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Effects of telemetry EMG implants on hindlimb locomotor recovery in thoracic spinal contused rats.

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EFFECTS OF TELEMETRY EMG IMPLANTS ON HINDLIMB LOCOMOTOR RECOVERY IN THORACIC SPINAL CONTUSED RATS

By

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ABSTRACT

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Spontaneous recovery in rats after spinal cord injury (SCI), and interventions that improve or impede spontaneous recovery are not well understood. During a study to characterize recovery landmarks after SCI using telemetry EMG, we discovered loss of function in telemetry implanted animals. To investigate this difference further, we implanted animals with leads in various hindlimb muscles to look for behavioral and kinematic differences in recovery. We found significant differences in both open field locomotor testing (BBB) and kinematics between implant and non-implant groups where implanted groups showed loss of coordination, as well as trends for animals implanted below the knee to show even greater loss of function than animals implanted above the knee. From these findings, we concluded the cause of loss of function to originate either from mechanical interference in animals' stepping; peripheral pain, inflammation, and unpatterned afferent input influencing post-injury, sensitized lumbar circuitry; or a combination.

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INTRODUCTION

Activity based therapy following spinal cord injury (SCI) is based primarily on findings from feline studies where treadmill training of the hindlimbs following transection of the spinal cord supported a recovery of weight-supported overground stepping in injured animals (Belanger et al., 1996; Boyce et al., 2007) Although animals achieved weight-supported stepping within weeks following injury, the same results have been difficult to replicate in human studies (Dietz et al., 1998; Dobkin et al., 2007; Nadeau et al., 2010). Similarly, rodent studies have shown only a very modest improvement in overground locomotion from activity-based therapies following SCI (Basso et al., 1995; Fouad et al., 2000; Multon et al., 2003; Heng et al., 2009; Guertin et al., 2011). Though it has been difficult to improve upon spontaneous recovery achieved in rodent and human studies using activity-based training, both possess the ability for pattern generation early after SCI. Although the lumbar spinal cord possesses the ability to generate a locomotor pattern independent of supraspinal input, the origin and mechanisms of the central pattern generator (CPG) are not well defined. With understanding of and accessibility to the lumbar spinal cord's inherent ability to generate locomotion, new training paradigms could be developed and implemented to further improve recovery in SCI patients.

In previous SCI studies using rodent models, spontaneous recovery has been observed by multiple investigators, where animals recovered weight supported stepping within a few weeks of injury (Jane et al., 1963; Basso et al., 1996;). Because this return to weight supported stepping has been found in the absence of intervention, interpretation of results in experiments that involve training paradigms is difficult. Retraining studies have been faced with the challenge of overcoming a potential ceiling effect from spontaneous recovery. In a 2000 paper, Fouad suggested the apparent spontaneous recovery was a result of in-cage retraining by rats with SCI. He proposed the animals' activity while moving freely about their cages was responsible for the recovery effect seen in these experiments (Fouad et al., 2000).

Past studies in our own lab have also been challenged by spontaneous recovery of locomotion in rats following SCI (Magnuson et al., 2005; Smith et al., 2006). As assessed by the BBB Open Field Locomotor Scale, a measure of hindlimb function during overground stepping (BBB; Basso et al., 1996), rats from injured (untrained) control groups achieved scores of 13-15 or 10-12 for moderate or moderately-severe injuries, respectively, and neither swim training nor shallow-water training improved BBB scores (Kuerzi et al., 2010; Magnuson et al., 2009; Smith et al., 2006). We first attempted to address the effects of in-cage retraining using a hindlimb immobilization model. In this study (Caudle et al., 2011), rats' hindlimbs were immobilized after a mild-moderate SCI at the T9 cord level. We found a drastic drop in hindlimb function when compared to injured but not immobilized animals. Furthermore, when removed from the wheelchairs after 8 weeks, these animals never regained the function of unrestrained, injured animals. This study suggested that hindlimb-immobilization after injury impeded recovery

following SCI by restricting feedback and input from afferent sources involved in hindlimb loading, cutaneous feedback from paw placement, and phasic limb movements, or by the introduction of aberrant afferent feedback in the form of noxious or unpatterend signals to the lumbar circuitry. With the sustained deficits after removal of hindlimb restriction, it appeared the inherent ability of the lumbar cord to recover locomotor ability was limited at that time point (8 weeks post-injury). If the cord can facilitate recovery of stepping to some extent, it is possible this capacity is at its maximum acutely, within the first few weeks following injury.

To further investigate the influence of in-cage activity, we developed an overnight activity monitoring system to measure in-cage activity following SCI. Since rats are nocturnal animals, the system uses infrared cameras and software for sampled recording of the animals' activity in their cages. Using tracking software, we were able to measure the distances our animals were moving overnight. Coupled with BBB assessments, we began to investigate the relationship with in-cage activity, measured as distance travelled overnight, with recovery over time after SCI. We found that both cage size and housing condition (single vs. double) influences in-cage activity and that there is a correlative relationship between BBB scores and distance traveled overnight (unpublished observations).

For some time, our lab has been concerned with gain and loss of function in our contusion model, primarily in the context of activity dependent plasticity and training paradigms. We consider gain of function to be an increase in overground stepping quality and ability beyond "spontaneous" recovery. Loss of function involves anything that would inhibit typical spontaneous recovery, such as hindlimb immobilization. We would

like to understand the physiological mechanisms involved for the purpose of promoting training that facilitates gain of function, and to eliminate activities that lead to loss of function. We have observed significant recovery and time point thresholds in rats' behavior using measures such as the BBB scale, and we believe these functional thresholds coincide with crucial processes happening within the lumbar circuitry, extrinsic influences on the lumbar circuitry, or both. Based on these observations, we hypothesize that there are "landmarks of recovery" that, when achieved, lead to rapid improvements in function. These recovery landmarks parallel the key functional steps outlined in the BBB Score (Basso et al., 1995, 1996). We feel that the best way to approach these issues is to closely investigate and characterize the hindlimb electromyogram (EMG) patterns expressed during in-cage activity in the first few weeks following SCI in our rat contusion model.

To characterize intrinsic and extrinsic recovery landmarks we designed a study using a radiotelemetry system (Data Sciences International, St. Paul, MN) to record EMG activity in ankle flexors and extensors of injured rats during overground stepping, as well as during overnight in-cage activity. Our goal was to monitor changes in the amplitudes and phase relationships of EMG bursts in the context of in-cage activity and the recovery of overground stepping abilities. We hypothesized that each of the key landmarks of recovery would involve characteristic changes in EMG activity (amplitude and or patterns) and that they would preceed increased in-cage activity. These changes would indicate the recovery or development of one or more abilities or capacities that are necessary for, or that would facilitate in-cage activity. Similarly, we hoped to measure the relationships between these changes and overall recovery of locomotion. Finally, we

wanted to establish time points to focus on further in regards to neural substrate, and promotion of loss/gain of function for experimental purposes.

When conducting these experiments and looking at the progress of BBB scores over time, we discovered a difference in BBB scores between injured animals that had been implanted for telemetry EMG recordings and animals that were injured, but did not receive implants. In the 3-5 weeks of assessment, it became clear that the two groups (implanted and un-implanted) were functionally distinct. Both groups had reached BBB scores of 11's by the 3 week time point which indicated weight-supported stepping, but in the 4-5 week period, non-implant animals experienced an increase in BBB scores to 15-17 which indicated forelimb/hindlimb coordination. Their implanted cagemates' scores remained in the 11-12 point range. It appeared that the EMG telemetry implants were having an effect on locomotor recovery. This effect would cause complications in interpretation of our data and would challenge our goal to characterize effects of in-cage activity and recovery landmarks.

The discovery that EMG telemetry implants were influencing locomotor recovery after SCI led us to shift our experimental question to an examination of the effects of the implants on recovery and to begin investigating the cause. The use of radiotelemetry to record EMG is a rather novel method that will allow us to record EMG in situations where we were not able to before such as during in-cage activity, swimming, or moving freely in-cage with immobilized hindlimbs. This would give us not only an outcome measure beyond behavioral testing, but a window into the processes governing recovery of stepping within the lumbar cord. If we could establish the cause of the loss of function from the implantation, we could either eliminate the effect all together, or have the ability

to design future experiments to account for the effects of implantation. Past studies using various EMG implantation techniques did not include non-implant controls (Antri et al., 2002; Feraboli-Lohnherr et al., 1999; De Leon et al., 1994; Pierotti et al., 1989; Berriere et al., 2008). Therefore, examining effects of invasive implants to measure locomotor outcomes after SCI is a novel idea and has the potential to lend new evidence and insight into effects of peripheral pain and inflammation on CNS circuitry and locomotor recovery after SCI. Investigation of the effects of the EMG telemetry implants also allowed us the opportunity to consider mechanisms involved in gain/loss of locomotor recovery following SCI.

There are multiple reasons to consider for the loss of function in our injured, telemetry EMG implanted animals. The implants consist of battery pack and transmitter modules that sit in the peritoneal space as well as a thick connecting cable that joins the 2 modules. Beyond the actual transmitter, EMG wires, with the tips placed in selected hindlimb muscles, must traverse from the transmitter module to the muscle. Depending on where the wires are placed, this could introduce interference with the animals' range of motion in hindlimb joints. Finally, pain and inflammation from the entire implant could also have an effect on the animals' recovery. In our original set of animals, EMG wires were placed in the tibialis anterior (TA) and lateral gastrocnemius (LG) muscles. This implantation required wires to course subcutaneously past the knee joint, introducing a potential source of inflammation, as well as restricting the range of motion about the knee. Since our lab also uses similar implants for measuring cardiovascular (CV) activity after SCI that includes a transmitter module placed in the peritoneal cavity, but involved no EMG wires in the hindlimbs, we looked to rats with the CV implant to

assess effects of the module in the peritoneal space on locomotor recovery. Two rats from the CV study received the same injury used for the in-cage activity/EMG studies, and their BBB scores showed locomotor recovery that was similar to non-implanted animals. Thus, it is unlikely that the peritoneal implantation of the transmitter module alone was a cause of the loss of function. After eliminating the module implant as a potential suspect, we focused on placement of EMG leads in the hindlimbs. Since the first set of animals had wires placed in ankle flexors/extensors with leads passing by the knee joint, we chose to implant a new set of animals with lead placement in hip and knee flexors/extensor muscles superior to the knee joint. Since we had found signs of inflammation and/or infection around the knees of previously implanted animals, we hypothesized lead placement above the knee would allow greater range of motion about the knee joint as well as decrease the potential for inflammation in the hindlimbs. With less impedance on range of motion and decreased potential for inflammation, we hypothesized recovery of hindlimb locomotor recovery more similar to that of non-implant animals.

METHODS

Study design and experimental protocol. A total of 10 female Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) with weights in the range of 255-280 grams were used in these experiments. Experiments were performed in accordance with the guidelines of the University of Louisville Institutional Animal Care and Use Committee. Animals were separated into 3 groups: Group 1: injured with telemetry EMG implants below the knee (n=2); Group 2: injured with telemetry EMG implants above the knee (n=3); and injured with no telemetry EMG implant (n=5). Group sizes were kept small due to the cost of the transmitters as well as the cost and logistics of more cages for overnight activity monitoring.

Data Systems International (DSI; St. Paul, MN) telemetry EMG implants and implantation surgery. The DSI 4ET dual module transmitter was used to collect EMG data in these experiments, as well as to test the effects of implantation above and below the knee on locomotor recovery. The 4ET transmitter weighs 12.8 grams and consists of 2 modules; one which houses the battery and transmitting apparatus and a sensing module which has the input for the EMG leads. These 2 modules are connected via a cable. 8 EMG leads are connected to the sensing module, allowing 4 channels of EMG to be collected with each channel containing both negative and positive electrode leads. The EMG signals from the sensing module are transmitted through the telemetry

module using radio frequencies that are detected by DSI receiving units. Data is collected and processed using the Ponemah (DSI) analysis software on a desktop PC.

