, df=6, 48, p=.05). Mild animals' hindlimb base of support was 13% wider at Week 2 than at Week 1 (p<.05), 12% wider at Week 4 than at Week 1 (p<.05), 13% wider at Week 5 than at Week 1 (p=.01), 12% wider at Week 6 than at Week 1 (p<.05), and 14% wider at Week 10 than at Week 1 (p<.01)(Figure 6). Table 5 exhibits differences across time for the Mild group at 7 cm/sec.

At 10 cm/sec, repeated measures ANOVA revealed no significant differences over time in the Mild injury group.

At 15 cm/sec, we were only able to consider four time points due to the small n in the Mild group at this speed. We chose to analyze the Baseline data in conjunction with Weeks 2, 4, and 10, in order to compare uninjured data with early, middle, and late time points. Baseline right hindlimb swing time was 55% longer than Week 2 (p<.01), 54% longer than Week 4 (p<.01), and 75% longer than Week 10 (p<.01). Figure 7 exhibits these right hindlimb swing differences. No other significant differences were gleaned from the 15 cm/sec data.

In the Moderate injury group, no significant differences were found across time in any of the three speed conditions.

Differences between groups

To assess the TreadScan® system's ability to differentiate injured from non-injured animals, Repeated Measures ANOVA with Tukey's HSD post hoc analyses were run between injury groups. At 7 cm/sec, the left (F=14.213, df=2, 18, p<.001; t=38.5, df=3, 18, p<.05) hindlimb swing times were 53% longer in the Sham group than in the Mild group at Week 5. Similarly, the right (F=16.523, df=2, 18, p<.001; t=43.8, df=3, 18, p<.05) hindlimb swing times were 65% longer in the Sham group than in the Mild group at Week 5. All other weeks were non-significant for hindlimb swing times. Right hindlimb stride length was 38% longer in the Sham than in the Mild (F=8.701, df=2, 18, p=.002; t=15.0, df=3, 18, p=.01), and 52% longer in the Sham than in the Moderate (t=18.8, df=3, 18, p<.05) at Week 5. Left hindlimb stride length was 41% longer in the Sham group than in the Mild group at Week 5 (F=8.254, df=2, 18, p=.003; t=15.7, df=3, 18, p<.05). Right hindlimb toe spread was 63% wider in the Sham group than in the Moderate group at Week 1 (p<.05), 61% wider in the Sham group than in the Moderate group at Week 3 (p<.05), and 45% wider in the Sham group than in the Moderate group at Week 4 (p=.05)(Figure 8). Rear track width exhibited a significant group difference in the repeated measures ANOVA (F=7.536, df=2, 18, p=.004); however, Tukey's HSD post hoc t-tests did not reveal any significant group differences at any time point (Figure 9). It is important to note that the relationship between injury and rear track width seems evident, and future studies will attempt to increase the sample size for each group, in order to reveal whether rear track width is truly affected by SCI (Figure 10). Right hindlimb stance percentage was 6% - 8% higher in the Mild group than the Sham group at Week 1 (F=20.172, df=2, 18, p<.001; t=0.05, df=3, 18, p=.05), Week 3 (t=0.06, df=3, 18, p<.05), Week 4 (t=0.05, df=3, 18, p=.05), Week 5 (t=0.06, df=3, 18, p<.05), and Week 10 (t=0.05, df=3, 18, p=.05). Similarly, left hindlimb stance percentage was 5% - 10% higher in the Mild group than the Sham group at Week 1 (F=29.338, df=2, 17, p<.001; t=0.08, df=3, 17, p<.01), Week 3 (t=0.04, df=3, 17, p=.05), Week 5 (t=0.05, df=3, 17, p=.01), Week 6 (t=0.05, df=3, 17, p=.01), and Week 10 (t=0.06, df=3, 17, p<.01). Left hindlimb stance percentage was also 7% - 10% higher in the Moderate group than the Sham group at Week 1 (t=0.05, df=3, 17, p=.05), Week 3 (t=0.07, df=3, 17, p=.01), Week 5 (t=0.07, df=3, 17, p=.01), Week 6 (t=0.06, df=3, 17, p<.05), and Week 10 (t=0.07, df=3, 17, p=.01). Due to the relatedness of stance and swing percentages, these differences in stance percentages are exactly the opposite of differences found in swing percentages at these time points.

At 10 cm/sec, repeated measures ANOVA indicated a difference between groups in left hindlimb stance percentage (F=9.474, df=2, 16, p=.002). Tukey's HSD post hoc t-tests revealed that left hindlimb stance percentage was 8% higher in the Mild group than the Sham group at Week 5 (t=0.06, df=3, 16, p=.05), and 8% higher in the Mild group than in the Sham group at Week 6 (t=0.06, df=3, 16, p=.05). Repeated measures ANOVA indicated a difference between groups in right hindlimb stance percentage (F=8.835, df=2, 16, p=.003); however, Tukey's HSD post hoc t-tests revealed no significant differences between any groups at any time point. Swing percentage differences between groups were exactly the opposite as the aforementioned stance percentage differences.

At 15 cm/sec, right hindlimb stride length is 27% longer in the Sham group than the Mild group at Week 4 (F=6.059, df= 1, 8, p=.039; t=12.4, df=2, 8, p<.05) and 30% longer in the Sham group than the Mild group at Week 5 (t=14.3, df=2, 8, p<.05). No other significant group differences were uncovered at this speed.

Electrophysiological Analyses

tcMMEP responses were recorded from the left and right gastrocnemius muscles at eight weeks post-injury. Of 12 animals in the Mild group, five showed no tcMMEP responses. One animal in the Mild group showed normal tcMMEP responses, and was therefore removed from the study. The remaining six animals in the Mild group exhibited a latency of 5.30 ± 0.56 ms, with a peak-to-peak amplitude of 0.198 ± 0.122 mV. Of 11 animals in the Moderate group, six showed no tcMMEP responses. The remaining five animals exhibited a latency of 5.14 ± 0.52 ms, with a peak-to-peak amplitude of 0.172 ± 0.135 mV. The average tcMMEP latency for a non-injured mouse is 4.79 ± 0.12 ms with a peak-to-peak amplitude of 2.65 ± 1.1 mV (Zhang et al., 2007a). Figure 11 illustrates the differences seen between pre-injury baseline levels and post-SCI tcMMEP data.

Histological Analyses

Following perfusion at ten weeks post-injury, Sham animals had 100% spared white matter, compared with 64.4% spared white matter in Mild injured animals and 37.8% spared white matter in Moderate injured animals (Figures 12 & 13). Results indicate that the Sham group had a significantly higher percentage of spared white matter than both the Mild group and the Moderate group. In addition, Mild animals had a significantly higher spared white matter percentage than Moderate animals.

Correlations

To discover how well performance on the TreadScan® system corresponds to spared white matter, several Pearson 2-tailed Correlations were run. As expected, the percentage of spared white matter correlated strongly with the final BMS score (Figure 14). The best predictors of spared white matter on the TreadScan® included right and left hindlimb swing time at both 7 cm/sec (Figure 15A) and at 10 cm/sec (Figure 15B). Rear track width also correlated well with spared white matter percentage at both 7 cm/sec (Figure 16A) and at 10 cm/sec (Figure 16B).

We were also interested in finding any strong correlations between gait parameters. During the initial recovery phase (Weeks 1 – 4) in the injured animals, right hindlimb swing times correlated well with right hindlimb stride lengths at 7 cm/sec in both the Mild and the Moderate injury groups; similarly strong correlations were found for the left side (Figures 17A,B). Interestingly, these correlations are not nearly as strong during later time points (Figures 18A,B). For example, right hindlimb swing times correlated with right hindlimb stride length at 7 cm/sec at a much weaker level for Weeks 5, 6, 8, and 10 post-injury. Similarly weaker correlations were found on the left side during later weeks.

Finally, we hoped to find a gait parameter that would correlate strongly with BMS scores. In the Mild group, rear track width correlated strongly with BMS scores for both the early time points (Weeks 1 – 4) and the later time points (Weeks 5, 6, 8, and 10) at 7 cm/sec (Figure 19).

DISCUSSION

In this paper, we describe the utility of the TreadScan® software system in conjunction with a motor-driven treadmill device in assessing locomotor function in mice. In naïve animals, we found that increased exposure to the treadmill prior to injury led to training effects. Animals receiving more than minimal exposure to the treadmill prior to injury were much less likely to perform the treadmill walking task (Figure 1), and had more variability in gait characteristics than animals in the lowest training group (Figures 2A,B). To our knowledge, this is the first study to look at training effects in mouse treadmill locomotion. It is our recommendation that other experimenters wishing to utilize a treadmill system be cautious in their training procedures, keeping treadmill exposure to a minimum prior to SCI.

To determine whether TreadScan® could detect differences within gait parameters across differing speeds, we looked at hindlimb stance times and hindlimb stride lengths at an early time point (Week 2) and a late time point (Week 10). Results indicate that the software is in fact sensitive to differences across speeds on such speed dependent gait parameters as stride length and stance time. It is interesting to note that Sham animals were capable of varying both of these gait characteristics in response to increasing speeds; injured animals, however, were impaired in this ability. It appears that early in the recovery process, SCI animals adapted to changes in speed by varying their hindlimb stride length, rather than hindlimb stance time. This is evidenced by the fact that at two weeks post-injury, both injury groups exhibited significant hindlimb stride length differences as speed increased (Figure 5A). However, during the late time point, the animals in both SCI groups no longer showed speed-dependent differences in hindlimb stride length (Figure 5B). Instead, their adaptation to increasing speed had shifted; at ten weeks post-injury, both Mild and Moderate animals decreased their hindlimb stance times as treadmill speed increased. Since Sham animals are able to adjust both stance time and stride length in the face of increasing speeds, it appears as though SCI has a negative effect on the relationship between hindlimb stance time and stride length. Thota et al. (2005) suggest that many locomotor deficits seen following thoracic spinal cord contusion are due to an interruption of supraspinal and propriospinal connections caudal to the injury. Ma et al. (2001) describe compensatory plasticity of remaining connections within the injured spinal cord as a possible mode of recovery following SCI. It seems that the primary phase of injury may lead to the deficits in hindlimb stance time adaptation in response to increasing speeds at two weeks post-injury; plasticity and recovery over the next eight weeks could then explain the animals' shift in adaptation to increasing speeds.

Previous studies into mouse behavior following SCI have found that injured animals exhibit a period of initial recovery that tends to plateau after two to three weeks (Kuhn and Wrathall, 1998; Ma et al., 2001; Basso et al., 2006). Consistent with these findings, our Moderate group showed an initial improvement on the BMS during the first two weeks, then displayed a plateau on BMS scores that did not improve for the remainder of the study (Figure 3B). This appears to indicate that the BMS is sensitive to early improvements in the repertoire of behaviors exhibited by injured animals. Interestingly, TreadScan® did not find any improvements over time in the Moderate group, indicating that perhaps the BMS is a better behavioral assessment for moderately injured animals than a treadmill-based system. This results from the fact that animals that received a moderate injury had difficulty performing the treadmill task, and many were simply unable to perform the requisite four to six consecutive step cycles.

In the Mild injured group, the BMS did not find any differences over the ten week study. Additionally, BMS subscores were unable to detect differences among these animals over time (data not shown). Indeed, this injury was very mild, and some animals were able to achieve a perfect BMS score of 9 following this injury. However, the TreadScan® was sensitive enough to show improvements in the mildly injured group over time. For example, rear track width, a measure of an animal's hindlimb base of support, showed significant improvement towards baseline levels beginning at two weeks post-injury (Figure 6). This is consistent with data from Ma et al. (2001), who reported decreased hindlimb base of support in C57Bl/6 mice in both a mild and a moderate injury. Our data suggests that even in an injury so mild that the BMS does not uncover behavioral deficits, TreadScan® is sensitive enough to discern initial deficits following injury, as well as subsequent improvements over time.

Strengthening the argument that TreadScan® may be more sensitive to mild spinal cord injuries than the BMS is the fact that mildly injured animals differed significantly from their own baseline hindlimb swing times (Figure 7). While the BMS failed to show any deficits in the Mild injury group at any time point, the TreadScan® system discovered that hindlimb swing times were significantly shorter following a mild injury than they were prior to injury. According to Thota et al. (2005), thoracic SCI can lead to deleterious effects in rhythmic locomotor activity, balance, and posture. It appears that in our study, both balance and posture were negatively affected; the animals seem to rush through the hindlimb swing phase due to a lack of balance, and their narrower hindlimb base of support suggests posture deficits. This is especially interesting considering the fact that while hindlimb base of support showed improvement towards baseline levels during initial recovery, hindlimb swing times did not improve toward baseline levels during the course of the study. This suggests that this gait parameter may provide a measuring stick for improvement in future studies. That is, any experimental treatment that leads to an increase in injured animals' hindlimb swing times toward baseline levels may prove useful in treating SCI.

It is interesting to note that the Sham group exhibited more significant changes in gait parameters over time than either of the injury groups. This seems counterintuitive, as one would expect the non-injured animals to perform consistently from week to week. However, our results indicate that most of the changes over time seen in the Sham group occurred at the slowest treadmill setting, 7 cm/sec. Non-injured animals do not prefer to walk at such a slow speed, as evidenced by the fact that baseline animals performed very inconsistently at speeds lower than 12 cm/sec (data not shown). The fact that the Sham animals were forced to walk at this slower speed may have led to the inconsistent gait measurements over time. Indeed, there does not appear to be a logical pattern of change over time; differences across weeks are seemingly random, with stride length differences existing between many weeks, while rear track width only shows a difference at one particular time point. The Sham animals were recorded at this slow speed in order to give as many comparisons between Sham and Injured as possible. However, we believe that this speed is not an accurate descriptor of the locomotor abilities of non-injured animals; it seems that the faster speeds of 10 cm/sec and 15 cm/sec are much more representative of the walking capabilities of the non-injured mouse.

Following spinal cord contusion, the BMS could only consistently distinguish between Sham animals and Moderate injured animals. No differences were found between Sham and Mild animals; differences between Mild and Moderate groups were only found at three time points (Figure 3A). This seems to suggest that the BMS is not as sensitive at uncovering behavioral deficits in mild injuries as it is in more moderate and severe injuries. Interestingly, the TreadScan® system was able to distinguish between Sham and Mild groups rather consistently, despite a lack of behavioral differences on both the BMS and the BMS Subscore (data not shown). For instance, hindlimb swing percentages were consistently higher in the Sham group than in the Mild group. This appears to be related to a decrease in balance in the mildly injured animals; these animals are forced to rush through the swing phase of the step cycle in order to bring the hindlimb back into contact with the treadmill surface, or else they could lose balance and either stumble or fall. As previously mentioned, this is consistent with the findings of Thota et al. (2005) that balance, posture, and locomotor control are all adversely affected by thoracic SCI. Sham animals do not face these balance problems, and thus are able to complete a more fluid, less rushed swing phase typical of a non-injured animal.

