ELECTROMYOGRAPHIC INDICATORS OF RECOVERY FROM SPINAL CORD INJURY

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Raquel Vanessa Santiago

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Research Thesis Advisory Committee:

Dr. John A. Buford, Advisor
Dr. D Michele Basso
Dr. Anne Kloos
ABSTRACT

Spinal cord injury (SCI) impairs locomotion and therefore diminishes quality of life. In the US, 11,000 new cases annually yield 175,000 to 275,000 people living with SCI. Many studies analyze recovery from SCI employing behavioral and anatomical measurements, but few measure muscle activity with electromyography (EMG) in the rat, the species most used for SCI research. EMG can measure muscle recruitment patterns as an indication of the organization of motor control patterns for locomotion. The purpose of this study was to analyze the changes in muscle activation of selected hindlimb muscles, determine which muscles best indicated differences between rats with mild SCI and normal rats, and determine which changes in EMG parameters best reflected the time course of recovery in the SCI rats.

Ten female Sprague Dawley rats were trained for three weeks to walk on a treadmill. Intramuscular EMG electrodes were implanted in the left hindlimb of four muscles per rat, including tibialis anterior (ankle flexor), lateral gastrocnemius (ankle extensor), gluteus medius (hip extensor/abductor) or sartorius (hip flexor/knee extensor), and semitendinosus (knee flexor/hip extensor). These muscles were studied for their unique EMG locomotor recruitment patterns based on previous studies in rats and in humans. As the subjects resumed treadmill training, EMG data were collected as a baseline. A mild spinal cord injury on the thoracic level was performed on half of the subjects. A standardized behavioral scale for quality of walking, the BBB Locomotor
Rating Scale, was administered pre-injury and at 1, 7, 14, and 21 days after injury. Throughout three weeks of recovery, treadmill walking and EMG recording continued 4 – 5 days per week. Average EMG patterns for ~50 – 150 steps per session were constructed from around the time of stance onset. Time latencies of EMG onset and offset, with respect to stance onset, along with EMG duration were analyzed for each step.

Four of the ten rats, two controls and two SCI, had high-quality recordings throughout the six week study for 3 – 4 of the muscles implanted; data from these subjects were the focus of analysis. The averaged EMG patterns suggested that SCI rats had increased overlap of activity between normally reciprocal muscles at the ankle. However, after statistical analysis this finding was not significant. Variation in latency for EMG onset time with respect to stance onset was higher for SCI rats than for normal rats after injury, indicating more variable timing of muscle recruitment. This was related to the significantly shorter swing duration in SCI rats. Recruitment patterns of multifunctional muscles showed the most apparent change after SCI, but returned towards normal after training. In parallel with the improved EMG recruitment patterns, BBB score also improved with the 3-weeks of training after SCI, as expected. However, some aspects of EMG analysis were independent of BBB scores.

These results show that changes in EMG during walking can be observed after SCI and return towards normal are evident during recovery. EMG is uniquely able to
reveal changes in muscle recruitment patterns that may underlie changes in walking behavior after SCI, and as such offers a window into functional mechanisms of recovery.
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CHAPTER 1

INTRODUCTION

1.1 Problem Statement

Worldwide, spinal cord injuries (SCI) as a result of an accident or disease can cause detrimental effects on the mobility of humans. In the United States alone, there are approximately 11,000 new cases of these injuries, yielding a total of 175,000 to 275,000 people living with SCI today (Basso, 2000; NSCISC, 2004). Although the costs a SCI patient can accrue may vary, those with an incomplete SCI can expect an expense of at least $200,000 in the first year (NSCISC, 2004). Quality of life for these individuals is greatly impacted, especially due to the impairment of their motor functions. Previous research studies have made attempts to determine measurements that may act as indicators of recovery from SCI using animal models. The study of the process of recovery from SCI using animal models with standardized behavioral and neuro-anatomical measures has proven effective. However, quantitative physiological measures of recovery from SCI are not yet standardized. Electromyographic (EMG) activity can be measured in the awake, behaving animal, and may provide an accurate means for detailed physiological assessment of muscle function as an index of motor recovery. Presently, the literature contains descriptive analysis of EMG; however there is no standardized EMG analysis approach that can be correlated with recovery from SCI.
A variety of experimental treatments for SCI are being developed in the animal model. For these treatments to be translated from the animal model to human beings there must be evidence of outstanding recovery of function. Because most SCI research occurs in the rat or mouse, there is concern that the high level of motor function required for the unique bipedal human locomotion may not be required for the quadruped to walk well (Capaday, 2002). EMG measures of muscle activity may offer a more exact view of the quality of recovery in the quadruped than visual observation alone. This additional detail may be required for research to recognize the most promising experimental treatments for possible translation to humans. A systematic investigation of the appropriate standards for quantitative EMG analysis aimed at developing standards would require a large number of SCI rats at various levels of severity, and the EMG analysis would need to be correlated with gold standards such as the behavioral score for recovery of walking and the anatomical measures of healthy tissue areas in the spinal cord (Basso, Beattie, & Bresnahan, 1995). This would be an extensive, multi-year project. Before such a project could succeed, an initial descriptive study is required to identify promising quantitative measures of EMG related to recovery in a small number of subjects with a range of relatively good recovery levels from mild spinal cord injuries. In addition to the exact aspects of EMG to measure, the extent of impairment within various leg muscles after SCI remains unknown. Knowing which muscles have EMG patterns most sensitive to the differences between normal walking and good, but not quite
normal, walking after SCI is also important for investigators to focus on which muscles to study.

The present project is designed to analyze a dataset of EMG during treadmill walking for normal and spinal cord injured rats. These data were collected in part by the student and are part of an ongoing research project in the advisor’s laboratory. The study analyzes the EMG data for walking in order to identify the most promising methods for quantitative analysis of EMG and the most important muscles to study for future studies that might attempt to develop a standardized approach of EMG analysis in the rat SCI model across various lesion severities.