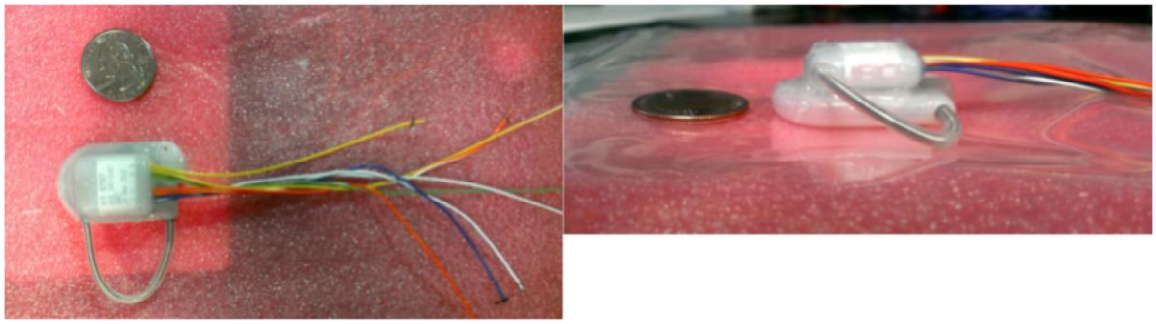


Figure 1. Images showing DSI telemetry EMG transmitter modules and EMG lead wires with respect to transmitter module size.

Animals were anesthetized using a peritoneal injection of ketamine (50mg/kg)/xylazine (2.4mg/kg)/acepromazine (0.5mg/kg) in 0.9% saline, and DSI 4ET transmitters were implanted using the DSI surgical protocol (DSI 4ET Device User Guide and Surgical Manual, 2010). The protocol was modified in our implantation with the 2 modules being attached with silicone adhesive and the joined unit implanted in the peritoneal cavity. This was done for use in other experimental paradigms in our lab including hindlimb immobilization and swimming experiments.

In group 1 EMG animals, the EMG leads were implanted into TA and LG muscles of both hindlimbs, with the electrode wires traversing the lateral sides of the hindlimbs, over the knees until they reached their placement muscles. The above the knee group (group 2) had their EMG leads implanted into vastus lateralis (VL) and biceps femoris (BF) of each hindlimb. Any excess electrode wire was coiled and tied with suture, and placed inside the peritoneal cavity just caudal to the 4ET modules. Since the implants can be sterilized and reused after experiments are concluded, the 3 4ET units

used in the above the knee group had all been used prior to this experiment. The lead wires must be trimmed after each use, and each 4ET unit used in the above the knee group had different length EMG lead wires. Lead wire lengths ranged from 8 inches to approximately 4 inches. This was important in determining if excess wire stored in the animals' peritoneal cavity was responsible in any way for recovery deficits.

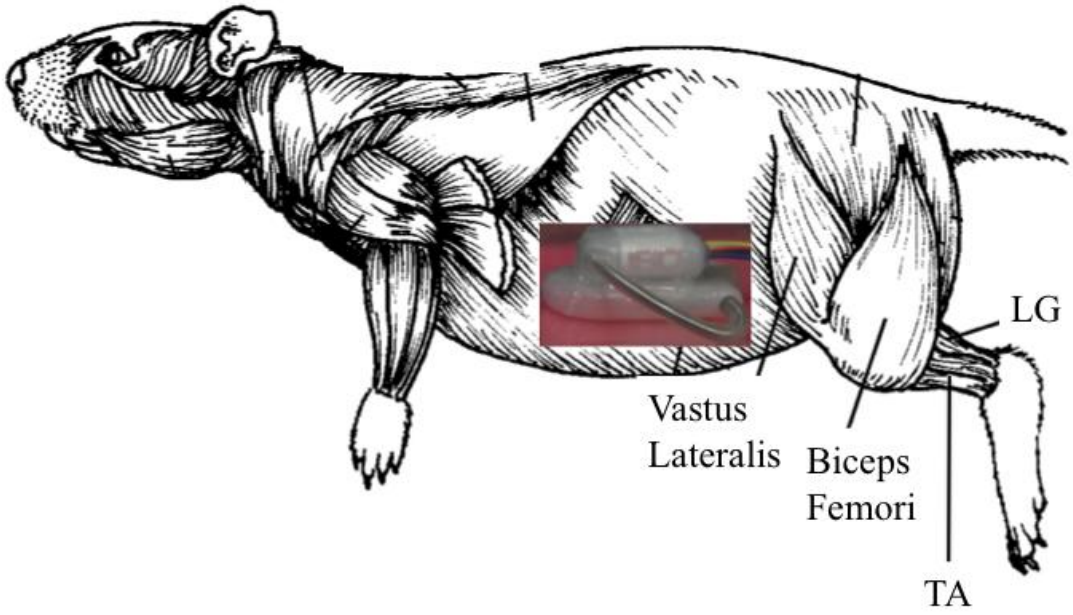


Figure 2. Diagram showing EMG telemetry transmitter module implant in peritoneal cavity as well as EMG lead implantation sites (diagram modified from http://biodidac.bio.uottawa.ca).

Following implantation, hindlimb incisions were closed with Surgical Suture (Henry Schein, Inc., New York, NY) while the abdomen was closed with surgical stainless steel clips (Buffalo Grove, IL). All incisions were coated with a mixture of New Skin (Medtech, Jackson, WY) and metronidazole (Mutual Pharmaceuticals Co., Philadelphia, PA), pulverized 500mg tablets for antibiotic action as well as to prevent the

rats from tampering with their incisions. For 7 days following surgery, animals were given 5ml 0.9% saline twice a day, 0.05ml gentamicin once a day, and 0.1ml Buprenex (.005mg/0.2kg; Recket & Colman Pharmceutical, Inc., Richmond, VA) twice a day. Ten days after surgery, all sutures and staples were removed under isoflurane anesthesia. Animals were single housed for 21 days following implantation surgery.

Spinal cord contusion surgery. After 14 days of recovery from 4ET implant surgery, all animals including non-implant animals were given an intraperitoneal injection of the same ketamine/xylazine/acepromazine cocktail used for implantation surgery, and brought to a surgical plane. Animals were given a 12.5g/cm injury at the T9 spinal cord level. Contusions were performed according to our standard protocol (Magnuson et al., 2009). Post operative care was the same as with the implant surgery. Seven days after contusion surgeries, animals were returned to double housing for the remainder of the study. Each implanted animal was cage matched with an injured, non-implant animal.

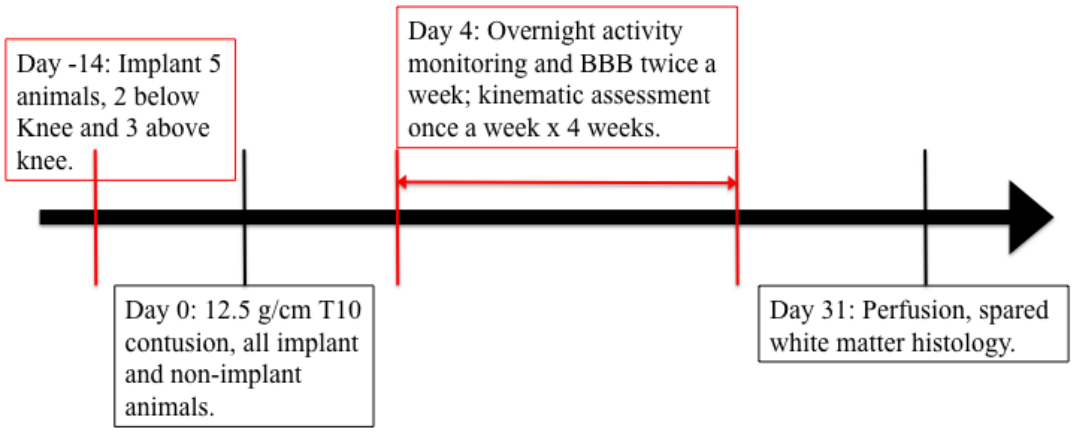


Figure 3. Experiment design and timeline.

Testing for recovery of overground locomotion (BBB). Starting 3 days after contusions, overground stepping ability was assessed twice a week using the BBB scale (Basso et al., 1995). Table 1 shows the major aspects of scoring for BBB testing.

BBB Score Range	Key Aspects of Score Range
0-8	Hindlimb movement without weight support.
9-14	Weight support without coordination.
15-21	Coordination and fine aspects of stepping.

Table 1. BBB scoring and corresponding descriptions for scores.

Assessment of in-cage, overnight activity. Overnight activity measurements were collected in the animal care facility where animals are normally kept at night. Breeding cages were customized with dividers that allowed the animals an amount of space equal to their normal cages. Clear plexiglass covers were created for overnight recording, and an infrared LED light and Basler ACA 645-100GM (Basler, Exton, PA) digital video camera system were mounted to record the animals in their cages. Video was collected at 4Hz using a program written in LabVIEW (National Instruments, Austin, TX). Previously, testing was done while developing the overnight system to determine what recording frequency (video frame rate) was necessary to accurately capture the rats' activity. Software was used to program recordings to capture the first of every 10 minutes for the 12 hour dark cycle in the animal care facility, totaling 72 recording loops a night. In-cage, overnight recordings were collected twice a week, beginning with the 3rd day post injury.

Figure 4. Overnight activity monitoring setup.

Overground 3-D kinematic assessment. On all days following BBB testing and overnight activity recording, overground 3-D kinematic assessments were completed on all animals. These assessments are used to measure range of motion in hindlimbs using peak/trough/excursion (PTE) of two angles based on points placed on the hip, ankle, and

toe (HAT) and the iliac crest, hip, and ankle (IHA)(Kuerzi et al., 2010; Caudle et al., 2011). Forelimb-hindlimb coordination was measured using plantar stepping index (PSI) and regularity index (RI), as described in the aforementioned studies. Furthermore, the coordinated pattern index (CPI) was used to analyze coordination patterns regardless of weight support or dorsal stepping.

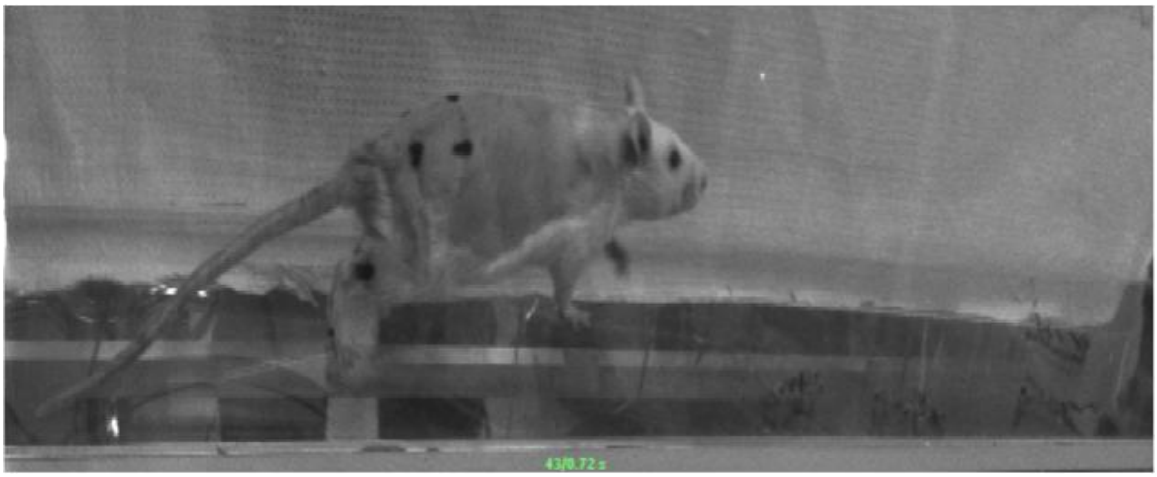


Figure 5. Single-frame image from video used for overground kinematic analysis.