While BMS uncovered a group difference at Week 1, Week 2, and Week 8, the TreadScan® system was not able to uncover any differences between Mild and Moderate injury groups at any time point. It is our belief that this lack of differences is attributable to the small sample size of the Moderate group. Many of these animals had to be removed from statistical analyses due to an inability to perform the treadmill task consistently. This resulted in a final sample size of only three animals for many of the analyses. Some gait parameters may indeed exhibit differences between Mild and Moderate injury groups, if more animals are included in the study. For example, right hindlimb toe spread seems to indicate a difference between Mild and Moderate injuries (Figure 8). In addition, hindlimb base of support appears to show a difference between Mild and Moderate groups at several time points, but not enough animals are included to insure that this difference is real (Figure 9). It is important to note that the sample size in BMS scoring was 12 for the Mild group and 11 for the Moderate; once sample sizes for TreadScan® are increased in future experiments, we expect to find more significant differences between injury severities.

An interesting correlation was found between hindlimb stride length and hindlimb swing time during early weeks post-injury in both the Mild and Moderate injury groups (Figures 17A,B). At later time points, these correlations are much weaker (Figures 18A,B). It seems that during the initial phase of recovery following SCI, the deleterious effects on the balance of injured animals led to a strong relationship between stride length and swing time. That is, the majority of the injured animals exhibited relatively short hindlimb stride lengths that correlated strongly with hindlimb swing times. After this initial recovery, injured animals exhibited more variable hindlimb stride lengths, while hindlimb swing times remained relatively stable. Thus, the correlation between these two gait characteristics seems to depend on the shorter stride length exhibited early during the recovery process.

We had hoped to find a gait characteristic that correlated well with BMS scores in injured animals. Interestingly, we found that hindlimb base of support correlated somewhat with BMS during the first four weeks post-injury (r=.49, p=.001, N=46). This correlation became much stronger during the later weeks (5, 6, 8, and 10) of the study (r=.70, p<.001, N=45). It appears that the variability in BMS scores during the initial four weeks post-injury led to the weaker correlation; once BMS scores became more stable, the correlation became much stronger. This observation strengthens the need for a higher sample size in both injury groups, in order to glean significant differences between injury groups from the hindlimb base of support data.

A relatively new measure of axon conduction in the injured spinal cord is the tcMMEP response. Depending on the severity of the injury, animals with SCI showed decreased or no tcMMEP responses at eight weeks post-injury. This measure provided information into the success of the injury; while some injured animals still had some tcMMEP responses, all had a longer latency and smaller peak-to-peak amplitude than non-injured animals (Zhang et al., 2007a). It is interesting that despite a lack of tcMMEP responses—and thus a lack of axonal conduction of descending motor pathways—injured animals were able to perform the treadmill task. Thus, it appears that our contusion injury was sufficient to eliminate tcMMEP responses in most animals while still allowing the injured animals to perform hindlimb locomotor tasks. In rats, Thota et al. (2005) suggested that recovery of hindlimb motor function can occur following incomplete SCI; this recovery seems to depend on the ability of the spinal cord to reorganize propriospinal connections. Thus, despite a lack of direct motor pathway connections, the injured mouse spinal cord may be able to sufficiently reorganize and allow the animal to perform complex locomotor tasks. This reorganization seems to occur rather quickly in animals with an incomplete SCI, as most recovery is seen during the first two to three weeks post-injury (Kuhn and Wrathall, 1998; Ma et al., 2001; Basso et al., 2006).

Spared white matter correlates well with various open-field assessment measures in rodents (Kuhn and Wrathall, 1998; Ma et al., 2001; Cao et al., 2005; Li et al. 2006). Consistent with these findings, the strongest correlation uncovered in the present study is between BMS and spared white matter (Figure 14). This suggests consistent injury severities, an argument strengthened by the significant differences between injury groups (Figure 13). However, the question remains: do any of the gait parameters measured by TreadScan® correlate with spared white matter? The encouraging answer is yes. At 10 cm/sec, hindlimb swing times at ten weeks post-injury correlate well with spared white matter percentage (Figure 15B). An even stronger correlation exists at this speed and time point between hindlimb base of support and spared white matter percentage (Figure 16B). We compared the terminal gait characteristics with spared white matter percentage because animals were perfused ten weeks post-injury and thus this time point is representative of the animals' locomotor abilities at this time. These findings are further examples of the deleterious effects of thoracic SCI on balance and posture in the rodent (Thota et al., 2005). Injured animals exhibited shorter hindlimb swing times and narrower hindlimb bases of support, leading to these relatively strong correlations.

TreadScan® offers more specific insight into the adverse effects of SCI than previous measures. Rather than pointing out the global differences between animals in terms of subjective measures of trunk stability or paw position on an ordinal scale, this software provides objective ratio data on numerous gait parameters relating to the injury. This is not to suggest that the BMS be discarded in favor of TreadScan®. Quite the opposite, in fact, as the BMS still provides the strongest correlation with spared white matter percentage. However, the wealth of information provided by TreadScan® should not be ignored. This software system is especially sensitive to gait changes in Mild to Moderate SCI. In an ideal world with endless time and resources, a combination of various assessment tools could be utilized to provide the most possible information about a SCI mouse. For instance, the BMS could be used to provide insight into the animal's open-field abilities, the Beam Walking task could offer information about the animal's fine motor functioning, TreadScan® could uncover fine locomotor differences in gait characteristics, and kinematic analysis could supply insightful information about hindlimb positioning and joint angles. Currently, very few labs will have the resources or time necessary to complete such a comprehensive study of SCI behavior. Thus, it is our recommendation that TreadScan® be used in conjunction with the BMS to provide reliable, reproducible, and specific insight into the locomotor abilities of mice following SCI.

TABLE 1. Discoveries using transgenic mice

Strain	Mutation	Results	References
C57BL/6	EphA4 -/-	EphA4 regulates axonal inhibition and astrocytic gliosis	Goldshmit et al, 2004
C57BL/6	EphB3 -/-	EphB3 inhibits neurite outgrowth following SCI	Benson et al, 2005
C57BL/6 x DBA/2 x 129sv	GFAP -/- Vim -/- double mutant	Both proteins important in astroglial reactivity	Menet et al, 2003
C57BL/6	LIF -/-	LIF involved in the microglial/macrophage response to SCI	Kerr & Patterson, 2004
MRL/+	MRL/lpr (Fas deficient)	Fas-mediated apoptosis following SCI leads to spinal cord damage and neurological injury	Yoshino et al, 2004
C57BL/6	Caspase-1 -/-	Caspase inhibition reduces post-traumatic lesion size and improves motor performance	Li et al, 2000
C57BL/6	TNF-α -/-	TNF-α mutants exhibited decreased white matter preservation	Farooque et al, 2001
C57BL/6	TNFR1 & R2 -/-	TNFR-NF-κB pathway limits apoptotic cell death after SCI	Kim et al, 2001
C57BL/6	tPA -/-	tPA is involved in secondary injury following SCI	Abe et al, 2003
C57BL/6	NOS -/-	Nitric oxide is involved in secondary injury following SCI	Farooque et al, 2001
C57BL/6	ICAM-1 -/-	ICAM-1 negatively affects functional outcome following SCI	Farooque et al, 1999
FVBn	MMP-9 -/-	MMP-9 involved in abnormal vascular permeability in SCI	Noble et al, 2002
C57BL/6	Nogo A/B -/-	Nogo involved in restricting axonal sprouting following SCI	Kim et al, 2003

TABLE 2. Behavioral assessment tools for mice

Assessment tool	Behavior measured	Example references
Subjective Open-Field Measurements		
Rearing Events	Rearing event - each time an animal assumes an upright posture shifts weight to hindlimbs	(Hsu et al., 2006)
Semi-Quantitative	Locomotor-like movements - flexion-extensions occurring alternatively in both hindlimbs	(Guertin, 2004; Guertin & Steuer, 2005; Guertin, 2005)
Open-Field Locomotor Task	Six-point scale focusing on gross aspects of hindlimb function	(Fehlings & Tator, 1995; Faulkner et al., 2004)
Basso, Beattie, & Bresnahan (BBB)	Wide array from no hindlimb movement up to coordinated locomotion	(Farooque, 2000; Mikami et al., 2002; Hsu et al., 2006; Stieltjes et al., 2006)
Modified BBB (mBBB)	Similar to BBB, but modified for mice; tail position is omitted	(Ma et al., 2001; Joshi & Fehlings, 2002a,b; Apostolova et al., 2006; Li et al., 2006)
Antri, Orthal, & Bathe (AOB)	Adapted from BBB to assess hindlimb movements in completely transected rodents	(Antri et al., 2002; Guertin & Steuer, 2005)
Basso Mouse Scale (BMS)	Wide array from no hindlimb movement up to coordinated locomotion; designed specifically for mice	(Basso et al., 2006; Jakeman et al., 2006; Li et al., 2006)
Observer Rating of Natural Behavior Tasks		
Righting Reflex	Animal's ability to right itself when dropped on a cushion; scores range from 0 (no righting reflex) to 3 (animal rights itself immediately after the drop)	(Farooque, 2000)
Inclined Plane	Maximum degree of incline animal can hold for 5 s when facing downward	(Kuhn & Wrathall, 1998; Li et al. 2006)
Ladder Rung Task	Animal walks across a horizontal or inclined ladder with rungs of equal or uneven spacing; observer counts the number of mistakes, misses or slips	(Apostolova, 2006; Farr et al., 2006; Hsu et al., 2006)
Beam Walking	Fine locomotor function assessed as animal traverses beams of varying widths	(Farooque et al., 2006)
Hindlimb Motor Function Score (HMFS)	Ten-point scale; animals rated 0-5 based on performance in open field; animals demonstrating normal movement (score = 5) then traverse steel bars with decreasing widths for additional points	(Farooque, 2000)
Recovery Index	Estimates gain of function as a fraction of the functional loss induced by the injury	(Apostolova et al., 2006)
Footprint Analysis	Paws inked and gait characteristics determined manually via prints left as animal traverses a stationary walkway	(Ma et al., 2001; Faulkner et al., 2004)

TABLE 2. Behavioral assessment tools for mice (continued)

Assessment tool	Behavior measured	Example references
Computer-Assisted Scoring		
Electromyographic (EMG) Recordings	Onset and burst duration of muscle activation during locomotion	(Fortier et al., 1987; Leblond et al., 2003)
SCANET	Infrared beam sensors scan small horizontal movements, large horizontal movements, and vertical rearing	(Mikami et al., 2002)
Computer-Assisted Footprint Analysis	Gait characteristics determined manually via video recordings from underneath as animal traverses a stationary walkway	(Clarke & Still, 1998; Hamers et al., 2001; Apostolova, 2006; Hamers et al., 2006)
Treadmill	Gait characteristics determined manually via video recordings from underneath as animal traverses a clear treadmill belt	(Heglund & Taylor, 1988; Leblond et al., 2003; Guertin et al., 2004; Herbin et al., 2004; Kale et al., 2004; Amende et al., 2005)
TreadScan®	Gait characteristics determined automatically via video recordings from underneath as animal traverses a clear treadmill belt	(Hampton et al., 2004; Wooley et al., 2005)
Robotic Step Training	Number of steps and quality of stepping on a treadmill assessed following training via manual placement of limbs by a robotic training algorithm	(Cai et al., 2006)
Rotorod	Posture, head position, limb lift, limb carry, limb advance, limb placement, and stride length measured manually as animal moves on a rotating drum	(Faulkner et al., 2004; Farr et al., 2006; Hsu et al., 2006)
Computerized Locomotor Activity	Force transducers detect animal movements and measure locomotor activity and whole-body tremors as animal moves in the open-field	(Fowler et al., 2001; Farooque et al., 2006)
Kinematics	Anatomical markers used to create stick diagram representations of hindlimb position during either locomotor or swimming tasks	(Leblond et al., 2003; Guertin 2004; Guertin & Steuer, 2005)

Table 3 - All training groups baseline data (15 cm/sec)

	High Training Baseline	Medium Training Baseline	Low Training Baseline
Animals Responding	**n = 9 / 15**	**n = 13 / 20**	**n = 19 / 20**
Left Hindlimb			
Stance Time	**238.97**	**243.96**	**219.38**
(Std Dev)	**(29.28)**	**(21.05)**	**(17.94)**
Swing Time	**117.26**	**109.05**	**103.50**
(Std Dev)	**(25.51)**	**(14.55)**	**(11.94)**
Stance %	**67.08%**	**69.11%**	**67.94%**
Swing %	**32.92%**	**30.89%**	**32.06%**
Stride Length	**56.38**	**55.90**	**47.67**
(Std Dev)	**(6.20)**	**(4.96)**	**(3.61)**
Right Hindlimb			
Stance Time Avg	**246.99**	**240.52**	**221.26**
(Std Dev)	**(27.78)**	**(23.83)**	**(16.40)**
Swing Time Avg	**112.43**	**113.35**	**100.07**
(Std Dev)	**(21.40)**	**(18.83)**	**(11.57)**
Stance % Avg	**68.72%**	**67.97%**	**68.86%**
Swing % Avg	**31.28%**	**32.03%**	**31.14%**
Stride Length Avg	**56.74**	**55.85**	**47.86**
(Std Dev)	**(6.57)**	**(5.05)**	**(3.61)**
Rear Track Width	**24.30**	**24.19**	**23.81**
(Std Dev)	**(1.92)**	**(2.00)**	**(2.14)**
Left HL Toe Spread	**8.03**	**8.35**	**7.87**
(Std Dev)	**(0.76)**	**(0.45)**	**(0.65)**
Right HL Toe Spread	**7.83**	**7.72**	**7.48**
(Std Dev)	**(0.61)**	**(0.55)**	**(0.45)**