1.2 Review of Literature

Numerous studies have been conducted regarding spinal cord research using animal models. Recently, rats have been used as typical subjects in SCI studies to analyze recovery (Young, 2002). The majority of these studies analyze the behavioral outcomes of SCI such as the BBB score, a standardized rating scale named after authors Basso, Beattie, and Bresnahan, 1995. This particular measure of SCI recovery is based on an ordinal rating system for the quality of walking in the rat. While the BBB is an excellent measure of walking that can be efficiently applied to the intact subject with repeated measures during recovery, it is a behavioral measure and therefore cannot directly address mechanisms of recovery. Others have researched the various physiological and neuro-anatomical factors involved in SCI recovery; most current
studies utilize anatomical methods that require sacrifice of the animal for histological processing of the neural tissue to address and understand mechanisms (Basso, 2000). This requires several subjects per group with sacrifice of various groups in various time points in recovery, greatly adding to the expense of the project, the number of animals used, and between-subject variability. EMG analysis offers a means to make physiological measurements that may be somewhat closer to the mechanistic level than behavior. EMG has an advantage over anatomical approaches because these electrophysiological measurements can be repeated throughout the course of recovery without sacrifice of the subjects.

Much of what we understand about human function is derived from such animal studies. The relationship between electromyographic activity of leg muscles and the neurological systems that control those muscles have been studied in quadrupedal animals like the rat and the cat (Capaday, 2002). Besides the differences from our bipedal gait, humans and such animals have subtle but potentially important differences in the feedback mechanisms our central nervous system uses to influence walking. Care and attention to the details of such differences must be applied for the eventual translation between animal and human walking. Further research is needed to better understand these similarities and differences for the field of spinal cord research.

Some SCI studies in rodent models have described EMG patterns of several muscles, during various activities, to determine which muscle(s) experienced the greatest impact from a contusion in the spinal cord. “Electromyographic activity associated with
spontaneous functional recovery after spinal cord injury in rats” by Kaegi et al., applied EMG recordings during the SCI recovery phase in rats to determine the detailed changes in stepping patterns (Kaegi, Schwab, Dietz, & Fouad, 2001). The hindlimb muscles studied in this particular SCI project were vastus lateralis, a knee extensor, and tibialis anterior, an ankle flexor. These muscles were chosen because of their known contribution during quadrupedal gait, as well as for their surgical feasibility. During SCI recovery, EMG data revealed a decrease in step duration, an increase in EMG amplitude, and an eventual decrease of activity overlap between the extensor and flexor muscles (Kaegi et al., 2001). These findings indicate that there are significant changes in EMG activity patterns during SCI recovery of a normal walking rat. However, now that the EMG changes in these muscle groups are revealed, perhaps additional muscles, such as hip flexors/extensors, need to be analyzed to determine which muscle groups are best suited to measure SCI recovery.

To understand EMG analysis, it is important to understand what the EMG signal represents and how it can be measured. EMG represents the electrical sum of action potentials in muscle cells located near the EMG recording electrodes. In animal models, the bioelectrical signals are usually measured by intramuscular electrodes (Whelan, 2003). EMG recordings of selected muscles from adult mammals after SCI may be able to provide information regarding the effect on muscle recruitment patterns, including the level of activation (Kaegi, Schwab, Dietz, & Fouad, 2001). These differences in muscle recruitment derived from EMG may be utilized to help more accurately determine the
degree of recovery from SCI and provide additional observations that could relate to mechanisms of recovery. The most reliable parameters are the timing of EMG activity onset and offset with respect to events in the step cycle such as paw off and paw contact. EMG amplitude can be compared within a muscle for single subjects under different conditions. However, because the EMG amplitude can vary greatly with the quality of the electrode, its placement in the muscle, and the size of individual muscles, it is difficult to compare amplitude among individuals (Whelan, 2003). In the human this can be overcome by having subjects perform maximal isolated contractions of each muscle and representing the EMG amplitude as a percentage of the amount recorded during that exertion. For animals, however, it would be nearly impossible to train subjects to perform such voluntary isometric contractions of individual muscles. Hence, amplitude can be compared over time for individuals with stable EMG implants, but not between groups. Another aspect of EMG that can be measured reliably is the timing of the peak level of activity in a burst (Whelan, 2003). This parameter helps reflect the shape of the recruitment pattern. Finally, the number of bursts per step cycle can be identified; some muscles have one main burst per step, others have multiple bursts. Other measures, such as the degree of overlap in activity between two muscles, are calculated from the primary data on muscle onset and offset.

Previous EMG studies explored certain unifunctional, simple muscles serving as flexors and extensors and the relationship with the swing and stance phases of an animal’s gait. As described above, certain muscles illustrate changes in timing and an
increased overlap in activity after SCI (Kaegi, Schwab, Dietz, & Fouad, 2001).

However, in basic studies of central pattern generators and forms of walking, muscles with complex functions at more than one joint, such the hamstrings group, often show some of the most considerable differences (Pratt, Buford, & Smith, 1996). It is important to include these more complex muscles for comparison of EMG patterns in normal and spinal cord injured rats.

The missing information that this study strives to address is to determine which muscles would be the most informative to help differentiate between normal and SCI walking. EMG activation patterns, timing, amplitude, and burst patterns are compared between normal and injured animals as well as within an animal over time. Ultimately, a standardized approach to EMG analysis for SCI rats would help investigators use this physiological measurement to decide how well an experimental treatment for SCI has worked. This information, when combined with other results, could help decide if a treatment is ready for a human trial.

1.3 Objectives

The purposes of this study are ultimately aimed toward determining the differences in walking of a spinal cord injured and an uninjured rat. With the use of electromyographic data, the processes of SCI recovery can be better analyzed by developing a set of measurements to best reveal differences between normal and SCI walking. With this information scientists can have better knowledge of which muscles to
target for EMG analysis during the recovery process. The expectation is that EMG recordings of selected hindlimb muscles of quadrupedal walking rats may serve as useful indicators of SCI recovery. Key aspects of EMG measured were the timing and pattern of activation between uninjured rats and SCI rats.