Perfusion and histology. Five weeks after injury, rats were given double the surgical dose of ketmine/xylazine/acepromazine cocktail and perfused intracardially with 200ml 0.1M phosphate buffer. Once perfused, entire spinal columns were dissected from animals leaving the vertebral column intact. The entire column was placed in 4% paraformaldahyde (PFA) in phosphate buffer for 4 days. 4ET implants were also extracted from the implanted animals at this time and processed for re-use. After 4 days of fixation in PFA, spinal cords were removed from columns and postfixed in 4% PFA overnight. Following postfixation in PFA, spinal cords were immersed in 30% sucrose in phosphate buffered saline for 3 days for cryoprotection. Once cryoprotected, epicenters were processed for spared white matter (SWM) quantification according to our standard

protocol (Smith et al., 2006). We also delineated the ventral half of the epicenters to measure only the spared ventral fiber tracts which include the ventral lateral funiculus,

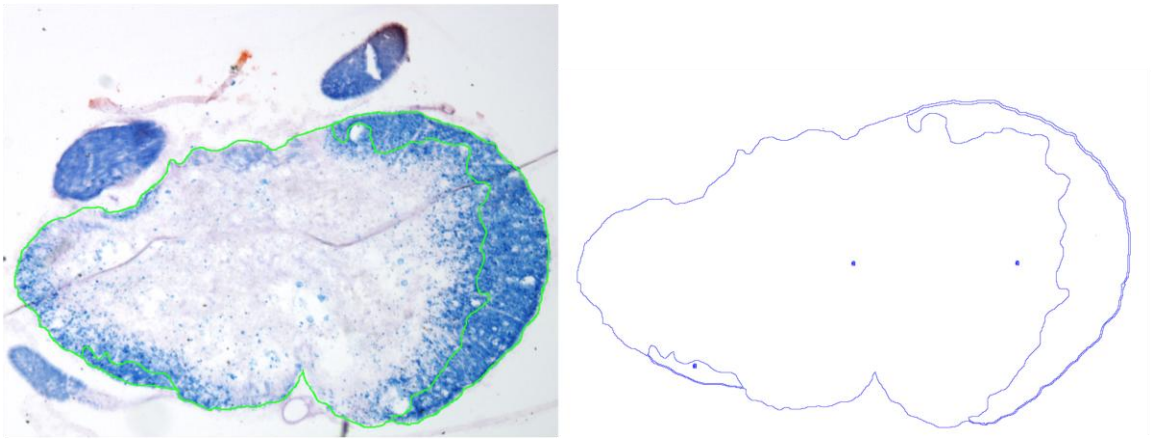


Figure 6. Representative image of stained epicenter showing spared white matter, original and traced images.

the fiber tracts of long distance propriospinal neurons involved in hind-forelimb coordination and timing.

Digitizing overnight activity videos. For overnight activity, points to be tracked and digitized were made on the animals using a permanent marker. Animals' backs were shaved from iliac crest to their tails, and a 2cm black circle was drawn dorsally and completely filled in. Recorded videos were played back in MaxTRAQ (Innovision Systems, Columbiaville, MI) software. This software allows the drawn points to be digitally tracked within the recording frame. Once tracked and digitized, the data was saved and exported as ASCII files for quantitative analysis.

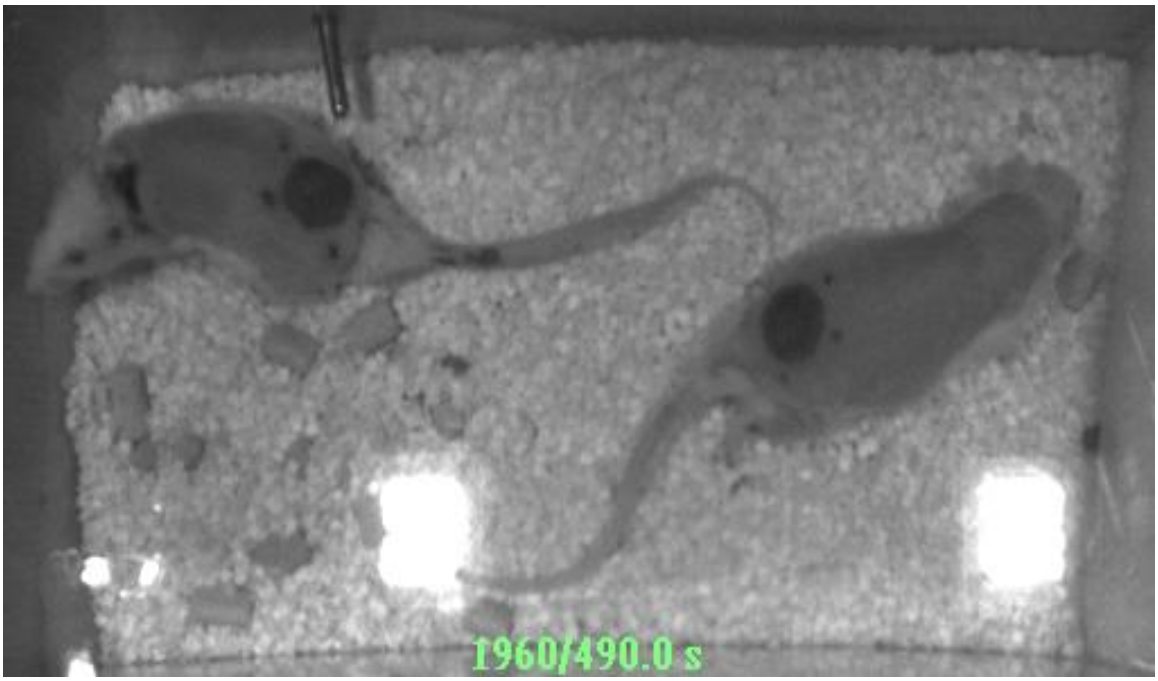


Figure 7. Single-frame image from overnight activity monitoring video.

Analysis of overnight activity. MaxMATE (Innovision Systems, Columbiaville, MI) software was used to collect raw numerical data in the form of coordinates of the digitized points. This data was imported into Microsoft Excel where a macro was used that calculated the distance between the digitized points, from frame to frame, then summed the distances to give the distance travelled by the animals during the 1 minute sampled loop. This calculation was performed for each 1-minute loop of a given night, then multiplied by 10 to estimate the total distance travelled during the 12 hour dark cycle. The distance is first determined in pixels and then calculated according to video calibrations to give the distance in meters.

Analysis of overground kinematics. Overground kinematics were analyzed for range of motion and limb movement/positions according to our standard protocol (Kuerzi et al., 2010; Caudle et al., 2011).

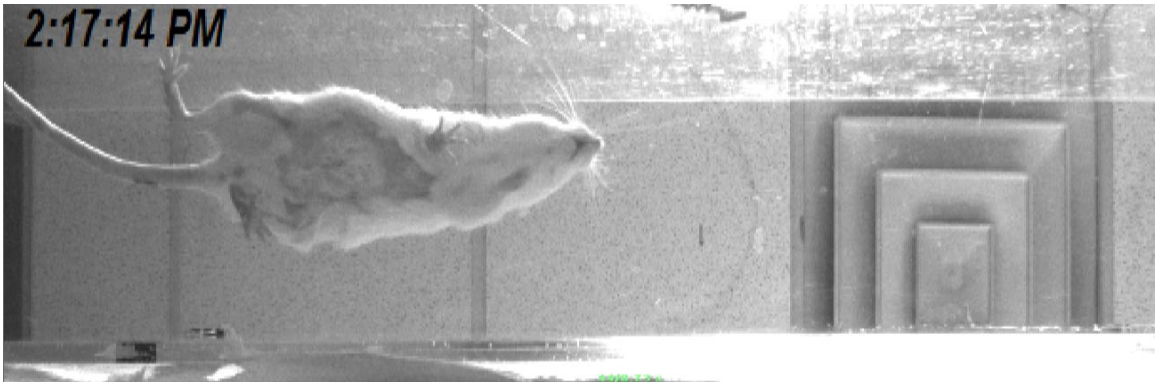
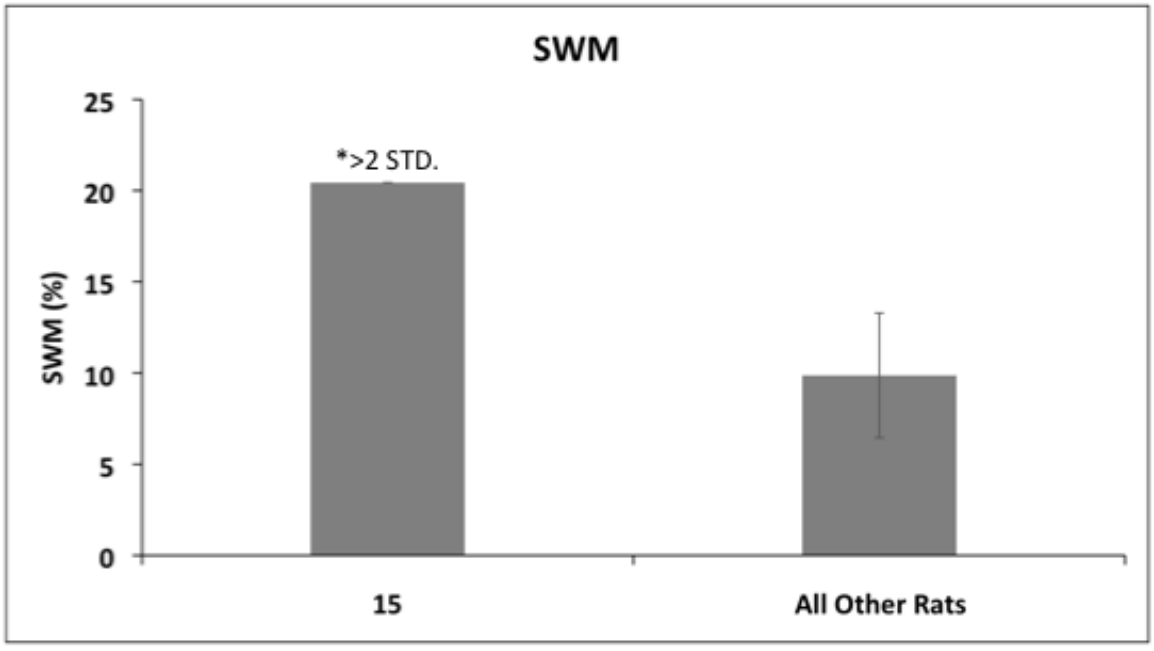


Figure 8. Single-frame image of bottom camera video during overground kinematics.

Statistics. An independent t-test was used to compare SWM between groups. Repeated measures analyses of variance (ANOVA) were used to compare BBB scores. Non-parametric Mann-Whitney U tests were used to compare PTE of the HAT and IHA. Mixed model analyses with pairwise comparisons were used to compare PSI, RI, and CPI. All data are presented as means ± standard deviation.