Table 4: Differences in Sham Group at 7 cm/sec

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8	Week 10
Animals	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9
Left Hindlimb								
Stance Time	339.29	423.82	357.91	404.26	393.84	367.09	398.45	386.15
(Std Dev)	(59.98)	(39.90)	(43.34)	(58.05)	(84.51)	(64.53)	(88.35)	(63.62)
	No significant change over time							
Swing Time	97.91	91.44	91.42	93.75	110.98	98.86	102.91	101.60
(Std Dev)	(15.11)	(15.29)	(8.52)	(11.19)	(16.70)	(20.03)	(18.29)	(18.65)
	No significant change over time							
Stance %	77.37%	82.26%	79.55%	80.99%	77.68%	78.70%	78.98%	79.12%
(Std Dev)	(3.29%)	(2.35%)	(2.05%)	(2.87%)	(3.19%)	(2.90%)	(5.11%)	(2.75%)
	Week 2 > Week 1, Week 5; Week 4 > Week 1							
Swing %	22.63%	17.74%	20.45%	19.01%	22.32%	21.30%	21.02%	20.88%
(Std Dev)	(3.29%)	(2.35%)	(2.05%)	(2.87%)	(3.19%)	(2.90%)	(5.11%)	(2.75%)
	Week 1 > Week 2, Week 4; Week 5 > Week 2							
Stride Length	37.86	40.67	42.17	47.43	53.85	50.67	46.19	45.69
(Std Dev)	(4.55)	(4.88)	(5.18)	(4.32)	(8.02)	(6.73)	(5.19)	(5.21)
	Week 4 > Week1; Week 5 > Weeks 1, 2, 3, 4, & 10; **Week 10 > Week 1**							
Right Hindlimb								
Stance Time Avg	347.42	400.42	356.36	396.26	408.81	380.16	401.40	386.59
(Std Dev)	(42.10)	(41.67)	(36.26)	(64.44)	(86.18)	(73.09)	(74.05)	(41.30)
	No significant change over time							
Swing Time Avg	98.65	102.07	100.29	97.63	111.42	93.90	96.71	103.84
(Std Dev)	(14.46)	(9.43)	(14.20)	(14.65)	(25.28)	(9.34)	(14.75)	(21.10)
	No significant change over time							
Stance % Avg	77.87%	79.65%	78.03%	80.09%	78.53%	79.77%	80.42%	78.94%
(Std Dev)	(2.06%)	(1.15%)	(2.33%)	(2.68%)	(2.31%)	(3.72%)	(2.34%)	(2.90%)
	No significant change over time							
Swing % Avg	22.13%	20.35%	21.97%	19.91%	21.47%	20.23%	19.58%	21.06%
(Std Dev)	(2.06%)	(1.15%)	(2.33%)	(2.68%)	(2.31%)	(3.72%)	(2.34%)	(2.90%)
	No significant change over time							
Stride Length Avg	39.59	41.46	43.59	45.23	54.67	49.81	42.90	45.20
(Std Dev)	(5.16)	(3.00)	(4.55)	(5.50)	(7.72)	(5.07)	(6.32)	(6.15)
	No significant change over time							
Rear Track Width	27.38	29.52	27.93	26.94	26.46	26.43	28.11	26.59
(Std Dev)	(2.28)	(2.35)	(2.30)	(3.05)	(4.04)	(2.91)	(2.66)	(2.45)
	Week 2 > Week 5, Week 6, Week 10							
Left HL Toe Spread	8.87	8.68	8.87	8.70	8.61	8.63	8.49	8.69
(Std Dev)	(0.68)	(0.84)	(0.68)	(0.68)	(0.74)	(0.70)	(0.66)	(0.83)
	No significant change over time							
Right HL Toe Spread	8.33	8.41	8.19	8.08	8.30	8.49	7.93	8.05
(Std Dev)	(0.47)	(0.41)	(0.67)	(0.81)	(0.74)	(0.43)	(0.79)	(0.56)
	No significant change over time							

Table 5: Differences in Mild Injury Group at 7 cm/sec

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8	Week 10
Animals	n = 10	n = 12	n = 12	n = 12	n = 11	n = 12	n = 12	n = 10
Left Hindlimb								
Stance Time	373.71	389.06	367.02	361.46	364.27	356.66	398.36	389.95
(Std Dev)	(43.72)	(69.09)	(50.35)	(66.59)	(81.50)	(46.36)	(58.65)	(67.48)
	No significant change over time							
Swing Time	66.26	72.09	68.01	71.32	72.57	75.08	74.41	70.29
(Std Dev)	(7.47)	(11.94)	(12.59)	(11.59)	(17.94)	(10.41)	(16.27)	(12.33)
	No significant change over time							
Stance %	84.78%	84.21%	84.31%	83.32%	82.86%	82.50%	84.21%	84.45%
(Std Dev)	(2.49%)	(2.35%)	(2.36%)	(2.40%)	(4.87%)	(2.45%)	(2.92%)	(3.69%)
	No significant change over time							
Swing %	15.22%	15.79%	15.69%	16.68%	17.14%	17.50%	15.79%	15.55%
(Std Dev)	(2.49%)	(2.35%)	(2.36%)	(2.40%)	(4.87%)	(2.45%)	(2.92%)	(3.69%)
	No significant change over time							
Stride Length	36.35	37.50	35.33	36.54	37.34	39.44	36.82	37.59
(Std Dev)	(2.95)	(3.97)	(7.28)	(4.89)	(6.47)	(6.66)	(3.73)	(5.09)
	No significant change over time							
Right Hindlimb								
Stance Time Avg	378.28	399.12	378.64	378.20	396.84	369.01	382.93	384.41
(Std Dev)	(63.15)	(90.20)	(60.32)	(70.23)	(94.35)	(54.58)	(56.38)	(78.56)
	No significant change over time							
Swing Time Avg	74.54	75.02	72.59	69.91	66.68	69.34	66.99	71.34
(Std Dev)	(11.73)	(10.18)	(18.04)	(14.90)	(13.79)	(10.41)	(7.94)	(10.78)
	No significant change over time							
Stance % Avg	83.17%	83.66%	83.80%	84.32%	85.12%	84.04%	84.94%	83.99%
(Std Dev)	(3.96%)	(3.71%)	(3.71%)	(2.06%)	(3.77%)	(2.52%)	(2.30%)	(3.21%)
	No significant change over time							
Swing % Avg	16.83%	16.34%	16.20%	15.68%	14.88%	15.96%	15.06%	16.01%
(Std Dev)	(3.96%)	(3.71%)	(3.71%)	(2.06%)	(3.77%)	(2.52%)	(2.30%)	(3.21%)
	No significant change over time							
Stride Length Avg	37.35	37.70	36.83	37.27	38.90	38.23	35.40	37.06
(Std Dev)	(3.73)	(3.91)	(6.60)	(4.27)	(7.13)	(6.68)	(3.26)	(4.36)
	No significant change over time							
Rear Track Width	21.34	22.05	21.94	22.59	23.48	22.32	23.21	23.60
(Std Dev)	(3.41)	(5.69)	(4.57)	(4.81)	(3.74)	(4.64)	(4.60)	(3.92)
	Week 2, Week 4, Week 5, Week 6, Week 10 > Week 1							
Left HL Toe Spread	7.32	7.65	7.57	7.75	7.81	7.71	7.34	7.70
(Std Dev)	(1.22)	(0.96)	(0.99)	(0.98)	(1.04)	(0.94)	(0.71)	(1.23)
	No significant change over time							
Right HL Toe Spread	6.66	6.90	7.19	7.27	7.42	7.34	7.24	7.55
(Std Dev)	(1.25)	(1.03)	(0.99)	(1.02)	(0.83)	(0.77)	(1.09)	(0.72)
	Week 4 > Week 1; Week 10 > Week 1							

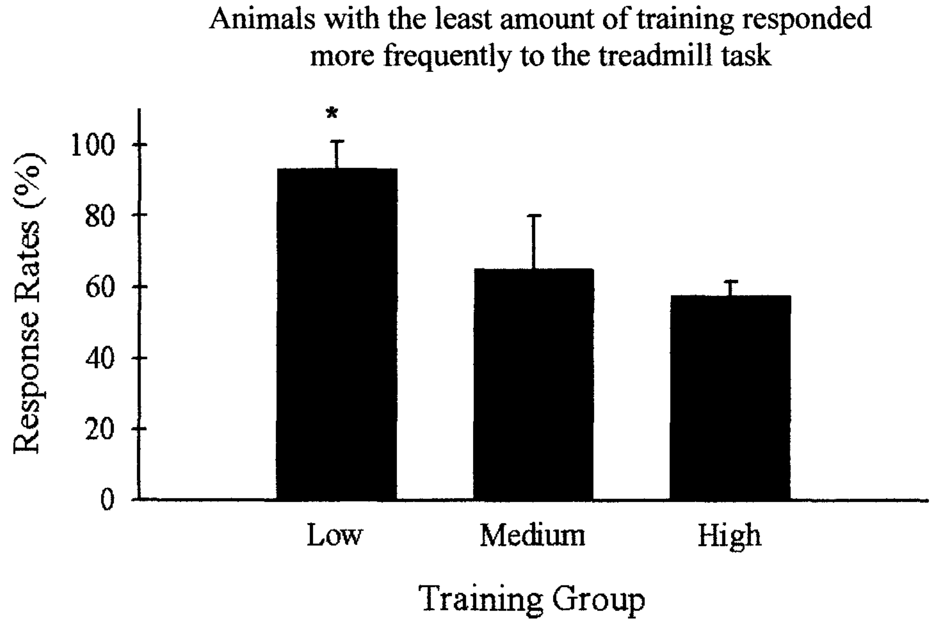


Figure 1: Animals in the Low Training Group had higher response rates to the treadmill task than animals in both the Medium Training and High Training groups ($\chi^2=7.1$, df=2, $p<.05$)

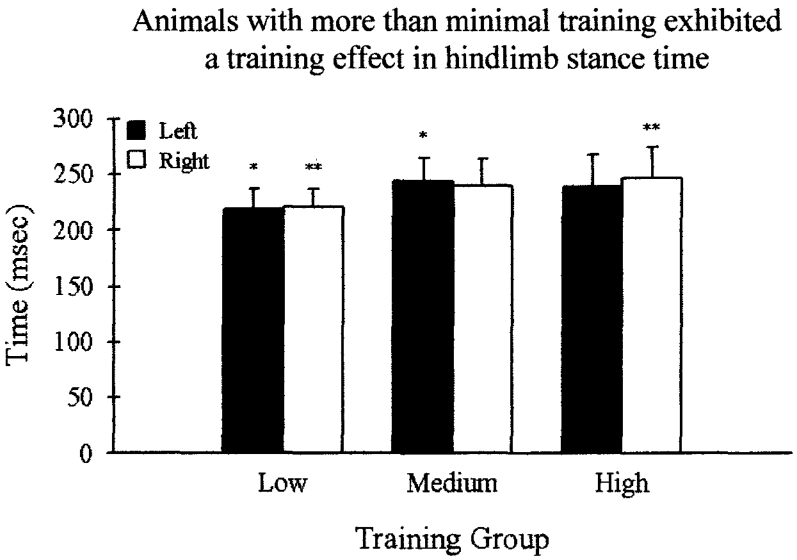


Figure 2A: A repeated measures ANOVA indicated training group differences in hindlimb stance times (F=6.649, df=2, 47, p=.003). Tukey's HSD post hoc t-tests revealed that left hindlimb stance times were longer in the Medium (t=24.6, df=2, 47, p<.05) group than in the Low group, whereas right hindlimb stance times were longer in the High group than in the Low group (t=22.6, df=2, 47, p<.05). The Medium training group approached a significantly longer right hindlimb stance time than the Low group (t=19.2, df=2, 47, p=.051).

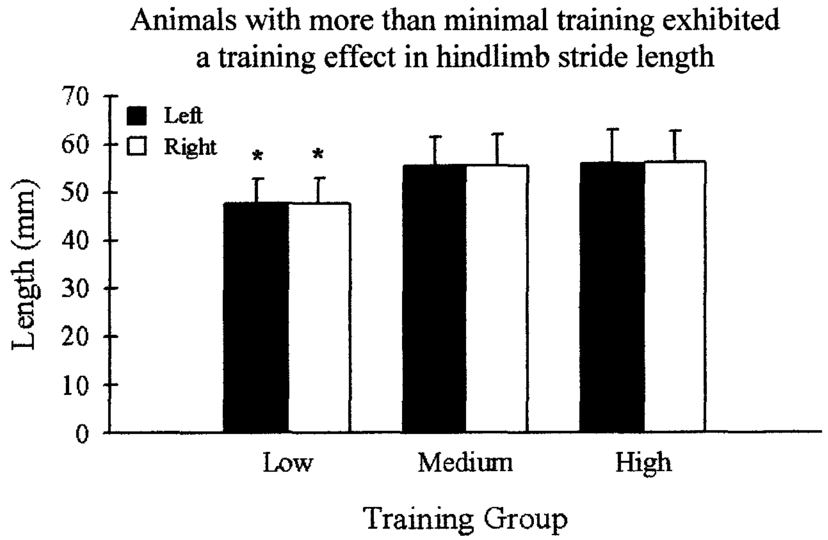


Figure 2B: Repeated measures ANOVA demonstrated hindlimb stride length differences between training groups (F=18.586, df=2, 47, p<.001). Tukey's HSD post hoc t-tests indicated left hindlimb stride length was longer in the Medium (t=8.2, df=2, 47, p<.01) and High (t=8.7, df=2, 47, p<.01) groups than in the Low group. In addition, Medium (t=7.9, df=2, 47, p<.01) and High (t=8.8, df=2, 47, p<.01) groups had longer right hindlimb stride lengths than the Low training group.

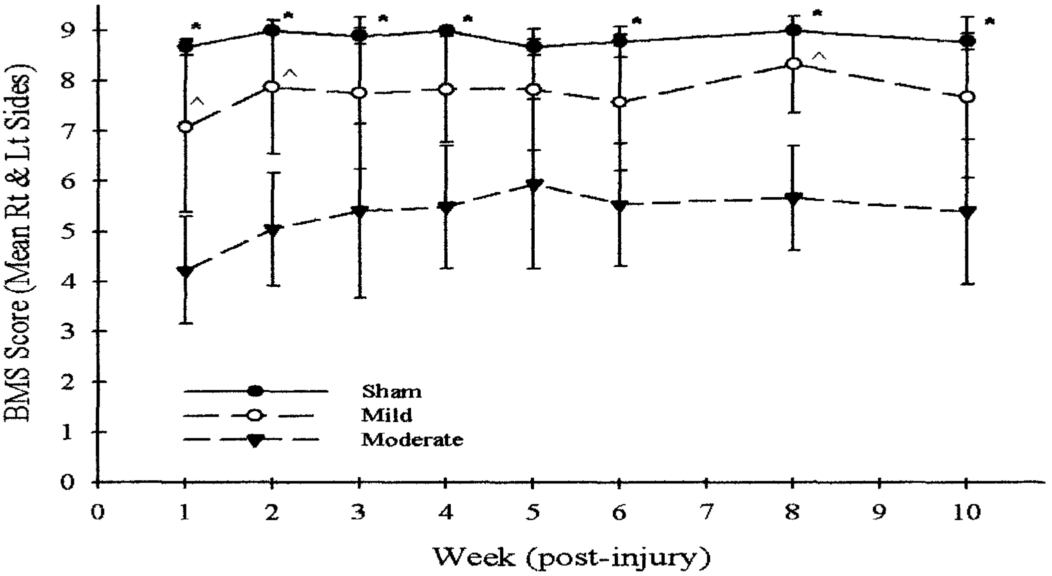


Figure 3A: A repeated measures ANOVA indicated a BMS difference between injury groups (F=41.973, df=2, 28, p<.001). Tukey's HSD post hoc t-test revealed significantly higher BMS scores for Sham than Moderate injury animals at Week 1 (t=4.5, df=3, 28, p<.01), Week 2 (t=4.0, df=3, 28, p<.01), Week 3 (t=3.5, df=3, 28, p=.01), Week 4 (t=3.5, df=3, 28, p=.01), Week 6 (t=3.3, df=3, 28, p<.05), Week 8 (t=3.3, df=3, 28, p<.05), and Week 10 (t=3.4, df=3, 28, p<.05). Mild injury animals showed higher BMS scores than Moderate injury animals at Week 1 (t=2.9, df=3, 28, p<.05), Week 2 (t=2.9, df=3, 28, p<.05), and Week 8 (t=2.6, df=3, 28, p=.05).