In addition to developing the proper measurements and choosing the proper set of muscles to study, it is important to determine which muscles and what measurements for those muscles are best to describe SCI recovery in relation with improvement of walking, revealed by BBB scores. It is hoped that the addition of these physiological measures can one day be applied along with other types of studies to identify the experimental treatment approaches most worthy of testing in human subjects so that effective rehabilitation services may be provided to individuals to improve the recovery process and thus the quality of life.

Predictions of this study were that there would be specific aspects of EMG analysis that would distinguish the motor patterns of normal compared to SCI rats. Parameters most sensitive to the difference between normal and SCI motor patterns in the rat were expected to be the correlations between the times of EMG onset or offset and paw off or paw contact on a step-by-step basis. Higher correlations were predicted for normal rats than SCI rats, indicating more consistent timing. The EMG burst pattern was also expected to differ between normal and SCI rats. Aspects of bursting pattern include the time of the peak activity in the averaged EMG waveform and the presence of a single or dual burst pattern per step cycle. In particular, semitendinosus was expected to have a
clear dual burst in normal walking and a tendency to become a single burst in SCI walking. Finally, the degree of overlap between antagonists LG and TA should be low in normal walking and higher in the SCI rats, as found by Kaegi et al.

Additionally, it was predicted that certain muscles that best show the differences between SCI and normal rats. We expected the multifunctional muscles, such as semitendinosus, to be most sensitive to the differences between normal and SCI rats. Important information was also expected from the ankle flexor and extensors as they relate directly to interaction with the ground for swing and stance.

Relationships between the time course of recovery in the SCI rats and changes in certain EMG parameters for specific muscles were also expected. This includes that the EMG parameters identified would change over the course of SCI recovery, moving more towards their normal values. Improvement of walking should be evident from more consistent timing of swing and stance as walking recovers. EMG parameters that improve along with walking behavior will be identified by multiple regression. Also, subjects with the highest BBB scores after recovery were expected have EMG measurements closest to normal, indicating that these EMG parameters are related to the extent of recovery.
2.1 Subjects and Design

Ten female Sprague-Dawley rats were subjects for the experiment. The study was conducted in two phases, six rats in the first group during one summer and four in the second. All methods were carried out the same way for both groups. To become familiar and comfortable with human contact, the animals were gentled and handled. Gentling proceeded with each rat being held for several minutes and presented to equipment. As it became evident the rats were accustomed to being handled, they commenced treadmill training. For approximately three weeks, all rats were trained to walk on a treadmill on a daily basis. At the beginning of training, they walked for 5 – 10 minutes in interval speeds from 10 meters per minute to 14 meters per minute. Once the rats appeared to be properly trained, EMG intramuscular electrodes were surgically implanted into four selected muscles in the left hindlimb: tibialis anterior (TA), lateral gastrocnemius (LG), sartorius (SART), and semitendinosus (ST). Because the sartorius implants rarely lasted as long as the study needed, the second group of subjects had gluteus medius (GM) implanted rather than SART. The rats resumed treadmill training daily for one week after implant surgery. EMG data were collected during treadmill walking while a video simultaneously recorded the behavior. After sufficient EMG data
were collected, five (half) of the subjects were designated to receive a mild spinal cord injury on the thoracic level. As recovery proceeded, all subjects returned to treadmill walking while EMG data collection continued for the duration of three weeks.

2.2. Treadmill Details

Columbus Instruments, model Custom Exer-4, provided an appropriately sized treadmill. The rats were contained in a reduced space towards the front the treadmill with an accessible reward delivery system (Cole-Parmer Instruments) presented directly in the front. When the rats walked in a desirable way, they received positive reinforcement with syrup produced by a feeder lixit.

The rats were trained to walk quadrupedally—with all four limbs in contact with the treadmill belt during gait. There was no area on the treadmill outside the belt where the rat would be capable of standing or setting its paw. The feeder was positioned at an appropriate level so it was easily accessible, allowing the animal to walk with its natural posture. Figure 2.1 is an illustrated example of a rat during treadmill walking and EMG data collection. At the beginning of training, it was necessary for the rats to become accustomed to the task. The rats walked in intervals of 2 – 3 minutes at a starting speed of 12 meters per minute. As training progressed, time intervals and speeds were increased or decreased depending on the need to modify behavior.
Figure 2.1: General illustration of rat during treadmill walking and EMG recording.
2.3 Implantation of EMG electrodes

Bipolar EMG electrodes were implanted in the left hindlimb of all subjects (for details of procedure, see Whelan, 2003). In preparation for the surgical intramuscular implantation, the rats were placed under anesthesia (ketamine and xylazine). For the associated study concerning the behavioral outcomes of a SCI, specific areas of fur were shaved and tattoos marked particular muscles of the lower portion of the rats’ body. The hindlimb muscles in this EMG study were the ankle flexor (TA), ankle extensor (LG), hip/knee flexor (SART) or hip extensor/abductor (GM), and hip extensor/knee flexor (ST).

Prior to the EMG implantation, a connector—which nine electrodes run from—was attached to each rat’s head. Using a scalpel blade, a small area of skin on the head was slit to expose the skull. For the stability of the wires, a trochar was used to run the electrodes subcutaneously along the left side of the rat’s body from the head to the pelvis. After the skull was cleaned with saline, four holes were drilled in the skull for the placement of screws necessary to hold the connector in place. The scalp was then coated with varnish and dental acrylic for the stability of the connector. Before the acrylic had completely hardened, the connector was put into place and more acrylic was applied.

An incision in the skin was made in the left hind leg, and the desired muscles were exposed and identified. Each pair of electrodes was previously labeled and measured for an estimated length necessary for the implantation and the ability of the animal to move freely. A Teflon material coated the stainless-steel wires; to expose the
wire, the tip of the coating was slit and stripped away about 2-mm from the end. The exposed wire was inserted at the end of a hypodermic needle. The needle was then implanted in the desired muscle, deep enough to secure the wire’s placement within the muscle, and the needle was withdrawn, leaving the wire in place. The second electrode of each pair was then inserted, using the same methods, in the same general location of the muscle—only a few millimeters apart—so that EMG could be measured.