RESULTS

Histology. One implanted animal from the above the knee group was excluded from all analyses due to being an outlier. This animal fell outside 2 standard deviations in SWM when compared to all animals in the study (20.436 vs. 9.865% ± 3.423). This animal also fell outside 2 standard deviations in BBB assessments when compared to all implanted animals (15.5 vs. 11). When ventral spared white matter was measured, this animal was determined to be an outlier compared to the implant group. Outlier status was designated via quartile calculations, 1.5 x 25Q (Ludbrook, 2008).



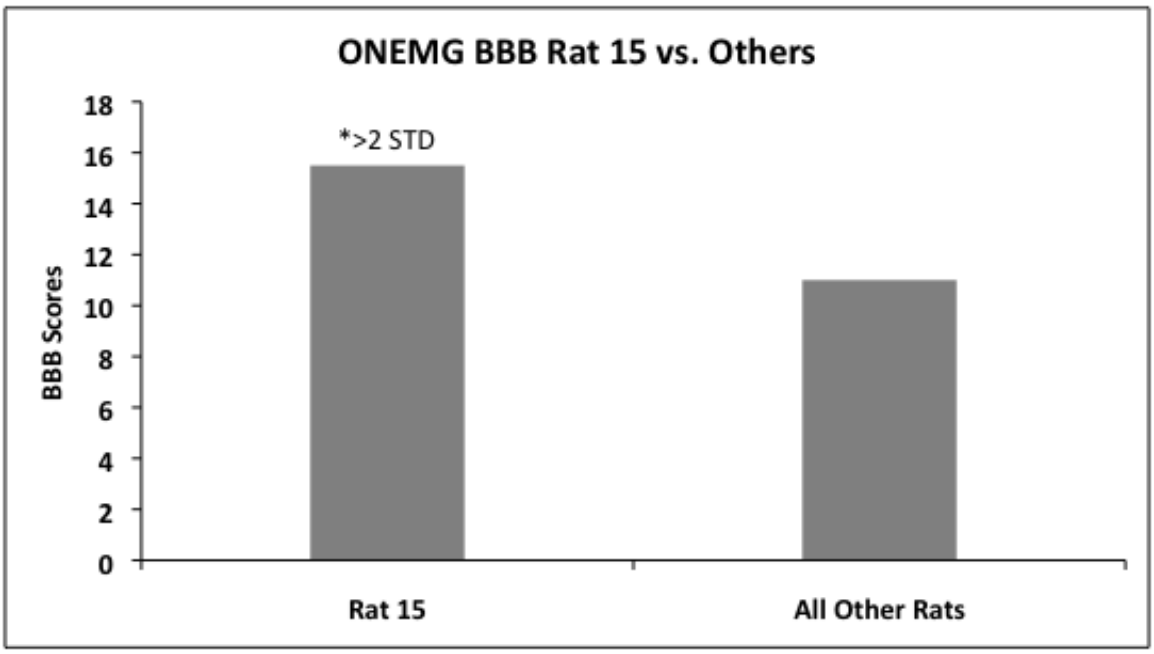
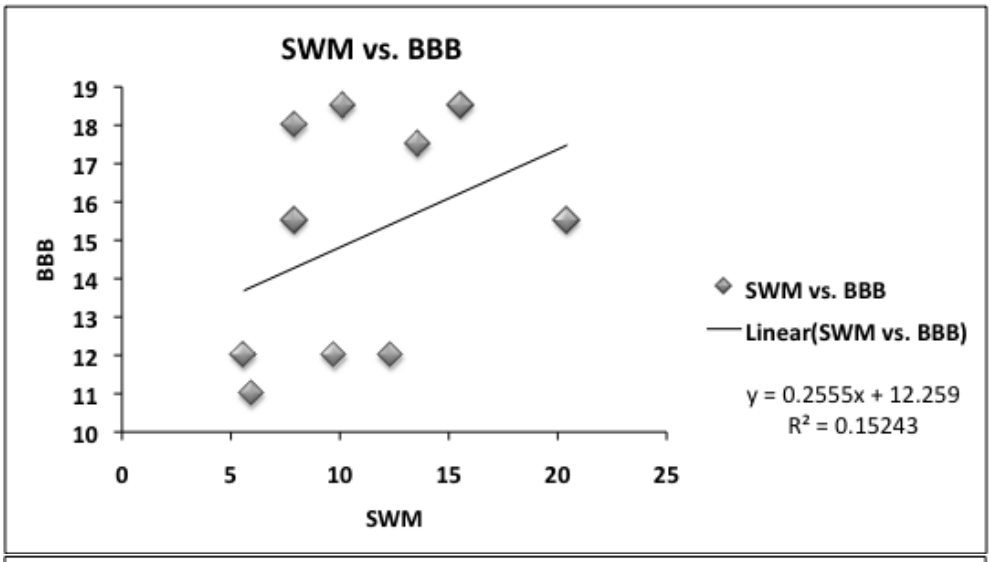
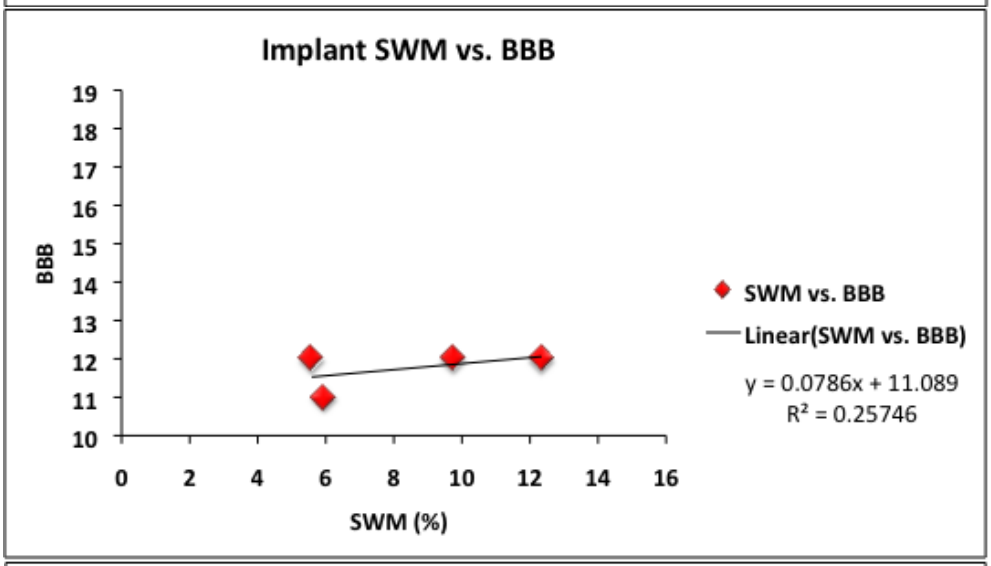


Figure 9. (ONEMG, Overnight EMG refers to name of the study) Graphs showing animal 15 as an outlier in respect to SWM when compared to all animals in the study and BBB in comparison to all imlpanted animals.

Epicenters of the spinal cord injury were analyzed for percent spared white matter (SWM) to eliminate fiber tract sparing as an explanation for any differences found in locomotor recovery. No differences were found in SWM between groups when analyzed either as implant vs. non-implant (8.408 ± 3.227% vs. 11.031 ± 3.435%; $p<0.281$), implant above knee vs. implant below knee vs. non-implant groups (9.146 ± 4.517% vs. 7.670 ± 2.941% vs. 11.031 ± 3.435%; $p<0.539$). When ventral white matter was analyzed, no correlation was found between ventral SWM and BBB scores in implant or non-implant groups ($r_{2=}.775$, $p<.225$, $n=4$; $r_2=.616$, $p<.269$, $n=5$).


SWM vs. BBB
BBB
SWM
SWM vs. BBB
Linear(SWM vs. BBB)
y = 0.2555x + 12.259
R² = 0.15243


Implant SWM vs. BBB
BBB
SWM (%)
SWM vs. BBB
Linear(SWM vs. BBB)
y = 0.0786x + 11.089
R² = 0.25746

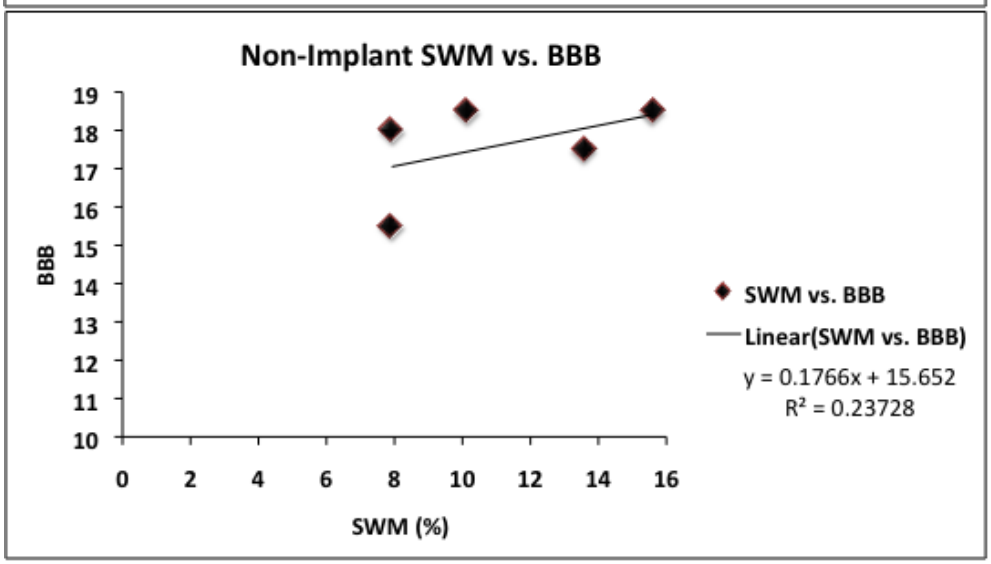

Non-Implant SWM vs. BBB
BBB
SWM (%)
SWM vs. BBB
Linear(SWM vs. BBB)
y = 0.1766x + 15.652
R² = 0.23728

Figure 10. Scatterplots showing correlations between SWM and BBB with all animals, as well as implanted and non-implanted separately.

Overground locomotor assessments (BBB). The final BBB assessment (day 28) revealed a difference between implanted and non-implanted animals (11.00 ± .0000 vs. 15.40 ± 0.4183; $p<0.05$). When analyzed as 3 groups, accounting for EMG lead placement, there were no differences between groups in recovery of overground locomotion.