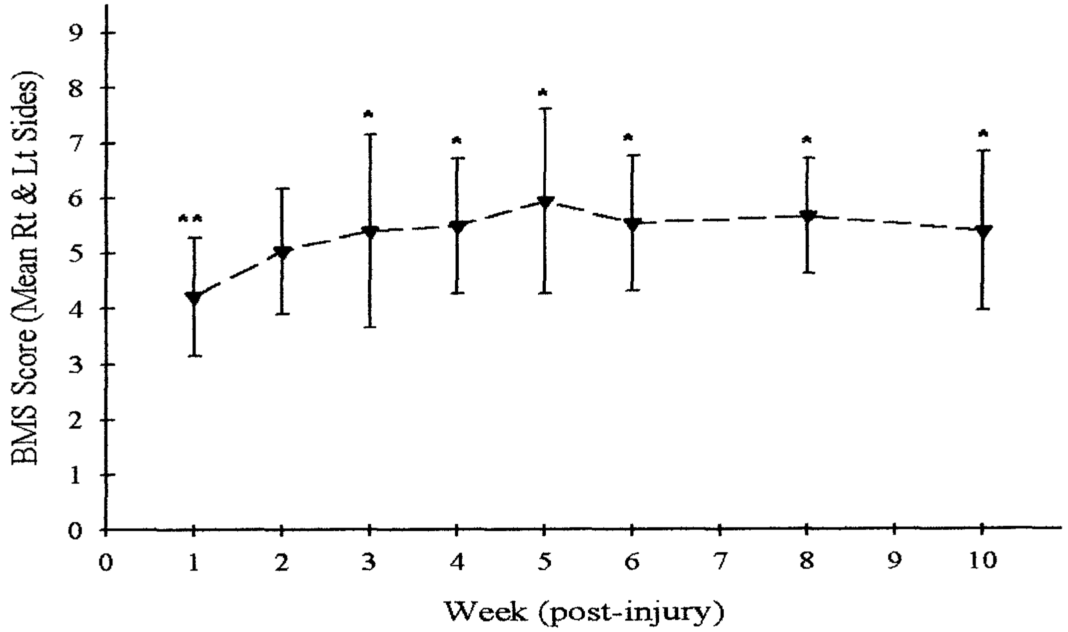
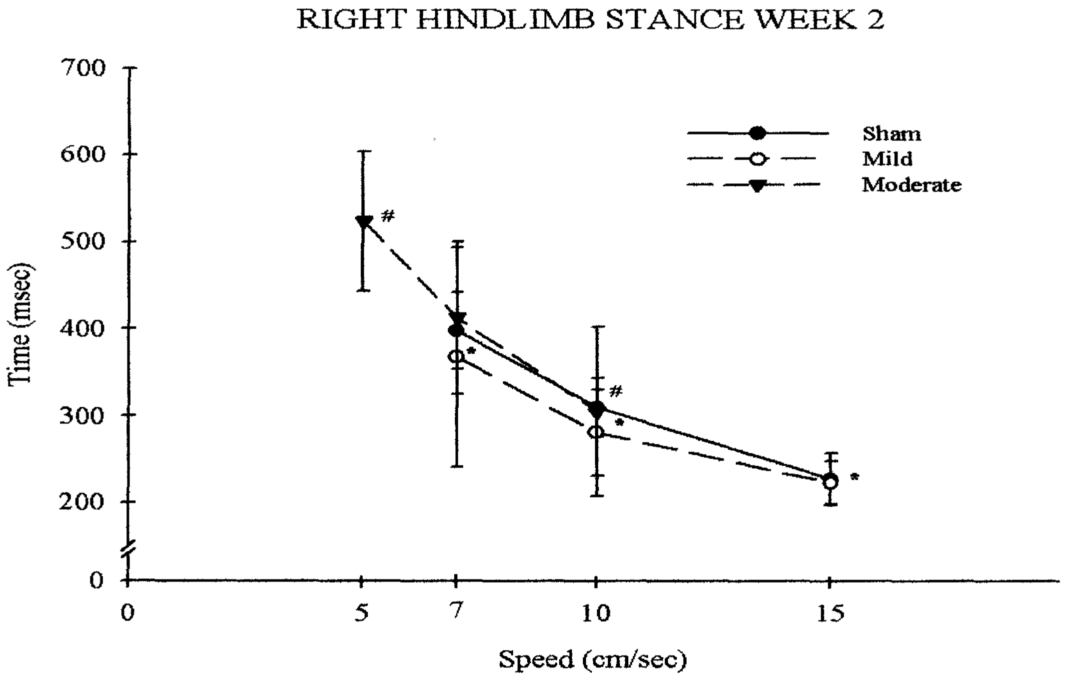
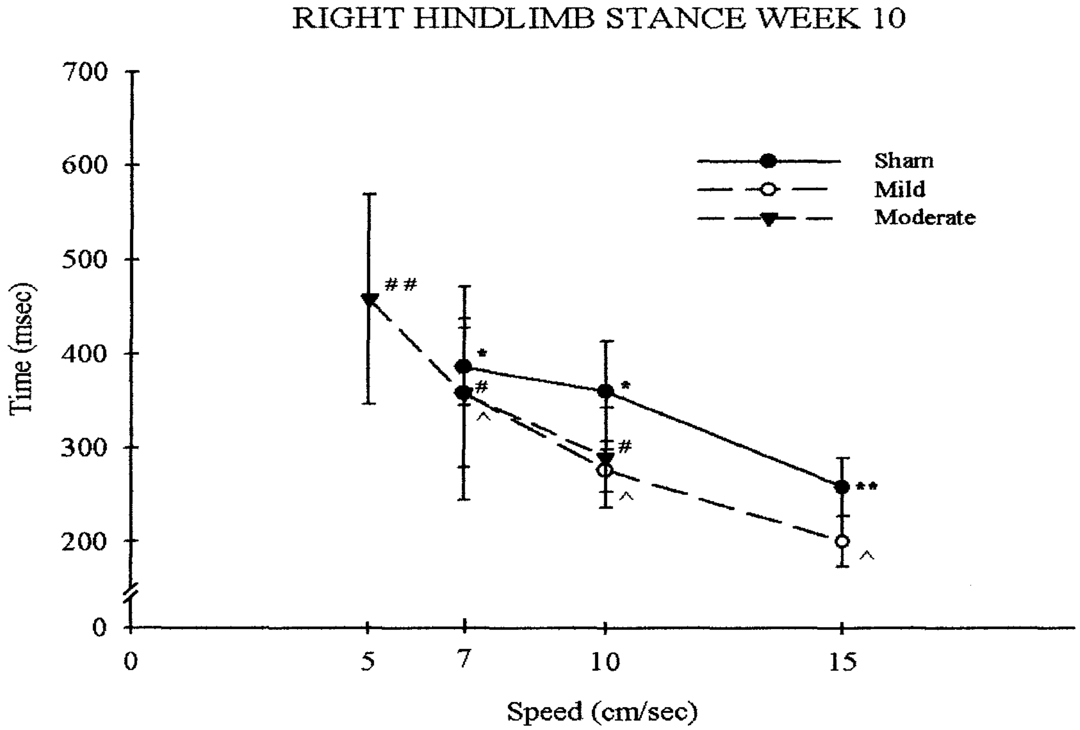


Figure 3B: Repeated measures ANOVA suggested that Moderate animals improve on BMS over time (F=3.132, df=7, 70, p=.006). Tukey's HSD post hoc t-tests revealed that Week 1 BMS scores were significantly lower than Week 3 (t=1.2, df=6, 70, p=.05), Week 4 (t=1.3, df=6, 70, p<.05), Week 5 (t=1.8, df=6, 70, p<.01), Week 6 (t=1.3, df=6, 70, p<.05), Week 8 (t=1.5, df=6, 70, p=.01), and Week 10 (t=1.2, df=6, 70, p=.05).



<u>Figure 4A</u>: At 2 wpi, a repeated measures ANOVA revealed differences across speeds on both right (F=59.389, df=2, 14, p<.001) and left (F=95.269, df=2, 14, p<.001) hindlimb stance times for Sham animals. Tukey's HST post hoc t-tests indicated that as speed increased from 7 cm/sec to 10 cm/sec, Sham animals shortened both their right (t=87.9, df=3, 14, p<.01) and left (t=98.1, df=3, 14, p<.01) hindlimb stance times. Similarly, as speed increased from 10 cm/sec to 15 cm/sec, Sham animals again shortened both their right (t=82.4, df=3, 14, p<.01) and left (t=85.2, df=3, 14, p<.01) hindlimb stance times. In Mild animals 2 wpi, repeated measures ANOVA indicated a left hindlimb stance time difference across speeds (F=8.470, df=2, 6, p=.018)(not shown). Tukey's HSD post hoc t-tests revealed that left hindlimb stance time was only significantly shorter for 15 cm/sec compared to 7 cm/sec (t=159.1, df=3, 6, p<.05). Repeated measures ANOVA indicated a speed difference in both right (F=14.907, df=2, 8, p=.002) and left (F=59.461, df=2, 8, p<.001) hindlimb stance times in Moderate animals. Tukey's HSD post hoc t-tests revealed that Moderate animals exhibited a shorter right hindlimb stance time for 10 cm/sec than for 5 cm/sec (t=218.4, df=3, 8, p<.01) at 2 wpi. Similarly, Moderate animals shortened their left hindlimb stance time as speed increased from 5 cm/sec to 7 cm/sec (t=49.4, df=3, 8, p<.05) and from 7 cm/sec to 10 cm/sec (t=132.2, df=3, 8, p<.01). Right and left hindlimb data are statistically similar for these speed differences; for simplicity, only right hindlimb data is depicted above.



RIGHT HINDLIMB STANCE WEEK 10

Figure 4B: At 10 wpi, repeated measures ANOVA indicated a speed difference for both right (F=44.403, df=2, 16, p<.001) and left (F=19.783, df=2, 16, p<.001) hindlimb stance times for Sham animals. Tukey's HSD post hoc t-tests revealed that right hindlimb stance times were shorter at 15 cm/sec than at 7 cm/sec (t=128.0, df=3, 16, p<.01); similarly, these times were shorter at 15 cm/sec than at 10 cm/sec (t=102.3, df=3, 16, p<.01). Left hindlimb stance times showed similar speed dependence; 15 cm/sec stance times were shorter than both 7 cm/sec (t=128.6, df=3, 16, p<.01) and 10 cm/sec (t=97.9, df=3, 16, p<.01) stance times. In the Mild group, repeated measures ANOVA indicated a speed difference in both right (F=18.225, df=2, 8, p=.001) and left (F=9.757, df=2, 8, p=.007) hindlimb stance times. Tukey's HSD post hoc t-tests revealed that right hindlimb stance times were shorter at 10 cm/sec than at 7 cm/sec (t=81.8, df=3, 8, p<.05) and shorter at 15 cm/sec than at 10 cm/sec (t=76.1, df=3, 8, p<.05). Left hindlimb stance times were shorter at 10 cm/sec than at 7 cm/sec (t=111.5, df=3, 8, p<.05), and 15 cm/sec was shorter than 7 cm/sec (t=143.0, df=3, 8, p<.01). In the Moderate group, repeated measures ANOVA indicated a speed difference in both right (F=20.928, df=2, 12, p<.001) and left (F=19.714, df=2, 12, p<.001) hindlimb stance times. Tukey's HSD post hoc t-tests revealed that Moderate injured animals exhibited shorter right hindlimb stance times at 7 cm/sec (t=100.6, df=3, 12, p<.01) and at 10 cm/sec (t=168.4, df=3, 12, p<.01) than at 5 cm/sec. Left hindlimb stance time was shorter at 7 cm/sec than at 5 cm/sec (t=87.0, df=3, 12, p<.05) and shorter at 10 cm/sec than at 7 cm/sec (t=81.5, df=3, 12, p<.05). Again, for simplicity only the right hindlimb data is depicted above.

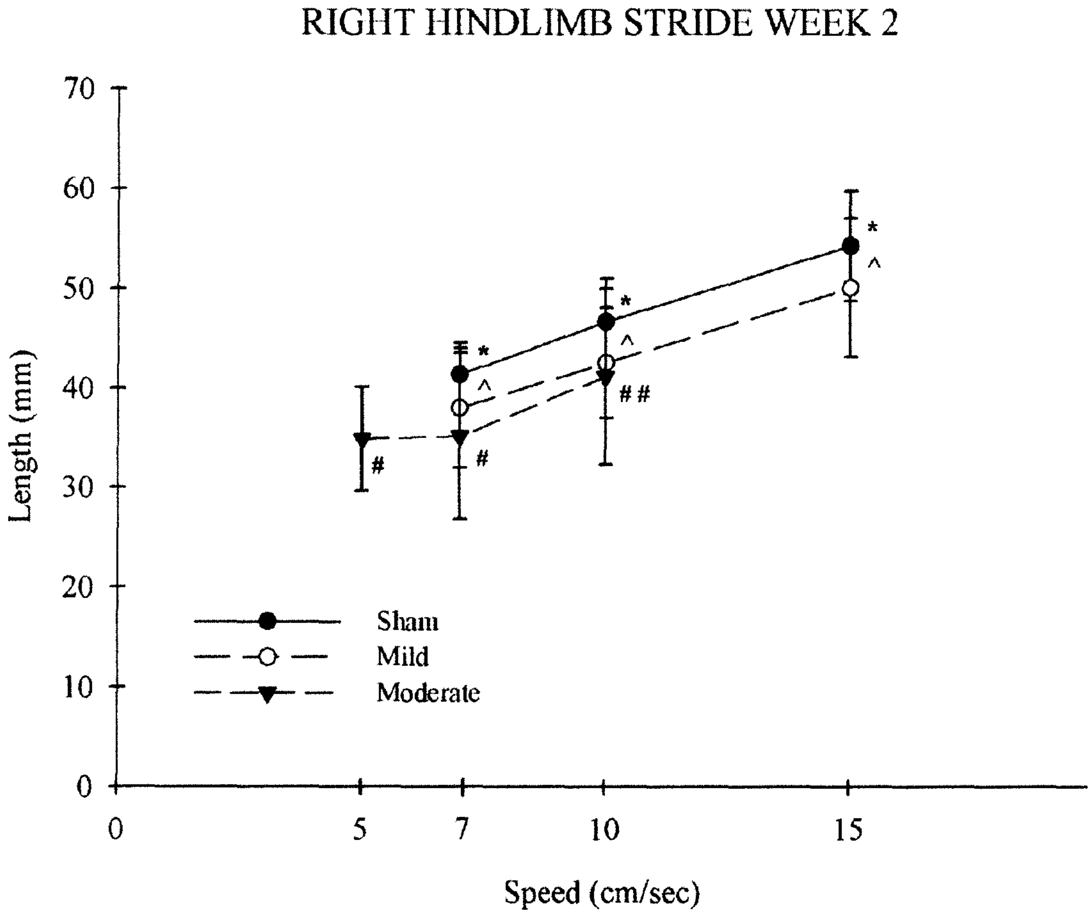


Figure 5A: Repeated measures ANOVA revealed a difference in hindlimb stride length across speeds on both the right (F=51.986, df=2, 14, p<.001) and left (F=41.941, df=2, 14, p<.001) sides. Tukey's HSD post hoc t-tests showed that in Sham animals, both right (t=5.3, df=3, 14, p<.01) and left (t=8.9, df=3, 14, p<.01) hindlimb stride length increased as speed increased from 7 cm/sec to 10 cm/sec. A similar stride length speed dependence was found as speed increased from 10 cm/sec to 15 cm/sec for both the right (t=7.6, df=3, 14, p<.01) and left (t=7.4, df=3, 14, p<.01) hindlimb. In the Mild group, repeated measures ANOVA indicated a difference across speeds on both the right (F=65.796, df=2, 6, p<.001) and left (F=8.135, df=2, 6, p=.02) sides. Tukey's HSD post hoc t-tests indicated that Mild animals showed longer right hindlimb stride length as speed increased from 7 cm/sec to 10 cm/sec (t=4.5, df=3, 6, p<.05) and from 10 cm/sec to 15 cm/sec (t=7.6, df=3, 6, p<.01), but the left hindlimb stride length was only longer for 15 cm/sec compared to 7 cm/sec (t=10.6, df=3, 6, p<.05). In the Moderate group, repeated measures ANOVA indicated a speed difference in both right (F=7.536, df=2, 8, p=.014) and left (F=5.594, df=2, 8, p=.03) hindlimb stride lengths. Tukey's HSD post hoc t-tests revealed that right hindlimb stride length was longer for Moderate animals in the 10 cm/sec condition than in both the 7 cm/sec (t=6.0, df=3, 8, p<.05) and the 5 cm/sec (t=6.3, df=3, 8, p<.05) conditions; left hindlimb stride length was longer for 10 cm/sec than for 5 cm/sec (t=7.8, df=3, 8, p<.05). Due to similarities in right and left side data, only right hindlimb data is depicted above.