Once all eight of the electrodes were implanted in the four muscles, the position of the electrodes was tested by running a current through wires. If the electrical stimulation caused an appropriate muscle twitch in the leg, depending on the muscle, this confirmed the correct placement of the electrodes. The ninth electrode served as the common ground wire and remained subcutaneously. All excess wire was placed underneath the skin and the incisions were then closed using sutures or wound clips.

2.4 Mild SCI

Preparations for all surgical procedures were consistent throughout the experiment (e.g. anesthesia). Half (5) of the subjects received the SCI; these were rats 10, 11, 14, 17, and 18. With the rats fully anesthetized (ketamine and xylazine), a laminectomy was performed on the eighth thoracic vertebra (T8).

In the laminectomy procedure, a midline incision was made above the vertebra. Using a scalpel, forceps, and probe, the T8 was located and identified. A self-retaining retractor was used to expand the midline and expose the vertebra. The muscles and
ligaments between T8 - T9 and between T8 - T7 were cut. Small cuts were made around the T8 until the bone was completely removed and the spinal cord was exposed.

After the laminectomy, an Electromechanical Spinal Cord Injury Device (ESCID 2000), operated by a technician, was used to induce the injury. The device uses the “dynamic capacity of an electromagnetic driver and a unique pattern generator to briefly compress the dorsal surface of the spinal cord at velocities that may mimic compression injuries seen in the human” (Stokes, Noyes, & Behrmann, 1992). Since the majority of SCI patients involve contusions of the cord, the device and protocols produces consistent and realistic results for injury procedures (Young, 2002). The rat’s vertebral column was stabilized by clamps with a chest pillow under the laminectomy site to hold the animal at the proper level. A retractor hook was used to open the incision to better expose the cord. A rostral clamp and a caudal clamp were positioned and locked onto the vertebrae. Once the injury probe was positioned directly above the cord, the technician initiated a hit, stimulating a blunt impact trauma.

During the first week of post-surgery, it was necessary that the injured rats received animal care to avoid any health risks, including infections or dehydration. Bladder expression was performed at least twice a day and continued until it was documented that the bladder was small or empty for three consecutive days. Rats were weighed daily and, based on weight, Gentocin antibiotics and 0.9% Saline were administered subcutaneously. Appetite and activity level were also noted during this first week of recovery.
2.5 BBB Locomotor Rating Scale

Locomotor recovery and functional ability was evaluated by assessing a score derived from the BBB Locomotor Rating Scale (Basso, Beattie, & Bresnahan, 1995). BBB scores are derived from on a 0 to 21 point scale based on hindlimb movement, paw placement, and paw usage. Observations are made within a four minute test period with rats walking in an open field. A score of 0 indicates no observable hindlimb movement, while a score of 21 describes coordinated, consistent locomotion. Aspects of locomotion described in categories involve movement of joints of the hindlimb, plantar placement, weight support, forelimb-hindlimb coordination, paw position, toe clearance, trunk stability, and tail position (Basso et al., 1995; Kaegi et al., 2001). BBB scores were assessed days 1 and 2 post-EMG implants, 1 day post-operation (dpo) of the SCI, 7 dpo, 14 dpo, and 21 dpo. Experienced raters with established reliability administered the BBB.

2.6 EMG Analysis

EMG data were collected during treadmill walking five days per week for three weeks after the SCI. Collection was through a differential AC amplifier (A-M Systems Model 1700). Filters were set with a bandpass from 10 Hz to 5,000 Hz and gains were set at 1000. Acquiring data to computer was controlled using Datapac 2K2 software (Run Technologies PCM-16S/16). The data were acquired with 16-bits resolution at a 2 KHz sampling rate. Throughout the treadmill walking sessions, EMG recordings and
video recordings were taken simultaneously (Canon NTSC ZR 65MC digital video camcorder). For behavioral analysis, videotaping proceeded on the left side of the treadmill during the gait activity. The parameters of the software were set to automatically synchronize the video recording with the EMG data (Figure 2.2). Set at a frame rate of 30 frames per second, video frames were deinterlaced for detection of paw contact and therefore swing-stance phases, yielding an effective frame rate of 60 Hz (16.6 ms/frame). The EMG were processed dynamically in the computer’s memory to remove DC offsets in the baseline, high pass filter the data at 48 Hz to remove movement artifacts, and rectify the data.
Figure 2.2: Example of synchronized video recording and EMG recording. Each video frame has a corresponding position on the EMG data display.
From the events created in Datapac for every step in each rat, average EMG data is calculated around the stance onset (refer to Figure 3.1). The averaged and rectified EMG recordings are compressed into a time series display; the graphs display EMG data from the muscles through channels, with the Y scale set at 1.0 mV. Gain parameters are set depending on the general EMG amplitude of each muscle. With the EMG data filtered, rectified, and averaged, the muscle activation bursts associated with walking can be made visible.

Once the general EMG pattern was evident, burst detection parameters are devised through the Datapac 2K2 software to detect individual EMG bursts for only good stepping using a reference interval. First, baseline periods of EMG during walking were identified as sustained (50 – 200 ms, depending on the muscle) EMG below 5 – 10% of the maximal EMG observed for each muscle during that bout of walking. From the baseline periods, a mean and standard deviation was calculated to determine a threshold, which was 2 standard deviations above the baseline mean. An interrupt parameter allowed EMG to dip below threshold for 30 ms without being counted as off. A minimal and maximal duration parameter was set for each muscle to capture bursts with durations reasonable for main locomotor bursts. These varied depending on the muscle, but were typically a minimum of about half the typical burst duration and a maximum of about twice the typical burst duration. Some manual adjustment of EMG onset and offset times or deletion/inserting of bursts was required after the software’s attempt at automatic detection. Figure 2.3 presents raw EMG data from a normal rat during gait. The square
wave labeled “A” shows the timing of paw contact and paw lift off during a series of consistent steps. EMG traces labeled 1 through 4 are from tibialis anterior, lateral gastrocnemius, semitendinosus, and gluteus medius, respectively. Each square wave labeled “G” through “K” shows when each muscle was found to be “on” or “off” based on the analysis parameters set through Datapac. Once bursts were detected from the software, a spreadsheet was constructed, containing temporal and amplitude measurements for each burst. In cases where a muscle had spurious activity in addition to its main burst, the main features evident in the averaged EMG were used to determine which bursts should be consistently accepted on a step by step basis and which EMG activity would be ignored for this phase of the analysis.
Figure 2.3: Example of raw EMG recordings of control rat 15 during treadmill walking. Each square wave represents EMG onsets and off sets for every paw on and off represented by square wave “A.”
2.7 Statistical Analysis