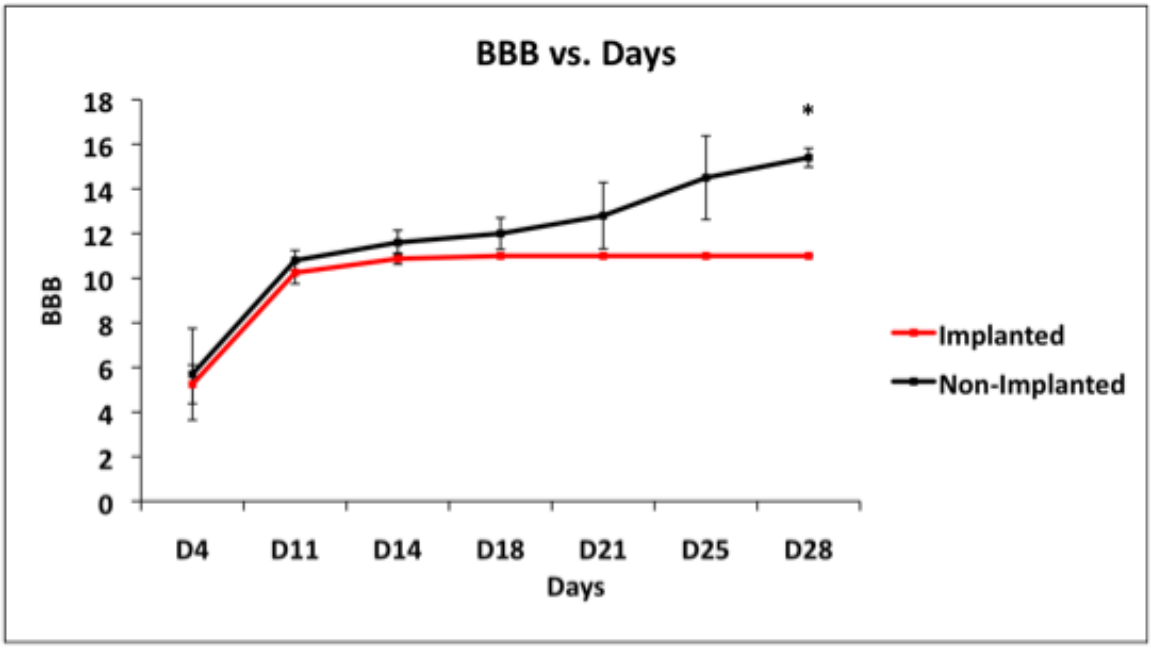
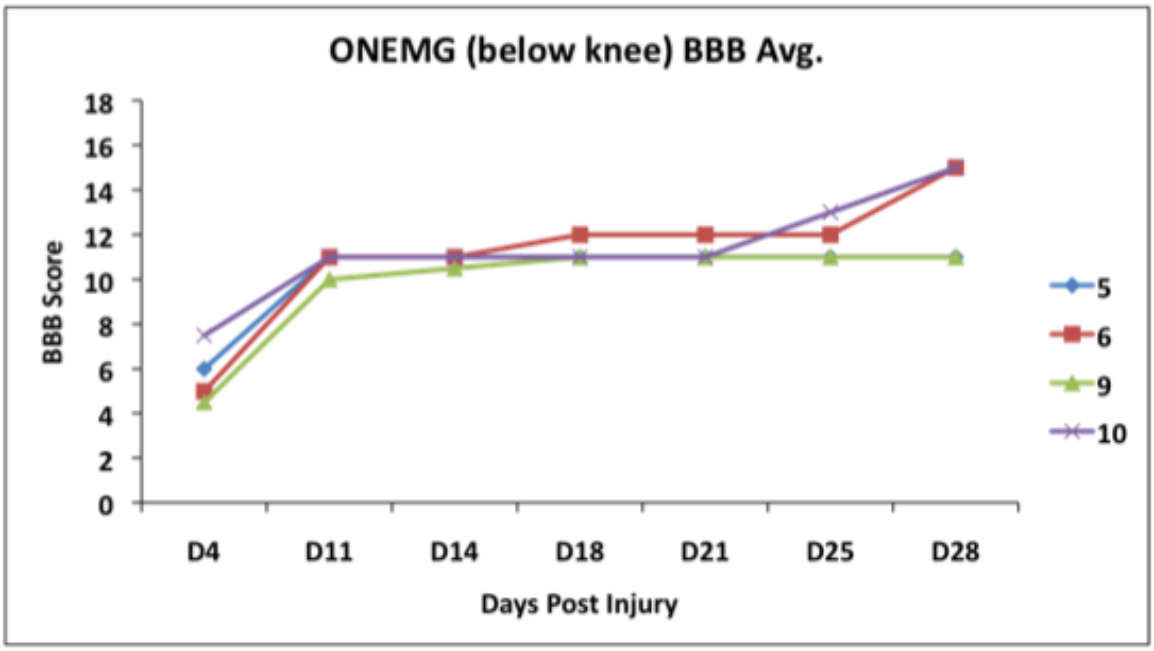


Figure 11. Average BBB scores between implanted and non-implant groups showing an increase toward weight-supported, coordinated hindlimb stepping in the non-implant group but not in the implanted animals (*$p<0.05$, one-way ANOVA with Tukey post hoc t-test).



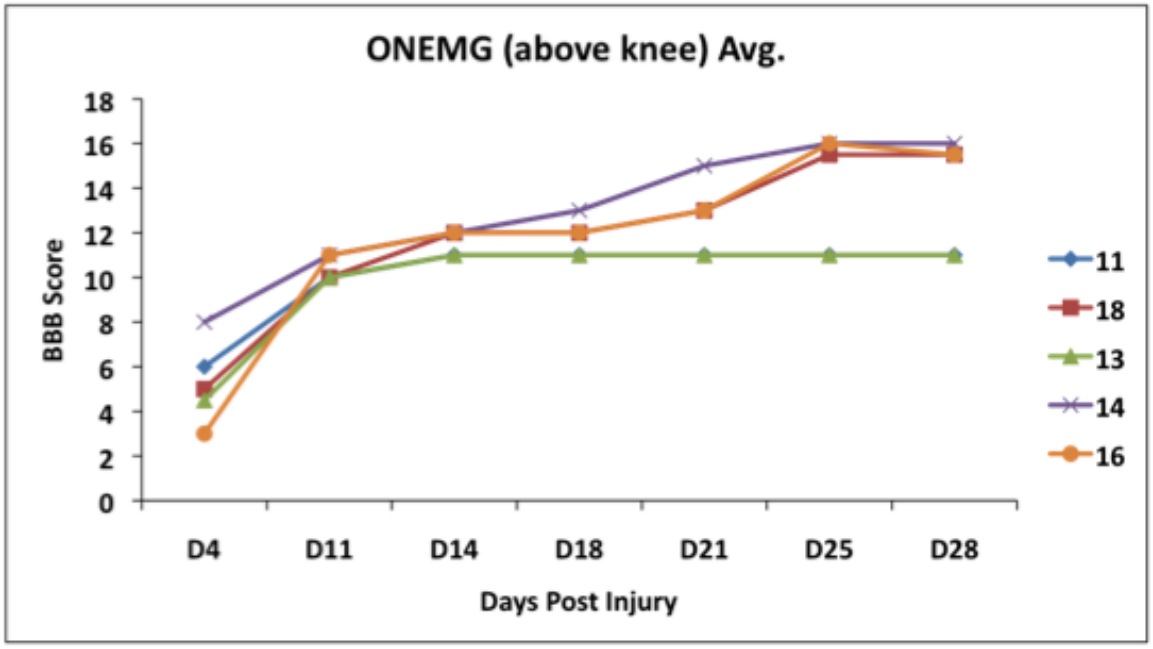


Figure 12. Below knee and above knee EMG implant BBB scores graphed separately with non-implant cagemates showing no difference in loss of function between the 2 implant groups.

Kinematic analysis. The peak/trough/excursion (PTE) of the angle with points on the hip/ankle/toe (HAT) and iliac crest/hip/ankle (IHA) was compared between implanted and non-implanted groups, as well as implanted below knee vs. implanted above knee vs. non-implant groups. No significant differences were found in these comparisons, although there was a trend for greater range of motion in the implant above the knee animals. There was also a fair amount of variability in the implant below the knee group that increased over time.

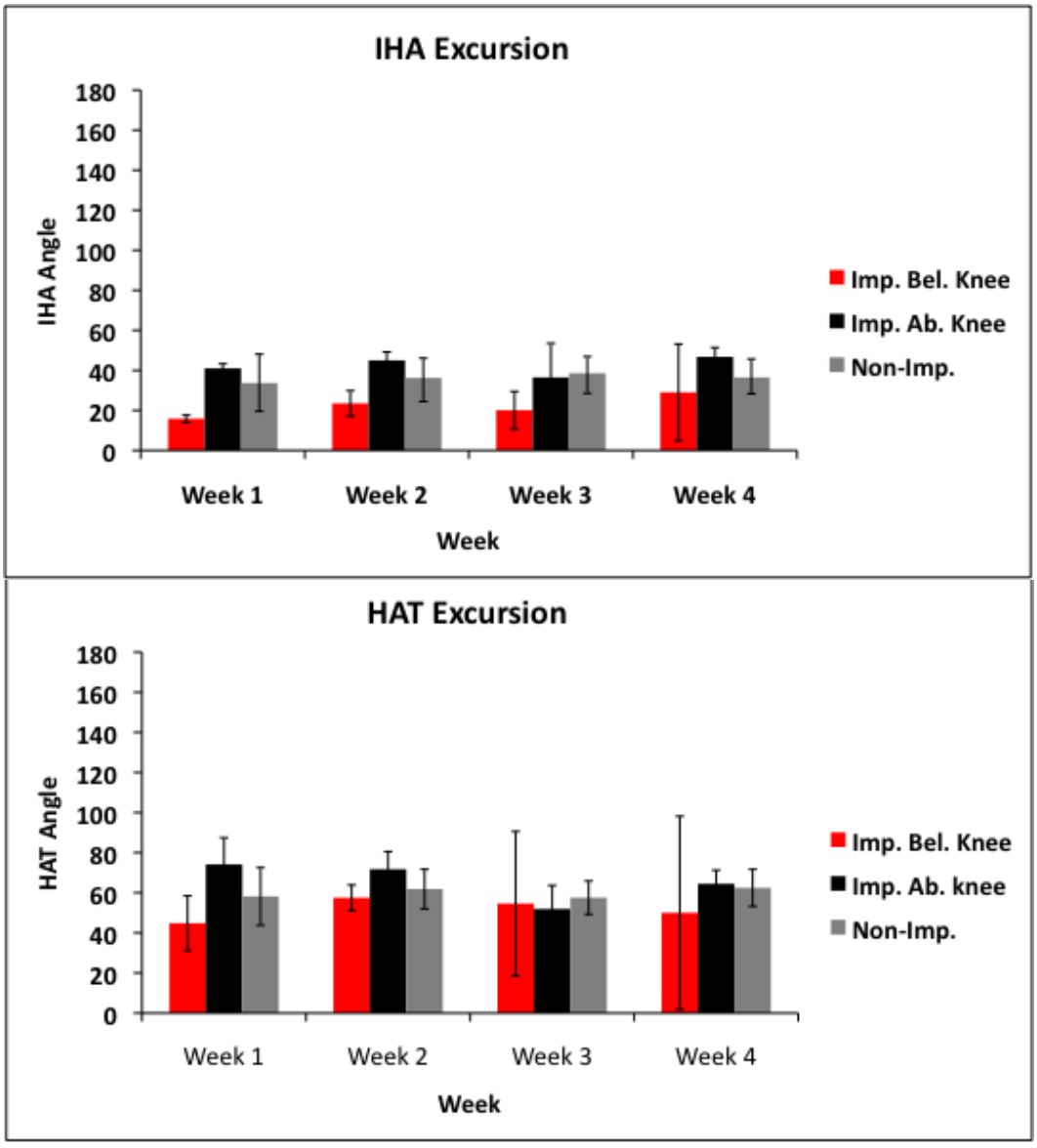


Figure 13. HAT and IHA plots (x 3 groups) showing no difference in average excursion about the knee and ankle between groups. However, a trend is present suggesting greater range of motion in implant above the knee vs. below the knee animals.

Analyses of bottom camera kinematics were used to examine coordination aspects of overground stepping between groups. When analyzed as 3 groups to look at effects of EMG lead placement, the only difference was found in the RI measurement between implanted below knee and non-implant animals at weeks 3 and 4 (20.9 ± 3.06% vs. 82.4 ± 15.03%, $p<0.01$; and 49.75 ± 12.13% vs. 86.64 ± 8.70%, $p<0.01$).