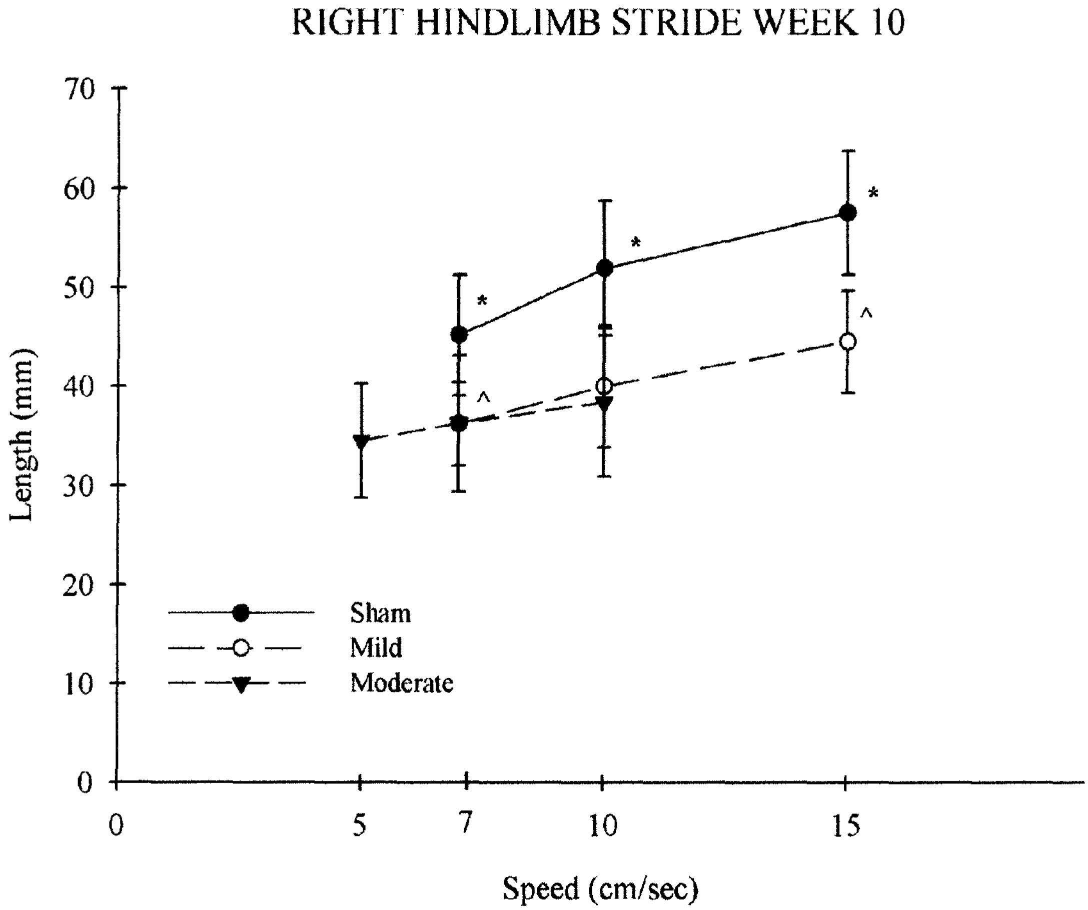


Figure 5B: At 10 wpi, repeated measures ANOVA indicated a speed difference for both right (F=18.602, df=2, 16, p<.001) and left (F=21.852, df=2, 16, p<.001) hindlimb stride lengths in the Sham group. Tukey's HSD post hoc t-tests revealed that right hindlimb stride length was longer at 15 cm/sec than at 10 cm/sec (t=5.6, df=3, 16, p<.05) and longer at 10 cm/sec than at 7 cm/sec (t=6.8, df=3, 16, p<.05). Similarly, left hindlimb stride length was longer at 15 cm/sec than at 10 cm/sec (t=5.1, df=3, 16, p<.05) and longer at 10 cm/sec than at 7 cm/sec (t=6.8, df=3, 16, p<.01). In the Mild group, repeated measures ANOVA also found speed differences between right (F=6.102, df=2, 8, p=.025) and left (F=6.744, df=2, 8, p=.019) and left hindlimb stride lengths. Tukey's HSD post hoc t-tests indicated that right (t=8.4, df=3, 8, p<.05) and left (t=9.8, df=3, 8, p<.05) hindlimb stride lengths were longer at 15 cm/sec than at 7 cm/sec in the Mild injury group. In the Moderate group, repeated measures ANOVA indicated a speed difference in left hindlimb stride length (F=6.337, df=2, 12, p=.013). Tukey's HSD post hoc t-tests revealed that left hindlimb stride length was longer at 10 cm/sec than at 5 cm/sec (t=4.4, df=3, 12, p<.05) in the Moderate injury group. Due to similarities in right and left side data, only right hindlimb data is depicted above.

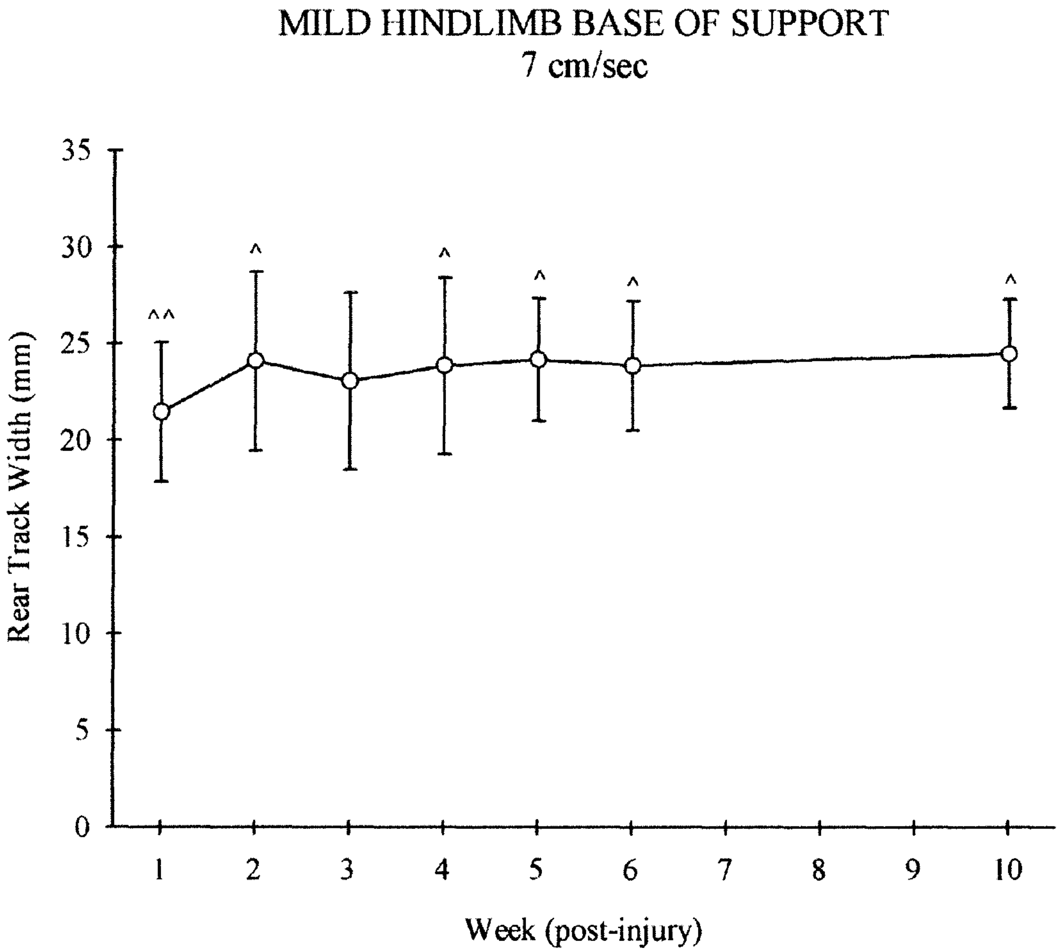


Figure 6: Repeated measures ANOVA indicated a rear track width difference over time in the Mild group at 7 cm/sec (F=3.741, df=6, 48, p=.004). Tukey's HSD post hoc t-tests revealed that Mild animals' hindlimb base of support was wider at Week 2 (t=2.7, df=6, 48, p<.05), Week 4 (t=2.5, df=6, 48, p<.05), Week 5 (t=2.8, df=6, 48, p=.01), Week 6 (t=2.5, df=6, 48, p<.05), and Week 10 (t=3.1, df=6, 48, p<.01) than at Week 1.

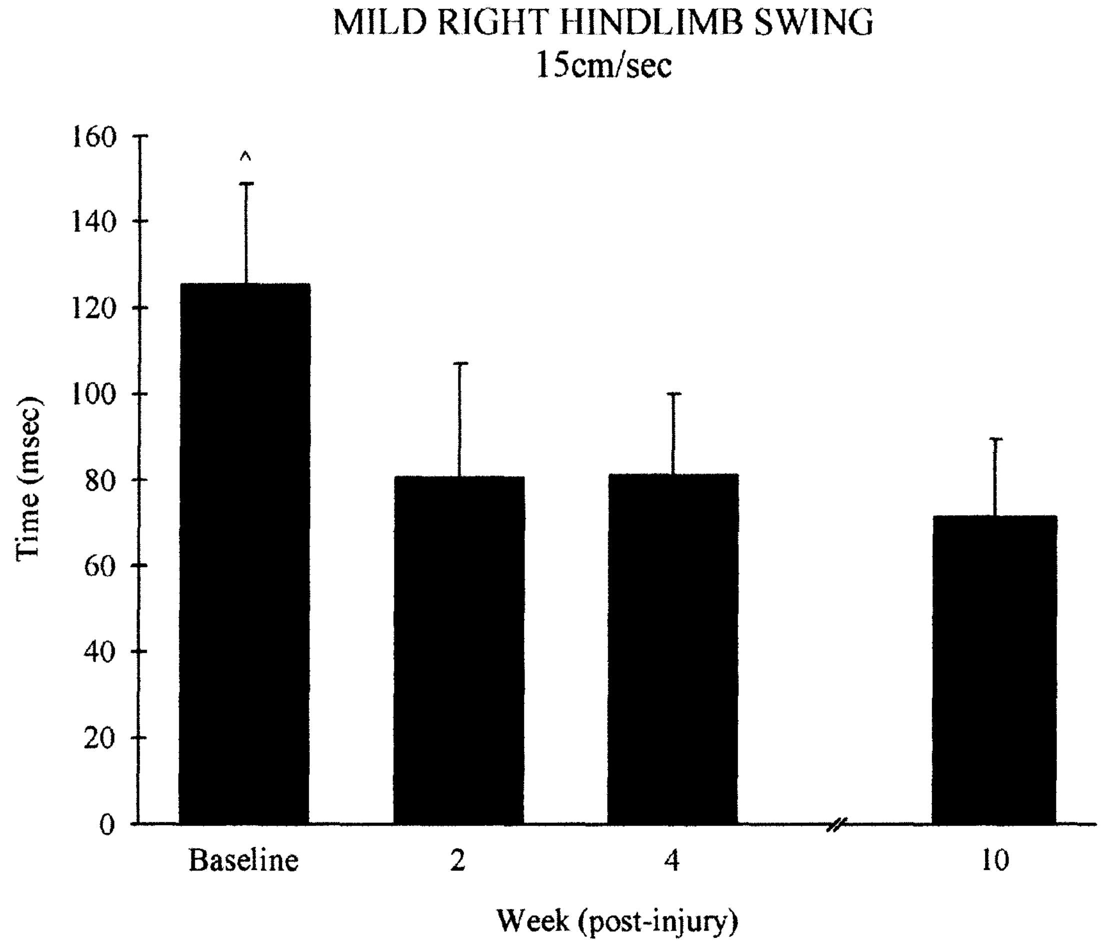


Figure 7: Repeated measures ANOVA indicated a difference between baseline and early, middle, and late time points in right hindlimb swing times in the Mild group (F=11.432, df=3, 9, p=.002). Tukey's HSD post hoc t-tests revealed that right hindlimb swing time was longer for the Baseline than for Week 2 (t=44.69, df=4, 9, p<.01), Week 4 (t=44.27, df=4, 9, p<.01), and Week 10 (t=53,87, df=4, 9, p<.01).