Using SPSS 15.0 software, descriptive statistics (minimum, mean, maximum, and standard deviation) were determined for all rats on a muscle-by-muscle, session-by-session basis. Muscle onset and offset times were expressed relative to the onset of stance. To compare normal to SCI rats, a 2-way ANOVA was used with time relative to the day of surgery for the SCI subjects as one level (pre-op vs post-op) and group at the other (normal vs SCI). The last post-op day was selected, when normal and SCI rats both walked at the same speed of 12 m/min. It is recognized that for a true ANOVA, one observation per rat would ideally be used, yielding a $n$ of 4 for this study. The purpose of this study was to determine what parameters might show differences for normal versus SCI rats. However, the ANOVA was run with multiple observations per step. This overly sensitive approach was used to reveal all relationships worthy of future study. Swing and stance phase duration were entered as covariates in the ANOVA to control for relationships between their duration and the EMG parameters. To determine what parameters were related to the BBB score, a stepwise regression was used with BBB score, swing duration, and stance duration as independent variables. This allowed estimates of the regression with BBB independent of variations in walking speed or step cycle period, also associated with SCI. For the same reason, swing and stance duration were covariates in the ANOVA.
RESULTS

3.1 Quality of walking

Towards the beginning of the study, one of the subjects, rat 16, died and therefore did not contribute any data. The incomplete spinal cord injuries within the 5 subjects were in the mild range, with a displacement of 0.5 mm from the spinal cord surface. Injured subjects started walking at 7 m/min while the control rats maintained speeds of 13 or 14 m/min. As recovery time progressed, SCI speeds increased to 10 m/min then to 11 m/min. By week 3 of recovery, all rats were walking at the same speed of 12 m/min. To determine the severity of the injury and the quality of the controls, functional abilities were observed and BBB scores were assessed.

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Table 3.1: BBB locomotor rating scores. Data in bold are SCI subjects.
All SCI subjects recovered with adequate motor function and locomotor ability. Table 3.1 shows the BBB scores given on days 1 and 2 post-EMG implants to determine the quality of walking in all rats and the effects of the EMG implant surgery. BBB scores were assessed on day 2 after implants if the rat did not perform well, such as rats 12 and 13. After the implant surgery, subject 12 had abnormal posture of the head, perhaps due to discomfort, contributing to an initial score of 19. It was also observed that subject 13 experienced a small infection along the EMG wires near her pelvis, again affecting discomfort and slight trunk instability. Throughout the three week time frame of recovery, the uninjured subjects retained stable BBB scores (Table 3.1).

The first day following the SCI (1 dpo), injured subjects received BBB scores in the 7 to 9 range. These scores are described as sweeping of the hindlimb, lack of weight support, plantar placement of the paw without weight support or during stance only, and/or dorsal stepping with no plantar stepping (Basso, D.M., Beattie, M.S. & Bresnahan, 1995). After one week of recovery, the SCI animals displayed slight improvement in locomotion ability, with BBB scores in the 11 to 12 range. Although the rats displayed capabilities of weight supported plantar steps, they lacked appropriate forelimb-hindlimb coordination. After 14 dpo, SCI rats 11 and 14 maintained stable motor function capabilities with good forelimb-hindlimb coordination, but still had difficulty with either toe clearance or paw positioning. SCI rats 10, 17, and 18 continued improving in locomotion throughout the recovery time frame. By 21 dpo, these animals displayed consistent forelimb-hindlimb coordination and toe clearance, and no longer dragged their
toes. Locomotion capabilities were, however, somewhat flawed due to paw rotation at lift off. In all SCI rats, these behavioral observations witnessed a regain of locomotion by the conclusion of recovery, but the return to full motor function ability was not accomplished within the recovery time frame.

3.2 EMG Analysis

Results based off of averages show typical muscle recruitment patterns. Normal rats maintained consistent and stable averaged EMG patterns over the three week recovery time frame (Figure 3.1). At the start of swing phase (about 200 ms before stance onset), activation bursts existed in the ankle flexor, TA, and in the knee flexor/hip extensor, ST. At the end of swing, the onset of stance, a clear activation burst was evident in the ankle extensor, LG; this existed in conjunction with the end of the TA burst. There was a dual burst in activation of sartorius during the initiation of swing and at the onset of stance. This behavior is similarly displayed in ST. Relative sizes of swing and stance phase bursts of ST changed over time, but an obvious pause between the bursts remained consistent throughout.

In SCI rats, however, average EMG patterns displayed changes in muscle activation throughout recovery (Figure 3.2). Prior to SCI, and displayed in normal subjects, hamstring (ST) activation patterns demonstrated a burst at swing onset. Following a clear, 100 ms pause was another burst with comparable amplitude associated with stance. After SCI, this dual burst pattern between knee flexor and hip extensor
activity of this muscle became less clear as the pause between bursts deteriorated. The swing and stance bursts blended and the stance phase portion of the burst became larger, making the distinction between swing and stance phase difficult. As recovery progressed, this pause between swing and stance had returned towards its baseline, but the pause between bursts remained slightly diminished (Figure 3.4).