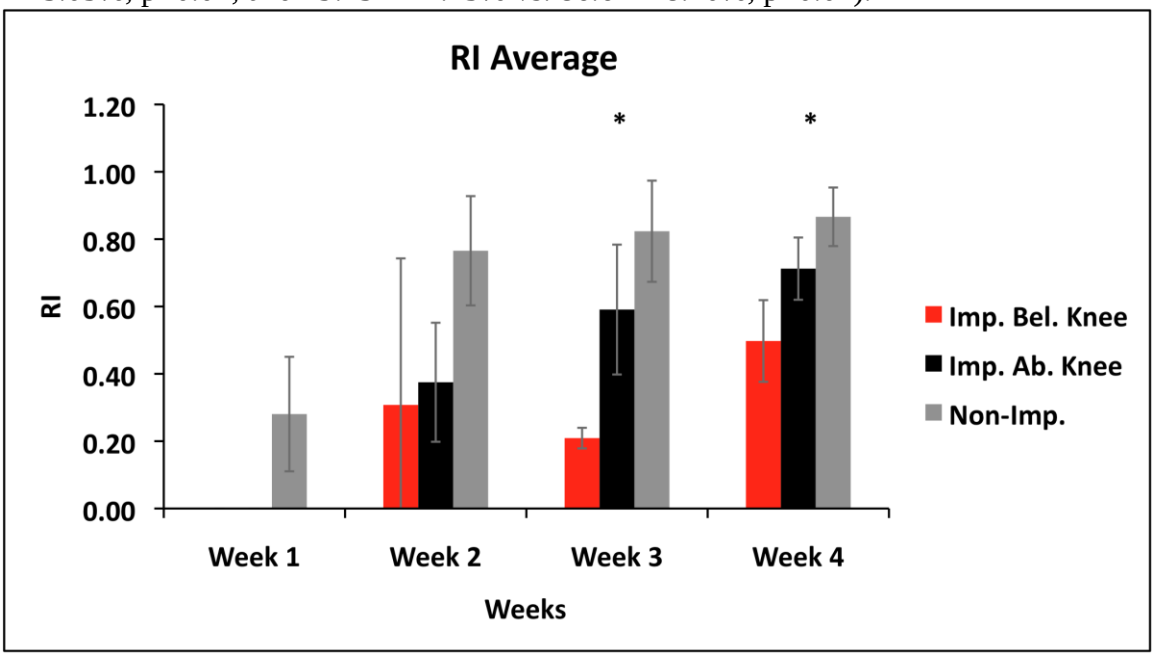


Figure 14. Plot of RI showing differences in weeks 3 and 4 between implant below the knee and non-implant animals. A trend is apparent between implant below knee and above knee animals at all time points, especially when considering the variability in the data (*$p<0.01$, one-way ANOVA).

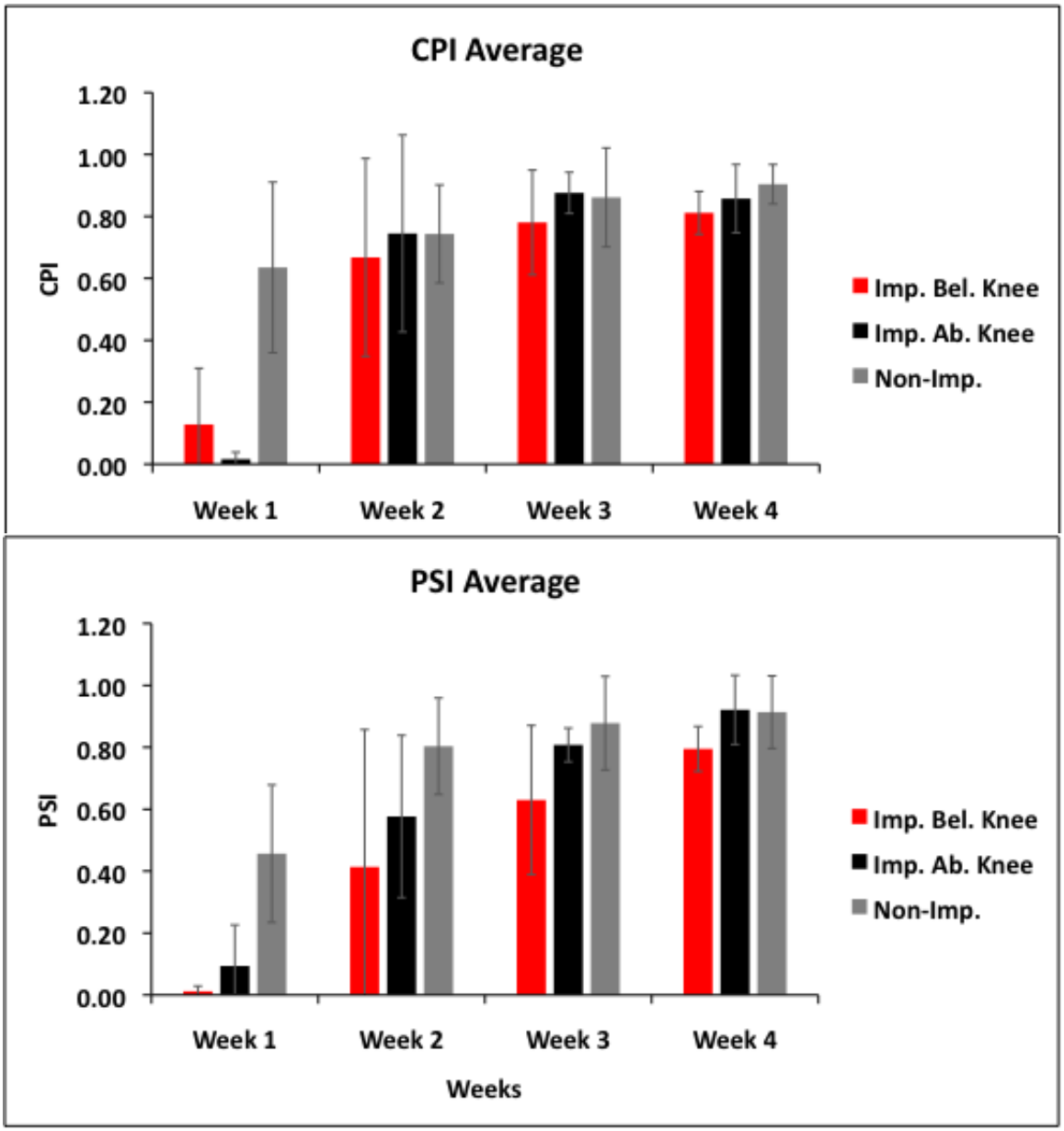


Figure 15. Plots of CPI and PSI averages between groups showing no statistical differences, but trends of greater CPI and PSI are present between implant above and below knee animals.

When analyzed as 2 groups to look at the effect of telemetry EMG implants in general, differences were found in RI between both implant and non-implant groups at all time points (Wk. 1: 0 ± 0% vs. 28.05 ± 17.00&%, $p<0.05$; Wk. 2: 34.13 ± 27.39% vs. 76.55 ± 16.22%, $p<0.05$; Wk. 3: 40.00 ± 24.76% vs. 82.35 ± 15.03%, $p<0.05$; Wk. 4: 60.50 ± 15.21% vs. 86.64 ± 8.70%, $p<0.05$). The non-implant group showed consistent improvement with an increased RI when compared to week 1 across time points while the implant group improved at weeks 3 and 4 when compared to week 1.

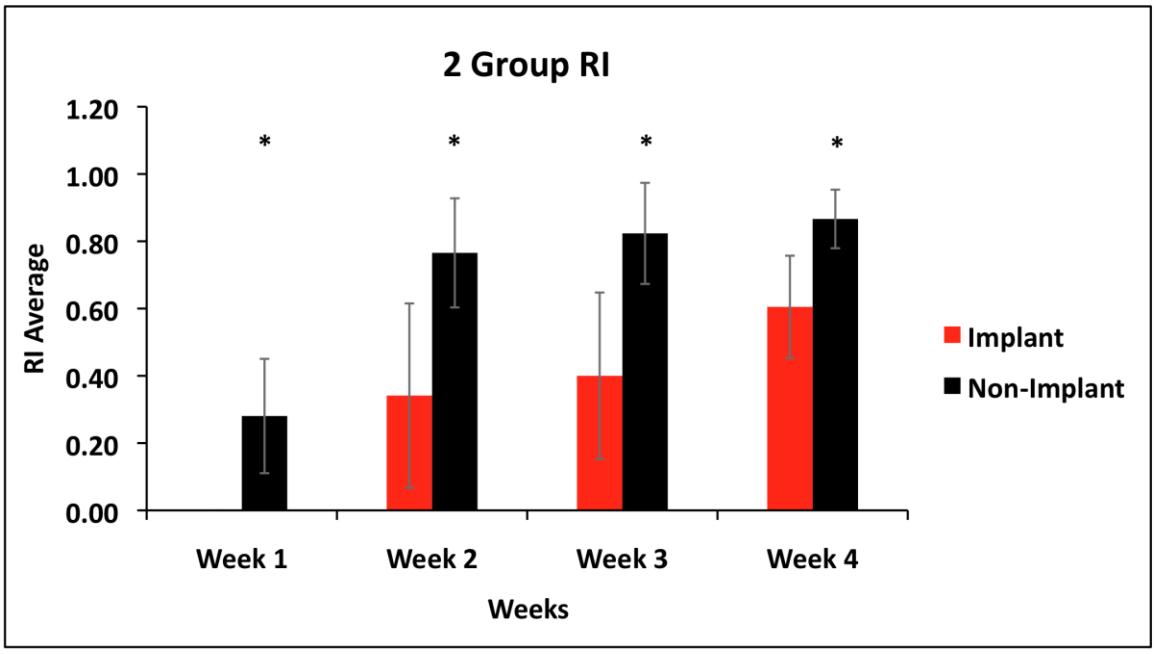


Figure 16. RI analysis between implant and non-implant groups showing group differences at all time points (*$p<0.05$, one-way ANOVA).

When analyzing CPI between implant and non-implant animals, there was a group difference at week 1 (7.22 ± 12.38% vs. 63.52 ± 27.54%, $p<0.01$).

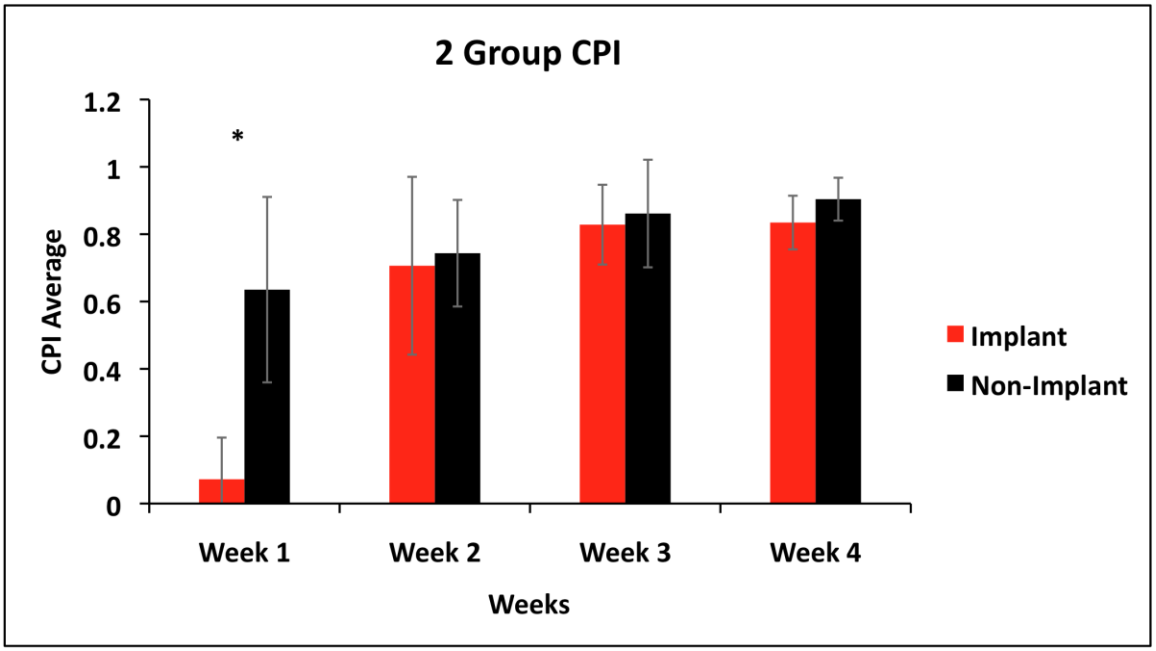


Figure 17. CPI analysis showing a between group difference at week 1 and a trend for greater CPI in non-implant animals across weeks 2-4 (*$p<0.01$, one-way ANOVA).