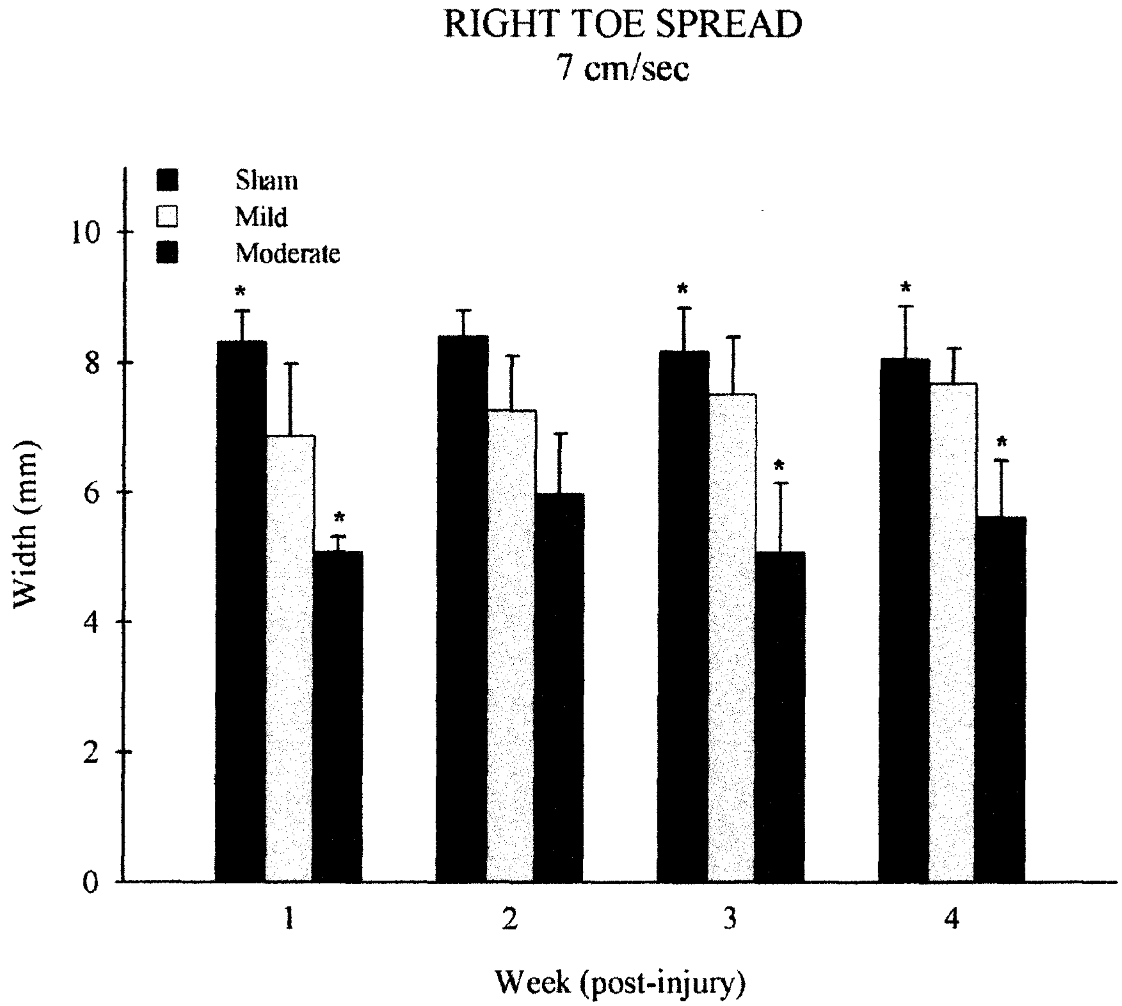


Figure 8: A repeated measures ANOVA indicated a significant difference between groups on right hindlimb toe spread (F=18.245, df=2, 18, p<.001). Tukey's HSD post hoc t-tests revealed that right hindlimb toe spread was wider in the Sham group than in the Moderate group at Week 1 (t=3.2, df=3, 18, p<.05), Week 3 (t=3.1, df=3, 18, p<.05), and Week 4 (t=2.5, df=3, 18, p=.05)

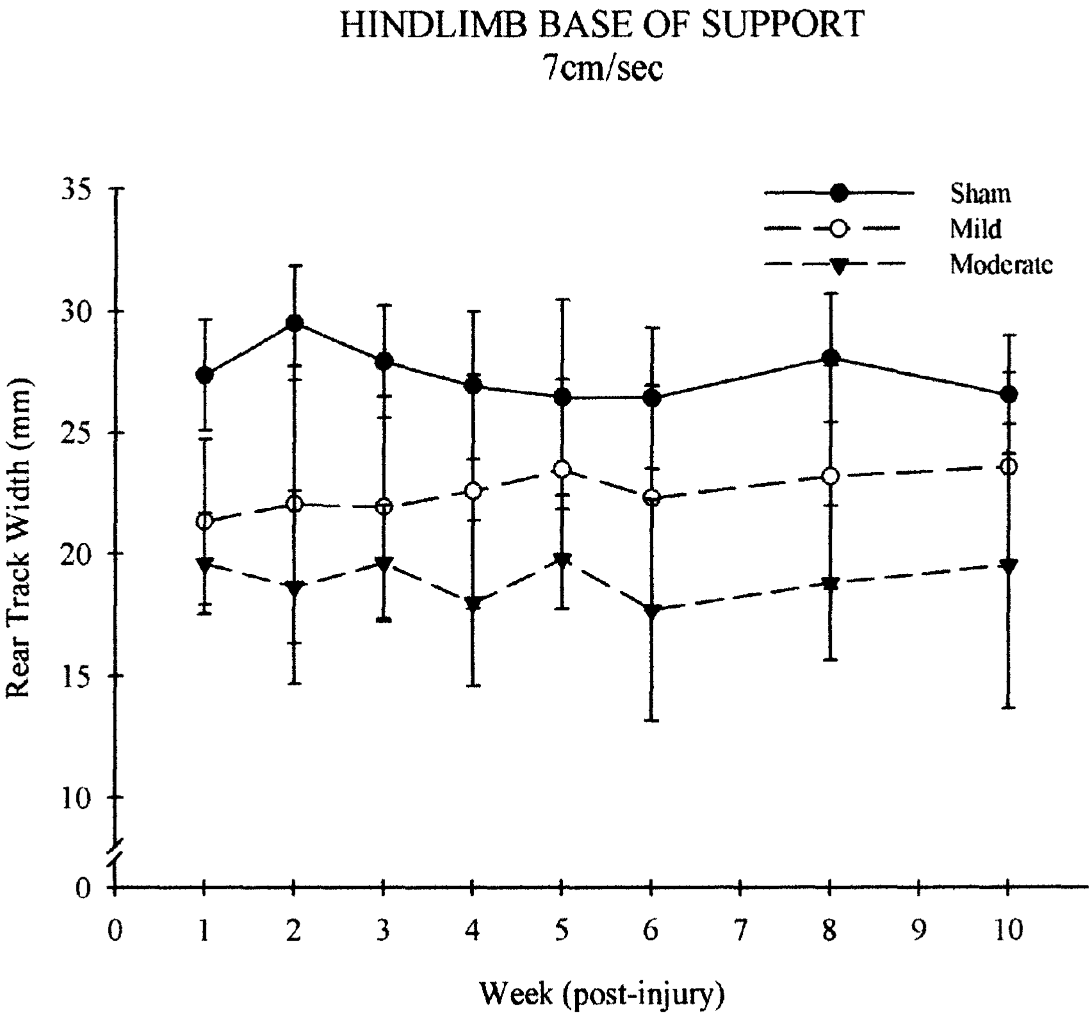


Figure 9: Despite a group interaction over time (F=7.536, df=2, 18, p=.004), Tukey's HSD post hoc t-tests revealed no significant differences in hindlimb base of support between injury groups. Future studies will increase sample size in an attempt to strengthen this observed trend.

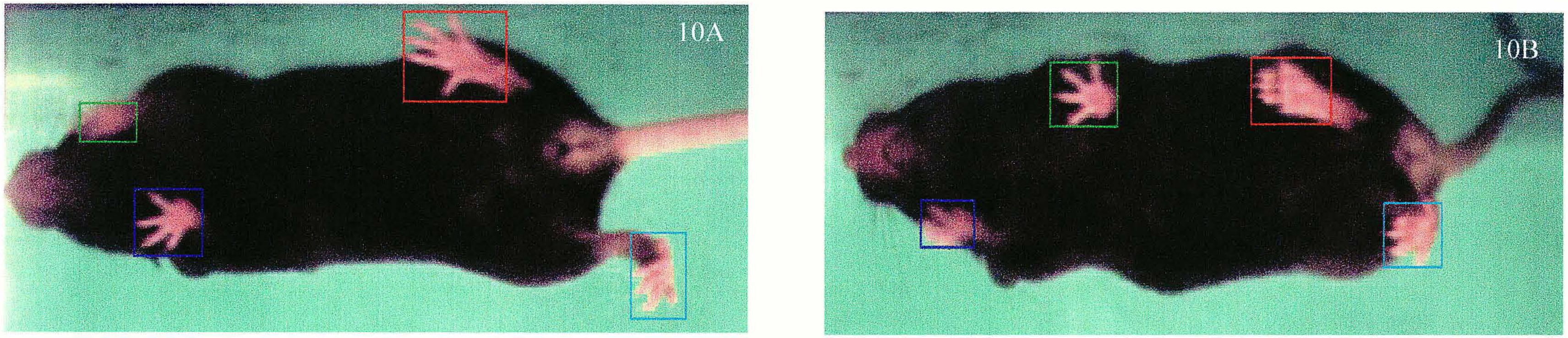


Figure 10: A sham injury animal (BMS=9) walking at 10cm/s on the treadmill four weeks post injury (10A). In TreadScan®, each paw is automatically color-coded and measured over 20 seconds of locomotion, and gait parameters are automatically calculated. Note the considerably narrower rear track width, decreased rear toe spread, and decreased rear stride length in a moderately contused mouse (BMS=5) walking at 10cm/s four weeks post injury (10B). According to our findings, a BMS score of 4 is mandatory for an animal to perform properly on the treadmill.

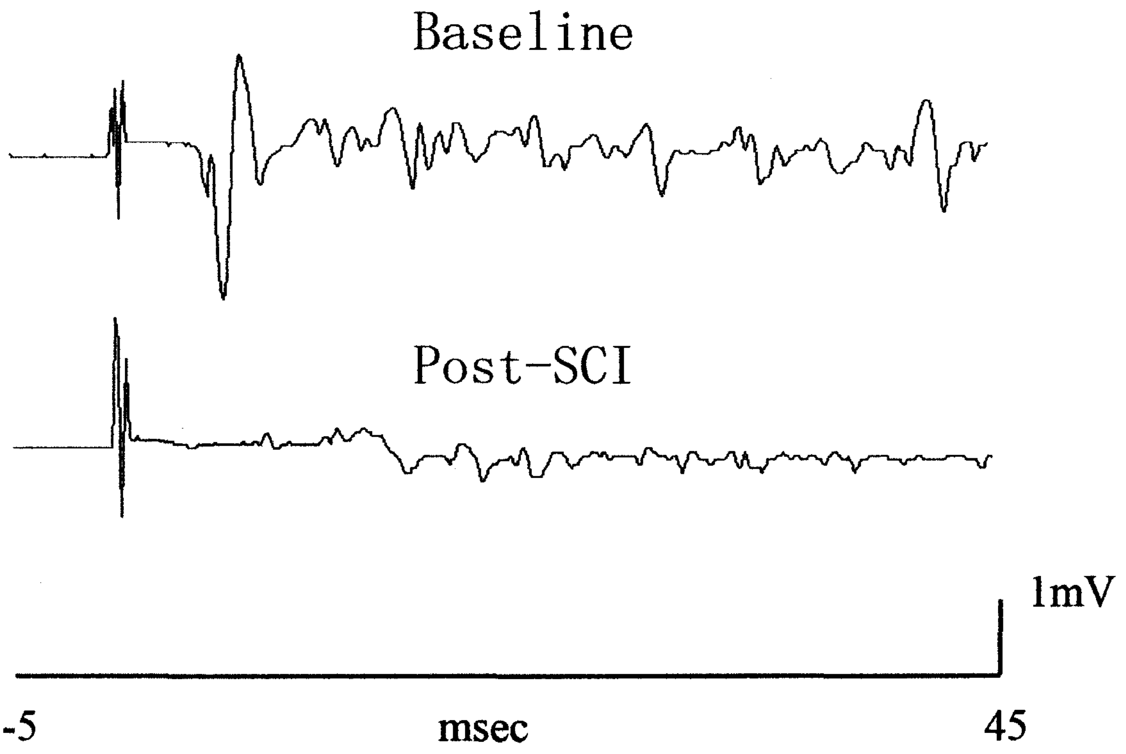


Figure 11: tcMMEP responses are absent in many animals following Mild and Moderate SCI.

Eriachrome Cyanine Staining Confirms The Lesion Severity Of Mild And Moderate Contusions

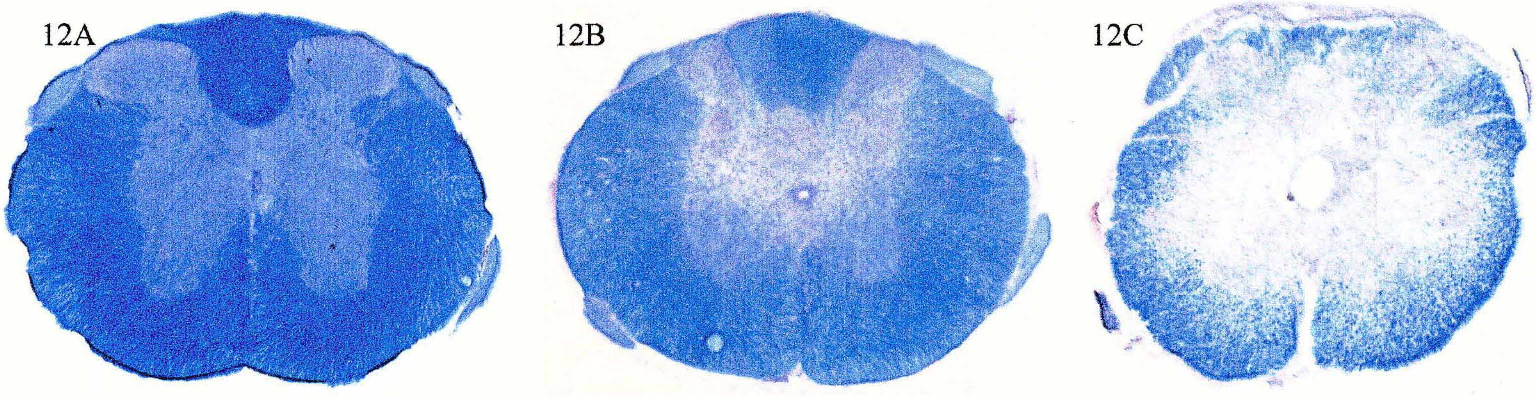


Figure 12: Sham injured mice show normal white matter staining at ten weeks post injury (12A). In mildly injured animals (0.25 mm displacement injury), a small loss of white matter is evident ten weeks post injury (12B). Moderately injured animals (0.40 mm displacement) show significant loss of white matter ten weeks post injury (12C).