Also, activity emerged in LG, the ankle extensor, in conjunction with the TA ankle flexor burst. This overlap between flexors and extensors is abnormal. In SART, the distinct flexor burst at the onset of swing is lost after SCI and only the late swing and stance phase activity remains. In ST, pause between the early pre-swing and pre-stance bursts is diminished immediately after the injury but begins to re-emerge as recovery proceeds.
Figure 3.1: Average EMG results from control subjects at pre-injury (A), 7 dpo (B), 14 dpo (C), and 21 dpo (D). Vertical line represents the onset of stance. Tick marks on the horizontal axis show 100 ms increments. The flexion burst initiating swing began about 250 ms prior to stance. The stance phase burst lasted about 400 ms. Thus, on the left of the graphs, the preceding stance phase is visible, and on the right, the following swing and subsequent stance are shown. Gains were adjusted for illustration purposes. Note how patterns remain consistent throughout the recovery phase of the study.
Figure 3.2: Average EMG results throughout recovery from SCI subject 14 at pre-injury (A), 7 dpo (B), 14 dpo (C), and 21 dpo (D). EMG averages formatted like the previous figure. Note how EMG patterns change, but return towards a normal pattern by then end of recovery.
Figure 3.3: Average EMG results comparing control subjects at pre-injury (A) and 7 dpo (B) to SCI subjects on the same dates (C) and (D). These results include GM, which lost some activity during stance after SCI. Note how the control subjects held a consistent ST pattern, maintaining the dual burst with the clear pause. In the SCI subject, this pause and thus the dual burst is lost.
Figures 3.1 to 3.2 show the changes in average EMG patterns in a control rat and a SCI rat in pre-injury and throughout the course of recovery. Figure 3.3 includes results from gluteus medius, showing differences in EMG patterns between a control subject and SCI subject pre-injury and 7 days post-injury. The purpose of this illustration is to compare the general differences in EMG between normal and injured rats. There is consistency in EMG patterns for all muscles in the control rat (Figure 3.3A,B). In the SCI subject, however, there are clear changes in patterns when comparing pre and post-operation days. Changes in EMG patterns for TA and LG after SCI are similar to those in Figure 3.2. Organization of gluteus medius recruitment also changed, but not as much as ST. GM activity during the stance phase for pre-injury and in normal rats was lost after the SCI (Figure 3.3D). The dual swing-stance burst of semitendinosus is completely lost on 7 dpo in the SCI rat. Figure 3.4 shows the changes in EMG patterns specifically for ST after SCI in comparison to a normal rat over time. This illustration includes the BBB scores to demonstrate the relationship between quality of walking and EMG. The lack of EMG activity immediately after the onset of stance in all muscles of (D) is the result of slower walking. However, EMG recruitment patterns in the ankle muscles, TA and LG, appeared relatively normal throughout recovery from SCI.
Figure 3.4: Average EMG patterns for semitendinosus for a normal versus a SCI rat over time. Note how the pause in ST deteriorates after SCI but begins to re-emerge with recovery. The vertical line marks stance onset. BBB scores are 21 unless otherwise noted.
Data from rats 13, 14, 15, and 17 (two controls and two SCI) were used for EMG and statistical analysis on a step-by-step basis. These yielded approximately 1,573 total steps and 7,474 EMG bursts from all subjects. A complete analysis of all rats would have been ideal, but not reasonable due to time constraints. Qualitative observations were made based off of horizontal bar graphs such as in Figure 3.5. These graphs illustrate average EMG onsets and offsets for each muscle including standard deviations. An interesting difference between injured and uninjured rats is the overlap between ankle flexor, TA, and extensor, LG, which appears after SCI. For normal walking rats, there is separation in time between TA offset and LG onset, due to their reciprocal relationship. However, after SCI, injured rats displayed time overlap in EMG between TA and LG. This change can also be due to the delay on the onset and offset of TA. Statistical analysis, however, showed no significant difference in the overlap between TA and LG after SCI. This and the decrease in activity for GM observed in Figure 3.3 may be due to the increased variability after the SCI.

Immediate observation after SCI is the extended duration of EMG for each muscle. This would be expected to be a result of slower walking and the need for each muscle to be activated longer. However, apparent differences after SCI were observed in swing and stance durations. The dual burst that the bi-functional semitendinosus normally displays was not as distinct immediately after SCI. Normal rats maintained their dual bursts for ST evident by the clear separation of EMG swing offset and stance.
onset. Figure 3.5 “A” through “C” shows this pause between the hip extension and knee flexion activity in ST and the decrease in this pause in “D.”

As recovery progressed, the dual burst in semitendinosus returned towards normal, but did not completely recover. In SCI rats, the overlap between LG and TA improved, showing more separation between time of TA offset and LG onset by the third week of recovery. The duration that each muscle was activated decreased with recovery time, as the rats walking speed increased. The standard deviations in Figure 3.5 are represented by the white boxes. The larger standard deviations revealed after injury in SCI rats can be the result of the variation in muscle recruitment. When comparing these bar graphs to EMG averaged patterns (Figure 3.2 & 3.3), it can explain why some bursts did not reappear after the onset of stance.
Figure 3.5: Horizontal bar graphs of average EMG onsets and offsets with standard deviation. Compare control rat at pre-op (A) and one week post-op (B), and SCI rat at same time points (C) and (D). Note the bigger standard deviations after SCI, indicating more variations in muscle recruitment. ST1 indicates the swing burst, and ST2 is stance.
3.3 Statistical Analysis

Using SPSS 15.0 software, an ANOVA analysis of variance was used to quantify significance in burst onset and offset latencies and duration as a function of pre-injury versus post-injury and SCI rats versus control rats. As explained in the methods, these were not formal statistics that analyzed only one observation per rat; the purpose was to use this analysis to identify what factors should be more carefully studied on a larger scale. Other aspects of EMG data that this study aimed to analyze were EMG amplitude, duration, time latencies with respect to swing and stance phases, and muscle overlap. For each subject, amplitude was normalized by converting it to a percentage of the highest amplitude observed for any single burst for each muscle. There was no systematic effect of SCI on burst amplitude, nor was burst amplitude related to the duration of swing or stance. Also, the predictions for higher correlations for EMG onset and paw off or contact over the duration of a treadmill walking session were all found to be ~1.0. Thus since all correlations were nearly perfect, no difference was found between normal and SCI groups. The TA-LG overlap difference is apparent in Figure 3.5, but was not found significant. It was slightly larger in both rats used for SCI, but there was no pre-op to post-op change in that variable.