Finally, a difference was observed between implant and non-implant groups in PSI analysis during week 1 (5.28 ± 9.05% vs. 45.66 ± 22.21%, $p<0.05$) with both groups showing improvement at weeks 3 and 4 compared to week 1 (Wk. 3: 71.87 ± 17.57% and 87.80 ± 15.09%; Wk. 4: 85.80 ± 10.59% and 91.37 ± 11.72%, $p<0.01$).

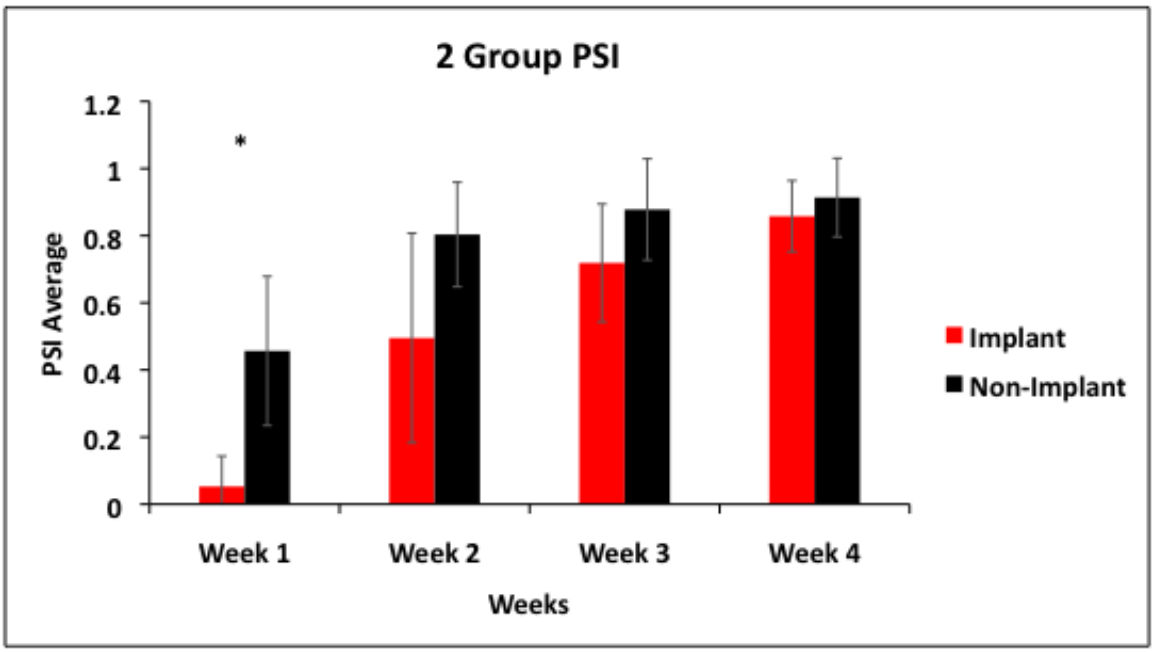


Figure 18. PSI analysis showing a between group difference between implant and non-implant animals at week 1 and a trend for greater PSI in the non-implant group over weeks 2-4 (*$p<0.05$, one-way ANOVA).

In-cage activity. There were no differences either between or within groups when looking at overnight, in-cage activity when analyzed as both 2 and 3 groups. However, there was a trend for the non-implant animals to have more overnight activity than implanted animals at every time point, from 20-60 meters a night more, though it was not significant. The data was rather variable between nights, but the week-to-week data pattern for each group was nearly identical.

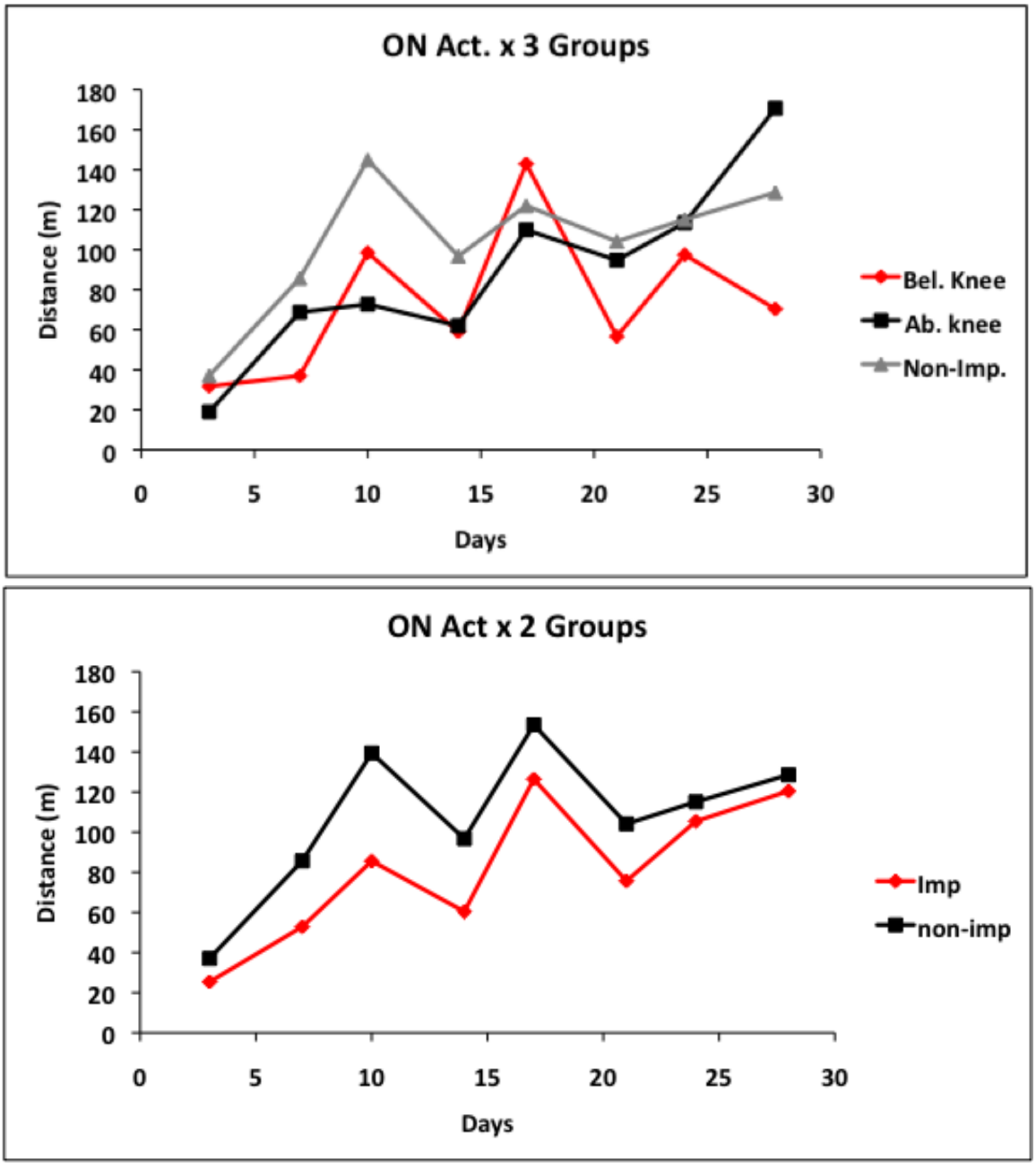


Figure 19. No significant difference in overnight activity was found when analyzed as 3 or 2 groups. However, a trend towards greater overnight activity in non-implant animals vs. implanted animals was observed.

DISCUSSION

During experiments intended to characterize recovery landmarks within the first few weeks following contusion in the T10 spinal cord, we discovered the EMG telemetry implants we had employed to record hindlimb muscle activity were having a negative impact on the recovery of hindlimb locomotor function. When we conducted further experiments to investigate the cause of the loss of function by implanting muscles with EMG leads both above and below the knee, we did not find statistical significance in measures of range of motion between below knee and above knee groups. However, there was a trend in the data for greater range of motion in the above knee group compared to the below knee group. The below knee group also had a great deal of variability in the data which appeared to increase over time. This deficit in range of motion could have been caused by mechanical interference in regards to the knee joint. There could have also been an effect from peripheral inflammation in the knee caused by the wires passing over the joint. This effect may be behind the increased variability over time. At least 2 animals showed signs of inflammation around the knee during dissection and implant removal. When analyzing behavioral data as 2 groups, implanted and non-implant, we showed there is a significant difference in recovery of hindlimb function between implanted and non-implant animals. The non-implant animals followed a typical recovery time course, achieving coordinated, weight-supported stepping (BBB-15-16) by 3-4 weeks, where the implanted animals regained the ability to weight-support (BBB-11-12) but did not achieve forelimb-hindlimb coordination. Kinematic analysis

supported a coordination difference between the groups, and although it was not significant, there was a trend for a greater PSI in implant above knee vs. below knee animals as well as non-implant vs. all implanted animals. At weeks 3 and 4, there was a significant difference between implant below knee and non-implant animals in the RI measurement, but not between the above knee group and non-implant. This suggests the above knee animals were closer to non-implant animals in RI at weeks 3 and 4 than below knee animals, providing further evidence for an effect of lead placement. With the large amount of variability, bigger sample sizes could likely reveal significant differences in lead placement on recovery. Histology comparing the SWM showed that the differences were not due to injuries with varying severity.

We also considered the presence of the EMG telemetry transmitter/battery pack as a source of disturbance in the animals' recovery of hindlimb function. Our lab also uses telemetry for collecting cardiovascular data from injured animals. Of two animals implanted with these transmitters and injured at T10, one achieved recovery to 18 points on the BBB assessment. However, the second animal only reached 11. The SWM of these animals were different, with the animal that scored higher terminal BBB scores having more SWM. Only 2 animals implanted with telemetry cardiovascular transmitters and T10 injuries makes any conclusions regarding effects on the transmitter implant difficult, and further investigation to examine the impact of the transmitter in the peritoneal cavity will be necessary to confirm or rule out that influence. It is also worth mentioning the difference in shape and size between cardiovascular transmitters and EMG transmitters. This size difference could account for a difference in impact of the

transmitters on the animals' recovery after injury either by mechanical restriction of normal movement, or through the introduction of peripheral inflammation.

Another reason for the loss of function experienced by the implanted animals could have been residual effects from the implantation surgery. Two weeks were given for animals to heal before contusions were administered. By this time, animals' sutures were healed, and animals had regained normal stepping per the BBB scale. Furthermore, an animal implanted early on to collect pilot data using the DSI system was injured 4 months following implantation. This animal exhibited the same loss of function as all implanted animals in the current study with a BBB plateau at 11 points by week 3 that remained until terminal assessment at 7 weeks. This showed that the surgery itself was not likely to be the cause for loss of function in implanted animals. Using an anti-inflammatory drug regimen after implantation should be tested for an improvement in recovery after implantation as well as following contusion.