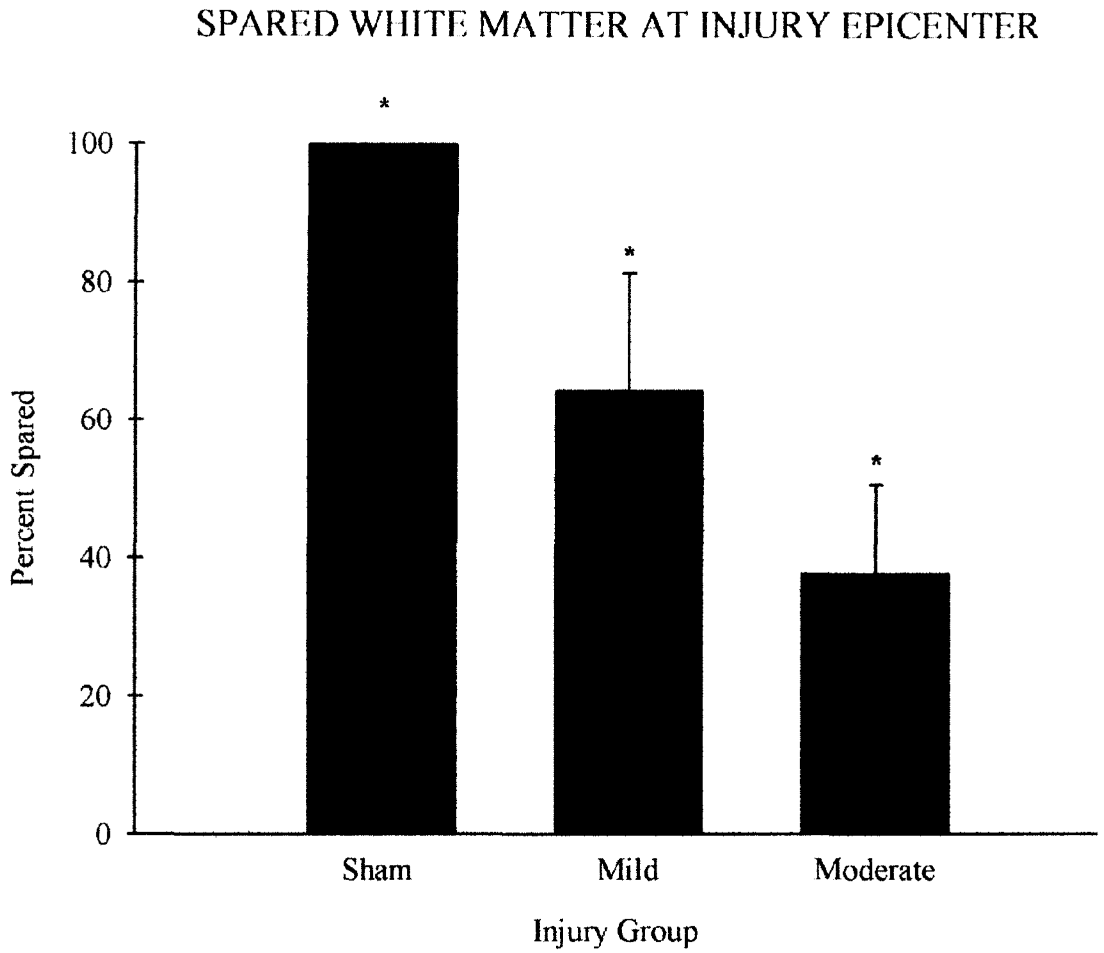


Figure 13: The Sham group had a significantly higher percentage of spared white matter than both the Mild group (F=57.547, df=2, 29, p<.001; t=35.6, df=3, 29, p<.001) and the Moderate group (t=62.2, df=3, 29, p<.001). In addition, the Mild group had a significantly higher spared white matter percentage than the Moderate group (t=26.6, df=3, 29, p<.001).

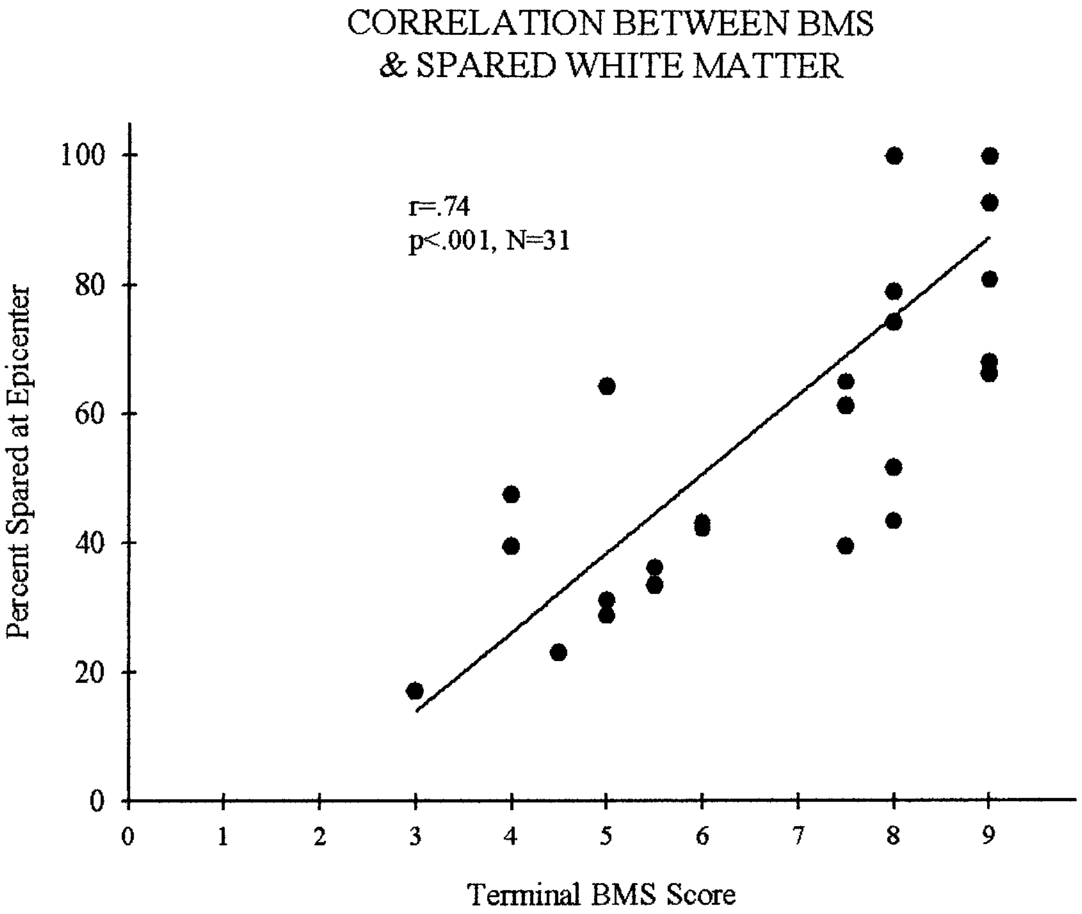
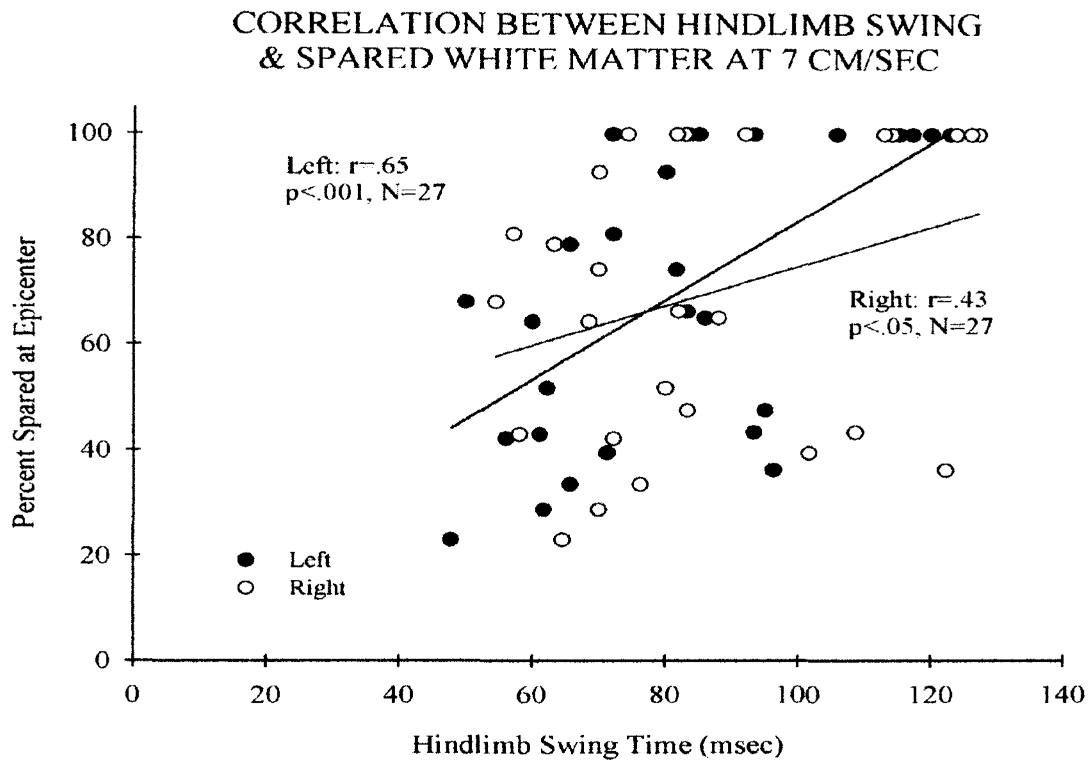
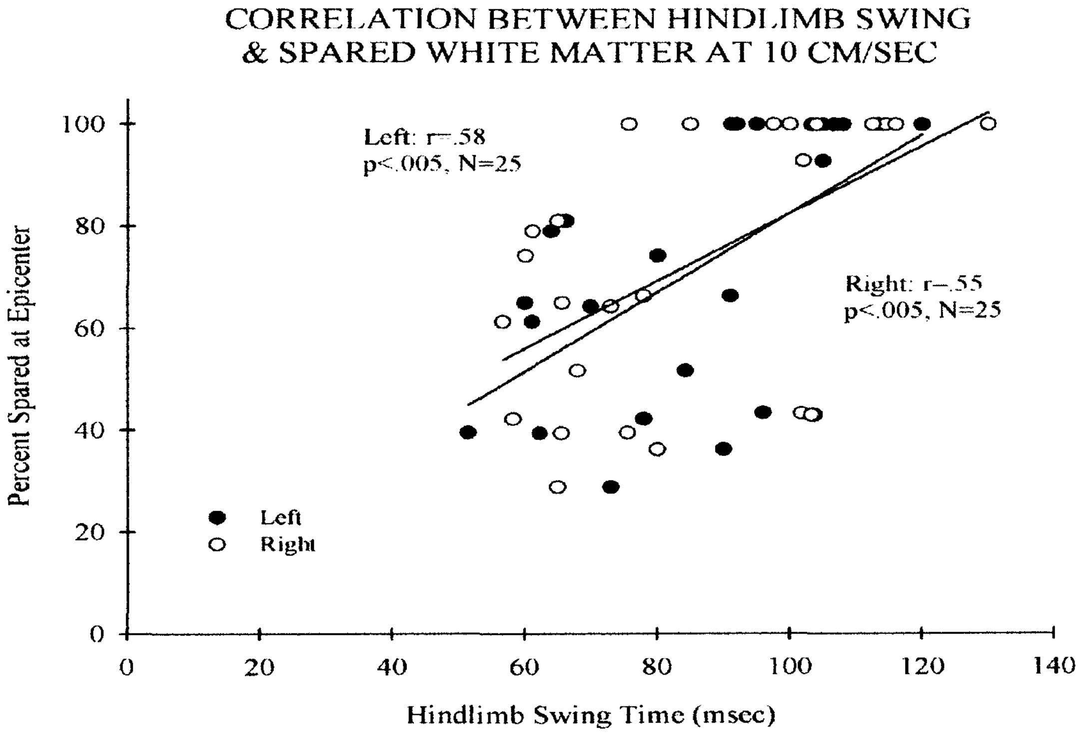


Figure 14: BMS scores correlate strongly with spared white matter percentage at the lesion epicenter



CORRELATION BETWEEN HINDLIMB SWING & SPARED WHITE MATTER AT 7 CM/SEC

Figure 15A: Hindlimb swing time correlates unevenly with spared white matter percentage at 7 cm/sec



CORRELATION BETWEEN HINDLIMB SWING & SPARED WHITE MATTER AT 10 CM/SEC

Figure 15B: Hindlimb swing time correlates much more evenly with spared white matter percentage when treadmill speed is increased to 10 cm/sec.

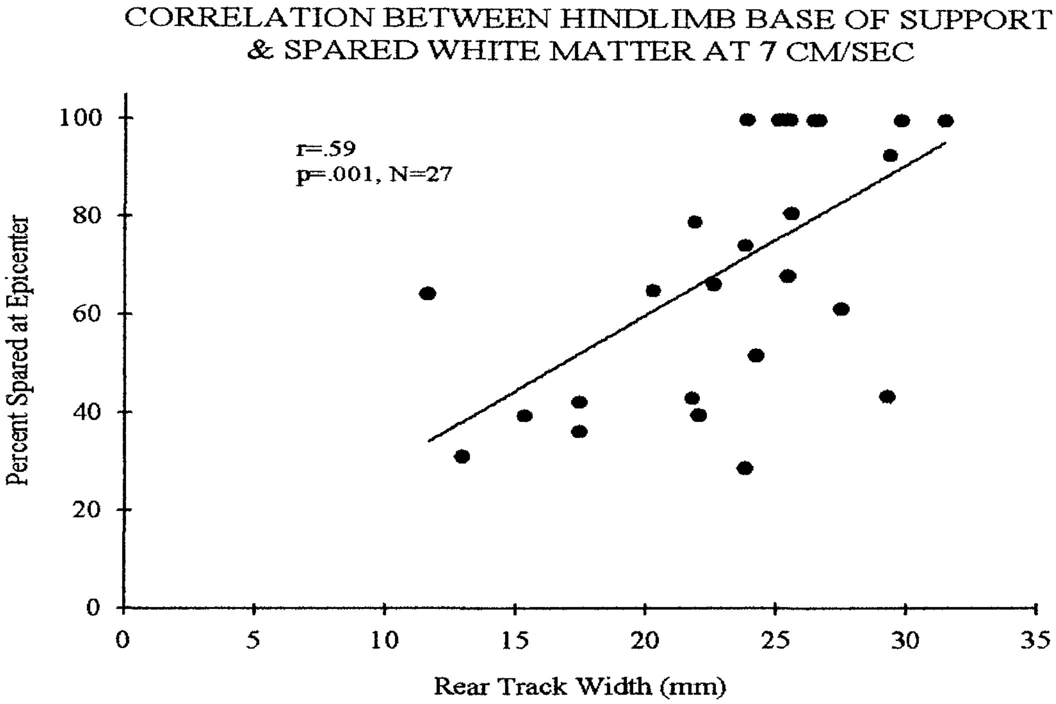


Figure 16A: Hindlimb base of support correlates well with spared white matter percentage at 7 cm/sec.

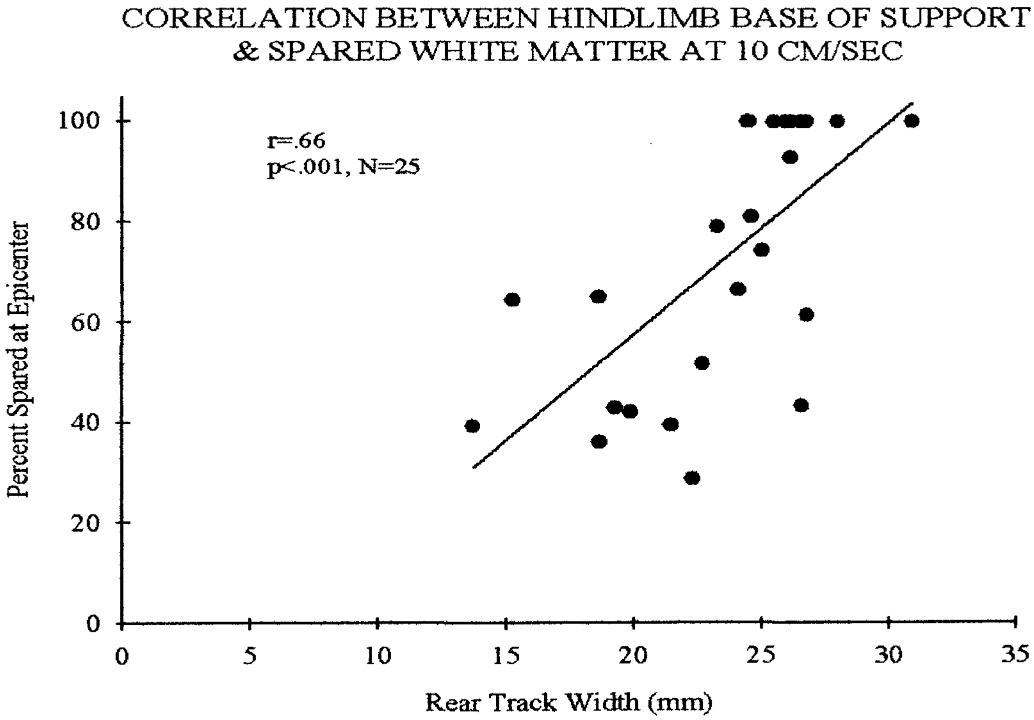


Figure 16B: Hindlimb base of support also correlates well with spared white matter percentage at 10 cm/sec.