Although immediately after SCI the rats were walking slower and there was a longer step cycle duration, the swing phase of the step was significantly shorter. Therefore, regardless of the walking speed, the duration of the swing phase was significantly affected. Figure 3.6 shows how the swing duration decreased. Swing was
shorter in absolute duration, and as a portion of the step cycle. Also, the duration of stance was slightly longer for injured rats, regardless of treadmill walking speeds (Figure 3.7). Figure 3.8 shows how the step cycle period did not vary throughout the recovery process for both control and SCI subjects.
Figure 3.6: Box plots of swing duration (A) and as a percentage of the step cycle (B). Smaller numbers on the Y-axis represent shorter duration of swing. Note how after SCI, injured subjects had a shorter swing phase. Each value on the box plots represents the timing of every good step of each group including the minimum, lower quartile, median, upper quartile, maximum, and outliers.
Figure 3.7: Box plots of stance duration (A) and as a percentage of the step cycle (B). Larger numbers on the Y-axis represent longer stance duration.
Figure 3.8: Box plots of cycle duration. Note how the cycle period remains stable regardless of subject (control vs SCI) or time in recovery.
Burst durations were not significantly different for any of the muscles when rats walked at the same speed. An interesting observation, however, are the differences in the timing of EMG onset of the flexor muscles in SCI rats. The time period between TA and ST onset and the onset of stance significantly decreased. In other words, the onset of these muscles was late. Figures 3.9 to 3.11 display this delay in onset latency.

TA onset latency with respect to paw contact was, as expected, significantly related to the duration of swing. In other words, TA onset latency was linked to the beginning of swing. However, even accounting for variations in swing duration, TA onset latency was significantly later after spinal cord injury. This is seen in absolute terms in Figure 3.9A, showing the later onset for TA as represented by the higher value for TA onset latency. In Figure 3.9B, the fact that the effect of the SCI was beyond variations in swing duration associated with SCI is shown. Here, TA onset latency is expressed as a percentage of swing phase duration, which normalized TA onset latency by the duration of swing. Even accounting for variations in swing duration, the relative onset time for TA was later after SCI. An ANOVA with group (SCI vs. control) by time point (pre-op vs post-op) using swing and stance duration as covariates to control for changes in step cycle temporal structure found an interaction between group and timepoint ($F_{1,522} = 57.39$, $p < 0.001$). Figure 3.12 displays a regression analysis done to represent the strong relationship between the TA onset latency and swing duration.

The change in onset latency for the swing-related ST burst (ST1) mimicked that for TA. The ST1 onset latency was later after SCI (Figure 3.10A), and this effect was
beyond that accounted for by the change in swing duration associated with SCI (Figure 3.10B). Again, this was significant in the ANOVA ($F_{1,428} = 32.46$, $p < 0.001$). The offset of ST1 was also delayed (Figure 3.11), however, the ANOVA revealed that the stance duration accounted for these differences. For the swing-related SART burst (SART1), onset was later after SCI in a manner that appeared to follow a pattern similar to that for TA and ST1. However, with only one control rat and one SCI rat with SART recorded, there were not enough degrees of freedom to test for an interaction between group and time point for this muscle.
Figure 3.9: Box plots of latencies of the onset of TA with respect to the onset of stance (A) and as the percentage of the step cycle (B). Later onsets are represented by the larger numbers on the Y-axis.
Figure 3.10: Box plots of latencies of the onset of ST-swing with respect to the onset of stance (A) and as the percentage of ST of the step cycle (B). Later onsets are represented by the larger numbers on the Y-axis.
Figure 3.11: Box plots of latencies of the offset of ST-swing with respect to the onset of stance. Later onsets are represented by the larger numbers on the Y-axis.
Figure 3.12: Regression between TA onset latencies and swing duration. Group 0.00 represents the controls and group 1.00 represents the SCI subjects.
A stepwise regression analysis was used to further explore the relationship between the onset latencies for TA and ST1 with swing phase duration and the locomotor ability of the animals as indicated by the BBB. A summary of the adjusted R-squared values for the significant factors in the regressions is presented in Table 3.2. For both flexor muscles, swing phase duration was the most significant factor in the regression, but BBB score was also a factor. However, the majority of the variance was accounted for by changes in swing phase duration. For TA, adding BBB score in on top of swing phase duration accounted for an additional 5% of the variance, bringing the adjusted r-squared up from 73% to 78%. For the ST burst, the BBB score was a stronger factor, improving the adjusted R-squared 21%, from 52% to 73%. However, the effect of the BBB score was strongly influenced by the presence of the normal animals with the BBB scores around 21. When the stepwise regression was repeated only for data including spinal cord injured animals after the injury, swing duration remained by far the most important factor in the regression and the influence of the BBB score was almost eliminated, accounting for less than 1% of the variance in this subset of the data (Table 3.2). Figure 3.13 shows the stepwise regression without the controls or pre-op days as variables.
<table>
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Table 3.2: Stepwise Regression for TA and ST1 Onset Latencies
Figure 3.13: Regression between BBB locomotor rating scores and time latencies of the onset of TA as a percentage of swing phase. This includes only SCI rats post-injury to demonstrate how the flexor onset latencies had no relationship with BBB scores represented by the R-square of 0.00.
There was also an apparent relationship between BBB score and ST-swing offset latency—when activation of the knee flexor was off—with the SCI group (Figure 3.8, Figure 3.11). However, once swing and stance phase duration were entered as competing factors, the variations in the duration of stance accounted for the difference in ST1 offset latency. Therefore, BBB score added nothing to explaining ST1 offset latency once the effect of stance duration was considered.
CHAPTER 4

DISCUSSION

This was an exploratory study with an objective to observe what further analysis can be done with certain aspects of EMG in future, larger-scaled SCI studies. The purpose of this experiment was to detect EMG data that could possibly indicate the level of recovery in a spinal cord injured rat. This study found qualitative differences in SCI rats through behavioral analysis and EMG patterns—including some that differed from previous studies. Quantitative measures of SCI recovery were analyzed, finding some EMG differences to be significant.