We also looked at the data as 2 groups, implant and non-implant, for global effects on recovery of function from telemetry EMG implants. As discussed above, we also ruled out mechanical impedance of range of motion from different lead implantation sites. This lead to 2 conclusions: the effect from implanted telemetry transmitters and EMG leads contributing to loss of function after SCI was affecting coordination while sparing the ability to load the hindlimbs; and the negative affect was either manifested in the CNS through hypersensitization effects of inflammation, originating peripherally as mechanical interference, or a combination of both. This loss of coordination with ability to weight-support was of great interest to us because of its similarity to results from the previously mentioned hindlimb immobilization study in our lab where acute hindlimb

stretching, similar to therapy with humans following SCI, without acute hindlimb immobilization, caused a sustained loss of coordination coupled with the animals' ability to support their weight while stepping. From the effect on recovery from stretching, it was concluded that an abundance of abnormal or noxious afferent stimuli was likely responsible for the loss of function observed.

In a review by Walters (2012), the author hypothesizes a sustained, hyperexcitable state is induced by SCI, and this hyperactivity of dorsal root ganglion neurons is not confined to the injury epicenter, but extends into uninjured cord levels including caudally into the lumbar enlargement. Walters' idea is based on a volume of past literature describing hyperactivity and central sensitization involving the nociceptive system following SCI, evidenced by behavioral measures of pain in experimental animals (for review, see Woolf and Salter, 2000; Ji et al., 2003). These studies show a hypersensitive system following SCI where processing of noxious and even normal afferent input is altered and exaggerated due to a loss of supraspinal modulation and inhibition to spinal cord neurons (Bruce et al., 2002; You et al. 2008). As a result of these changes, the Grau group has shown noxious stimuli causing impaired recovery of locomotion. In one study, noxious shocks administered in an uncontrollable manner, after SCI, lead to a drop in BBB scores throughout the 6 week study. Furthermore, this effect could only be observed if the stimuli were presented within days following SCI. However, if the stimulus was controllable or not administered until days after injury, the effect on locomotor recovery was diminished (Grau et al., 2004). In our study, telemetry EMG and transmitter implants were a source of constant uncontrollable, and likely noxious stimuli. Considering the critical influence of afferent input on locomotion (for

review, see Rossingnol et al., 2006) while assuming a hypersensitive state in circuits for processing afferent input, transmitters in the peritoneal cavity and the EMG lead wires inserted into the various muscles could have introduced a similar effect seen in the instrumental training experiments (Grau et al., 2004). From previous studies showing the influence of afferent input on spinal interneurons and presynaptic modulation from supraspinal inputs onto interneurons (Sillar and Roberts, 1992; Sillar and Simmers, 1994), and our observations using behavioral measures for the effect of implanted leads and transmitters, we would hypothesize the disruption of coordination in our implanted animals after SCI is a result of abnormal or noxious afferent input being introduced into an already hypersensitive and overactive lumbar sensorimotor circuitry.

The primary weakness of the current study is the low sample sizes. Limitations involved in the DSI telemetry system include expensive transmitters, an invasive implantation surgery that requires intensive aftercare, and limited recording capabilities. Only four receivers can be used at one time for recording EMG signals. This means total capacity for recording is 8 animals at 1 time. Since we used unimplanted, cage-match controls, we could only record 4 animals simultaneously, which was required for recording EMG during overnight activity monitoring. Although we found statistical significance in some measures, others show trends that could be statistically confirmed or eliminated by using greater sample sizes.

Another limitation of the current study is a lack of anatomical and quantitative observations in regards to changes in markers of noxious input to the spinal cord. Immunohistochemical staining for changes in markers of noxious input such as c-Fos or pERK along with changes in glycine, GABA, or glutamate levels in the dorsal horn could

be compared between implanted and non-implanted animals to confirm noxious influence form the implanted transmitters and EMG leads. Changes in glycine, GABA, and glutamate levels within laminae VII and VIII could also be quantified between implanted and non-implanted animals to investigate the influence of nociception and sensitization on ascending long-distance propriospinal neuronal populations responsible for coordination of hindlimbs and forelimbs. Looking for these markers may provide some idea of mechanism responsible for the loss of function in our telemetry EMG implanted rats after injury and could help to establish noxious input as the target for the loss in recovery between implant and non-implant animals. Using multiple time points could also be of benefit to look for changes in these systems in the acute time period following injury.

EMG was collected during the entire course of these experiments during overnight, in-cage activity as well as during overground kinematic sessions. Analysis of the EMG data from these experiments could reveal a great deal in regards to hindlimb alternation and synergistic/antagonistic muscle groups. EMG patterns in implanted animals could provide insight into activity at the lumbar motoneuron level in regards to inhibition and excitation.

From a methods standpoint, elucidation of the effect of telemetry implantation is critical for its future use as a tool for measuring EMG in our SCI model. Because of the methods used to investigate activity dependent changes, and gain and loss of function in our lab, telemetry for recording EMG is not only beneficial, but necessary in a number of our protocols where having animals hard wired to an acquisition computer would not work. The effect of the telemetry transmitters and EMG leads on hindlimb locomotor

recovery in our SCI model introduces challenges to interpretation of the data collected. Although it may be possible to limit our research questions to within 2-3 weeks following SCI, or the time to reach weight-supported stepping prior to coordination, some of the aforementioned anatomical and immunohistochemical techniques along with thorough kinematic analysis would be necessary to evaluate any differences between implant and non-implant groups within that timeframe. Further development and improvements in the telemetry transmitter devices would be optimal to eliminate or significantly decrease the effects from implantation for future studies in our lab and other labs that could potentially benefit from the use of the DSI system. These improvements should include utilization of smaller diameter EMG lead wires, as well as development of a smaller and less intrusive transmitter module. From data in our lab using the DSI telemetry cardiovascular monitor that uses a significantly smaller transmitter module, we have evidence that shows a smaller transmitter module for EMG could reduce the impact from implantation on functional hindlimb recovery following SCI in our rat model. If these improvements could significantly reduce the impact on recovery, the improved devices would have wide ranging applications in biomedical animal research.

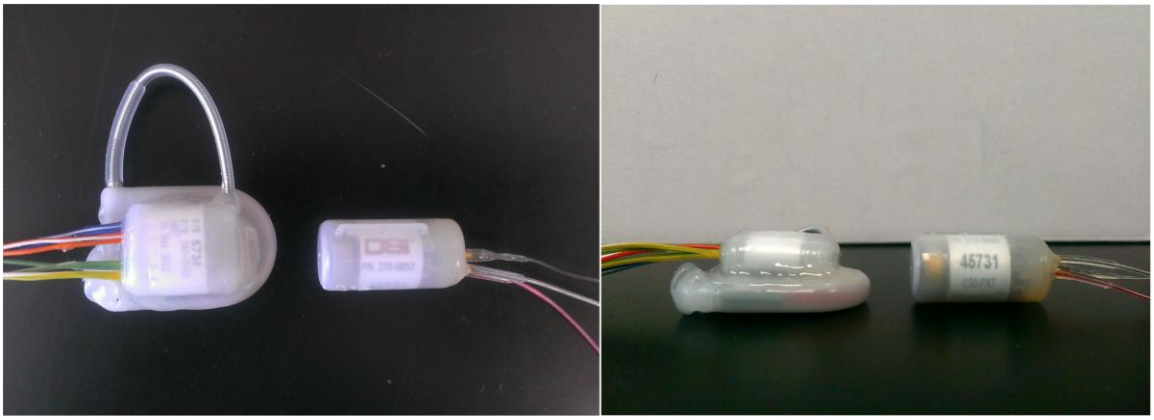


Figure 20. Images showing size differences between EMG (left) and cardiovascular (right) telemetry transmitters.

Discovery of an influence of implantation on recovery also highlights the importance of unimplanted matched controls in experiments where implantation is used for collection of EMG. As was mentioned in the introduction, previous studies using EMG implants have failed to show whether or not their implantation protocol has an effect on the recovery of their animals after injury (Antri et al., 2002; Feraboli-Lohnherr et al., 1999; De Leon et al., 1994; Pierotti et al., 1989; Berriere et al., 2008). Two major differences between the telemetry implants and most other EMG implants are the diameter and rigidity of the wire electrodes, and the sizeable transmitter implanted in the peritoneal cavity. Our study shows the potential for such an effect and the magnitude of the impact it could have on recovery after SCI.

Clinically, the results and discussed implications of the current study mirror and reinforce conclusions from our lab's stretching and hindlimb immobilization data (Caudle et al., 2012), that acutely after SCI, there is a capacity for the generation of locomotion within the lumbar cord that is independent, to an extent, from supraspinal input. And, with the loss of supraspinal modulation, the locomotor circuitry, especially regarding afferent influence on that circuitry is left in a hypersensitive state. Further investigation is necessary to characterize this acute, post-injury state and its time course. However, if this phenomenon is present in humans, it would emphasize the importance of evidence-based care after SCI along with some form of standardization of care that accounts for sensitivity/plasticity that can contribute to loss or gain of function and the changes in these factors over time after injury. In the hindlimb immobilization study (Caudle et al., 2011), a review (Harvey et al., 2009) was cited pointing out inconclusive and inconsistent data regarding outcomes of therapies practiced on patients following SCI. It is worth

calling attention to the point once again in light of growing evidence for a locomotor system that is hypersensitive to afferent input after SCI and highlighting the critical nature of a thorough understanding of therapeutic influence on the locomotor system after injury.

This study provided further evidence for effects on hindlimb locomotion originating from afferent input after SCI. Taken with the results of our previous studies involving hindlimb immobilization and stretching after SCI, the importance of attention to and understanding of effects of afferent input introduced to hindlimb locomotor circuitry is emphasized. Further characterization of the post-injury state of the lumbar circuitry and how it changes over time from acute to chronic time points will help us to understand the challenges and complexity of therapeutic intervention post-injury, as well as provide the ability to tailor therapy for the greatest benefit to SCI patients. Better understanding of changes in spinal cord circuitry following SCI will also help in experimental design and interpretation of results stemming from experiments using animal models of SCI and potentially beneficial tools such as the DSI telemetry system.

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Undergraduate Research Grant, University of Louisville **2007-2008**
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Dean's List (4 semesters)

TEACHING EXPERIENCE

University of Louisville school of Medicine
Teaching Assistant, Medical Neuroscience Sequence **2011-2012**
Tutored and assisted first year medical students in the gross laboratory for Neuroanatomy.

PRESENTATIONS

University of Cincinnati
Seminar in Perception **2008**
Presented undergraduate research in slant perception to a perception research group.

University of Louisville
Honors Thesis Defense **2008**
Defended honors thesis research in front of thesis committee

University of Louisville School of Medicine
Sensory Systems Journal Club **2008**
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University of Louisville School of Medicine
KSCIRC Noon Seminar **2012**
"The Final Common Path: Effects of SCI and activity dependent plasticity on lumbar motoneuron input and morphology."

PROFFESSIONAL DEVELOPMENT

Graduate Teaching Assistant Academy
9 month seminar for educators in higher learning through University of Louisville Delphi Center.
Certificate of successful completion awarded. **2011-2012**

PLAN Workshops *"Grant Writing"; "Writing a Literature Review"; "Mentor/Mentee Training"; "CV/Resume"; "Branding Yourself"; "Photoshop"; "EndNote"*
University of Louisville SIGS **2010-2012**

MEMBERSHIPS

Psi Chi-Honors Society in Psychology
Kentucky Academy of Sciences
Sierra Club