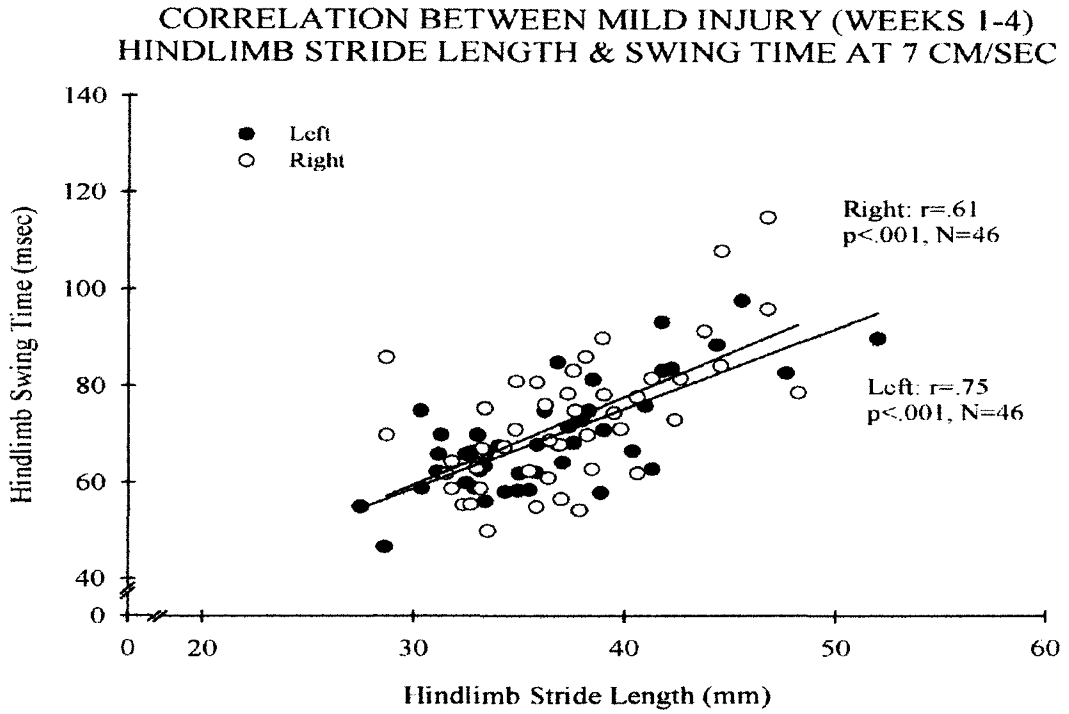


Figure 17A: During the initial recovery phase in the Mild injured animals, right hindlimb swing times correlated well with right hindlimb stride lengths at 7 cm/sec; similarly strong correlations were found for the left side.

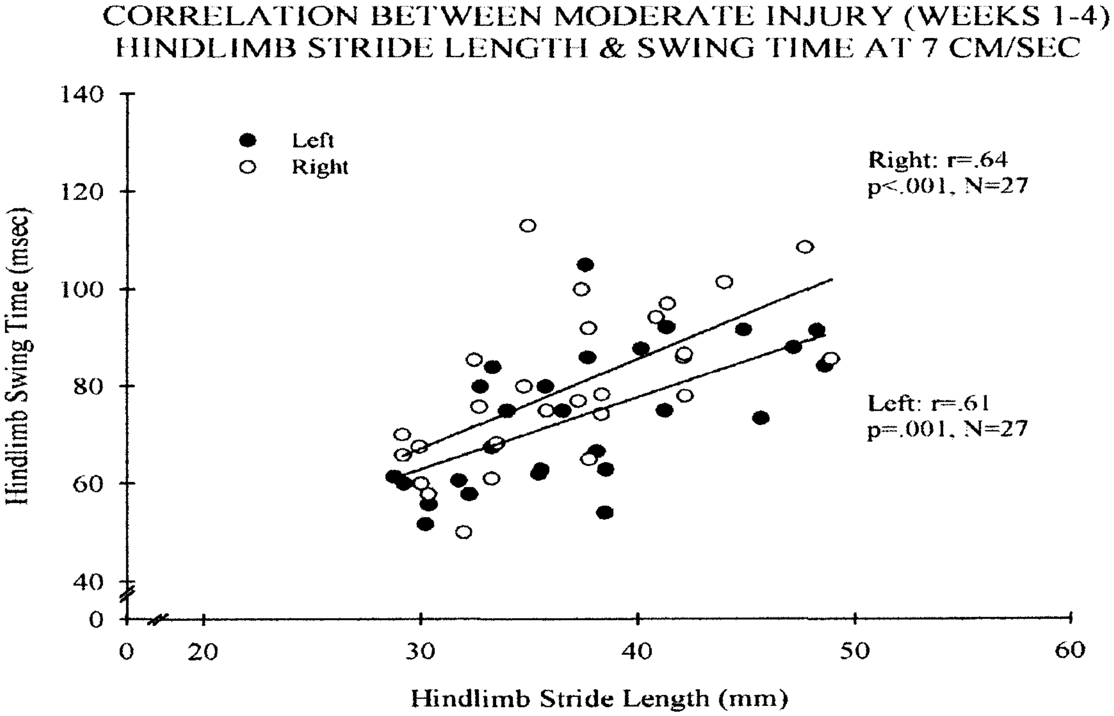


Figure 17B: During the initial recovery phase in the Moderate injury group, right hindlimb swing times correlated well with right hindlimb stride lengths at 7 cm/sec; similarly strong correlations were found for the left side.

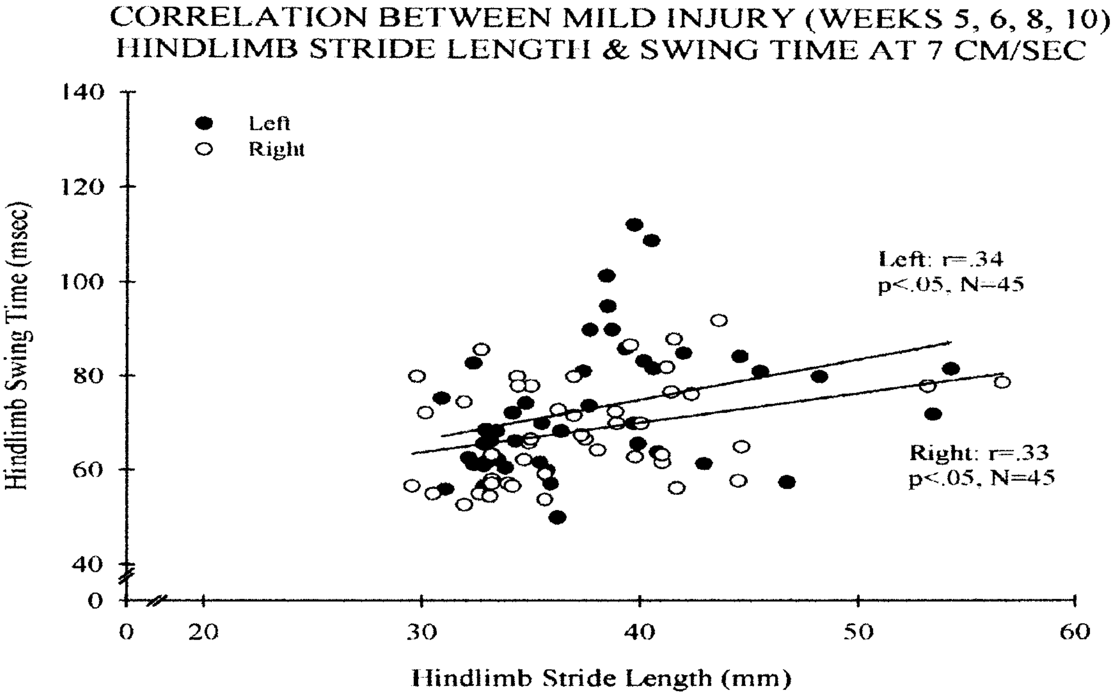


Figure 18A: Right hindlimb swing times correlated with right hindlimb stride length at a much weaker level for Weeks 5, 6, 8, and 10 post-injury in the Mild group. Similarly weaker correlations were found on the left side during these later weeks.

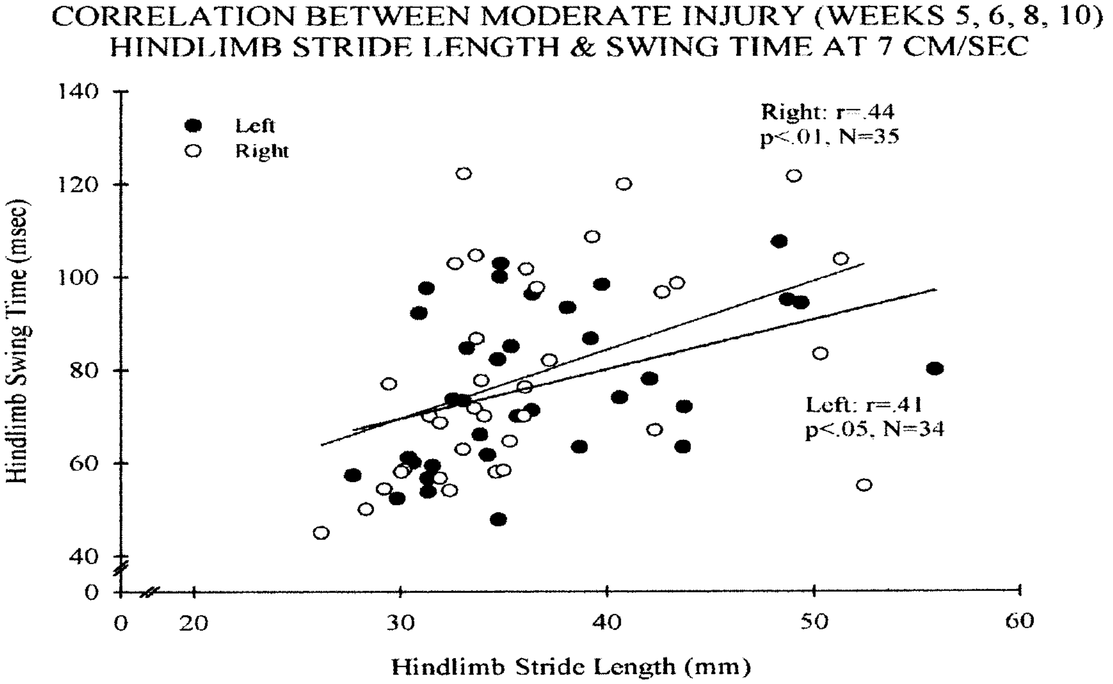


Figure 18B: Similar to the Mild injury group, right hindlimb swing times correlated with right hindlimb stride length in the Moderate injury group at a much weaker level for Weeks 5, 6, 8, and 10 post-injury than for earlier time points. Similarly weaker correlations were found on the left side during these later weeks.

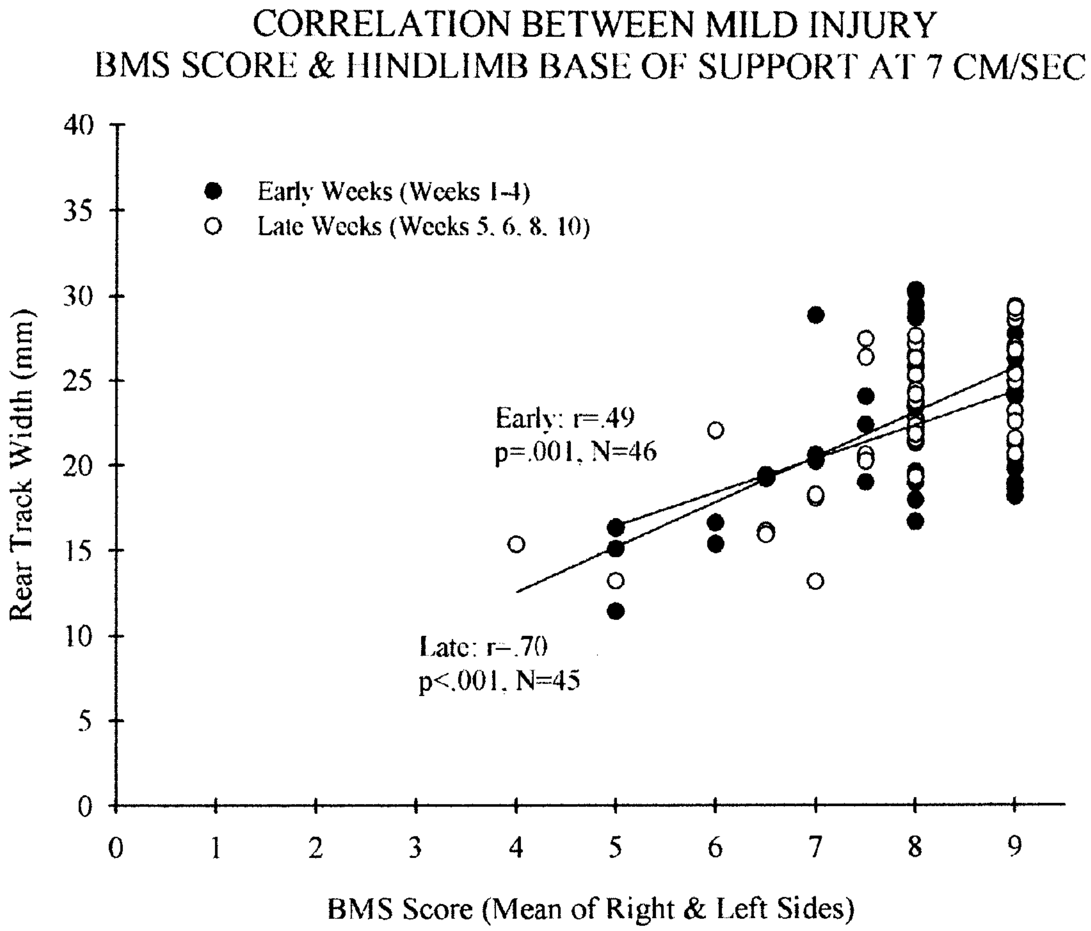


Figure 19: In the Mild injury group, rear track width correlated strongly with BMS scores for both the early time points and the later time points.

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