In the SCI subjects, the loss and recovery of motor function ability evidenced in the BBB scores was also displayed in the changes in patterns of activation, derived from EMG, in the selected hindlimb muscles. As recovery progressed, the recovery of muscle activity and improvement of BBB scores may represent an indicator of the degree of motor function recovery. EMG provided a way to analyze the recovery process from SCI outside behavioral observations. Further analysis of the relationship between these two assessments needs to be studied along with anatomical methods to determine whether recovery of EMG can be correlated with regeneration and repair of neural pathways in the spinal cord.
In continuation of SCI experiments, EMG analysis may be applied to other combinations of hindlimb muscles. The results demonstrate that the complexity of the muscle seemed to affect the extent of differences in the EMG patterns. Analysis of additional bi-functional muscles involving hip and knee flexion and extension, such as rectus femoris and iliopsoas, may be useful to future studies. Muscles such as these yield a transition in activity between flexors and extensors during locomotion—consistent with limb kinetics—and therefore may be more applicable to analyzing the process of SCI recovery (Pratt, Buford, & Smith, 1996). Additional muscles that may be worthy of study could be those important during the swing portion of a rat’s step, since the results show that this was a significant change in SCI subjects. Also, since this study found semitendinosus showing important changes after SCI, it may be valuable to consider its reciprocal muscles in conjunction with ST, such as those from the quadriceps group.

The relevance of SART activation patterns has yet to be confirmed, due to the variation of EMG results across the subjects and the unusual activity throughout stance. Seen in other studies involving various forms of motion, SART is not normally active during stance phase (Pratt & Loeb, 1990; Pratt et al., 1996; Quevedo et al., 2005), giving reason to believe additional research with SART activity and SCI may be necessary. This could be due to the complexity of its functions as well as the small size of the muscle in the rat, possibly resulting in crosstalk from the underlying quadriceps (vastus lateralis), which is commonly active during stance (Kaegi et al., 2001; Pratt & Loeb, 1990). Studies of motorneurons of SART and other muscles in the walking cat have
shown the significance of this muscle as a part of the motor pattern for locomotion, even in the fictive locomotion preparation (Quevedo, Stecina, Gosgnach, & McCrea, 2005). Further studies involving EMG are needed to analyze the reliability of the SART activation patterns associated with SCI. Perhaps SART EMG may be more accurately recorded by using patch electrodes, rather than intramuscular, on the fascial surface of the muscle; this method has proven effective in other studies of SART (Pratt & Loeb, 1990).

The EMG patterns involving TA, LG, GM, and ST appear reliable, as the results demonstrate consistency with other studies. The ankle flexor, TA, displays no considerable difference in patterns throughout the recovery process from SCI subjects; however, the relevance of this muscle is its predictable association with swing, making it applicable to determining the duration of each step. The observed overlap in activity with the extensor, LG, may be analogous to “typical gait changes observed in SCI patients” (Kaegi, Schwab, Dietz, & Fouad, 2001). This may be a result of the stretch reflex existing at this ankle joint—perhaps an indicator demonstrating the spasticity experienced in SCI patients today. The present study did not find a significant difference in the amount of overlap in between the reciprocal ankle muscles, TA and LG after SCI. However, since the study by Kaegi et al. found this result for tibialis anterior and vastus lateralis (knee extensor), it may be worth exploring this concept of overlap for different muscles in future studies.

The relevance of ST and SCI has also appeared fascinating, as the relationship between changes in EMG recordings and forms of locomotion has displayed sensitivity in
the activity of this muscle. Other studies involving fictive locomotion and backward walking display continuous activation between swing and stance phases (Buford & Smith, 1990; Quevedo et al., 2005). This differs from the pause between activation revealed in the EMG results for normal rats in the present study. Sensory inputs to ST may provide powerful modulation of this muscle’s activity for various forms of motion (Pratt, Buford, & Smith, 1996). Analysis of the kinematics of the hindlimb along with ST activity associated with SCI recovery may contribute to a better understanding of the value of this particular muscle.

It could be assumed that the decreased latency between TA onset and stance onset could be a result of the increased duration of stance due to the slower walking speeds. However, analysis of this relationship took into account stance duration and showed that the muscles’ EMG onset was still later after SCI. This is because higher BBB scores indicate that stance duration should be close to normal (Basso, Beattie, & Bresnahan, 1995). Overall, the regression analysis taken together with the ANOVA indicates that SCI had a categorical effect on TA and ST1 onset latencies. For both injured and uninjured animals, the duration of swing was the strongest factor related to the onset of the flexor muscles. This is expected since the onset latency of the flexors was calculated relative to stance onset but their function is to initiate swing. However, the novel finding was that after SCI, the flexor onset was relatively late within swing phase. This had a weak relationship to the BBB score, and mainly seemed to be a categorical difference in the timing of flexor onset relative to swing onset after spinal
cord injury. There was an observed difference in the ST1 offset latency as well, but was not significant. Thus, changes in ST offset latency after SCI were due to the influence of walking speed. In a subsequent study it may be valuable to keep all rats at the same walking speed throughout recovery (i.e. normal and SCI rats walk at 7 m/min at 7dpo) for better comparison, perhaps making these findings more reliable.

Essentially, the onset of ST and TA was later for the SCI rats, in accordance with their shorter swing phase duration. These results of the significant changes in the flexor muscles could be more closely analyzed in correlation to the decreased duration of swing. By the end of recovery, all rats were walking at the same speed of 12 m/min. This was factored into the statistical analysis and it was still found that SCI subjects had consistently shorter swing duration. Therefore, the duration of swing phase was shorter after SCI, regardless of step cycle period. Another study looked at this relationship and results were opposite (Pépin, Norman, & Barbeau, 2003). However, rats in that study were walking bipedally while in this study subjects were quadrupedal during treadmill walking. This interesting difference could be further examined in an additional study that could include groups of rats that walk quadrupedally and another walking bipedally.

The observed changes in individual muscle function revealed in EMG may contribute to understanding mechanisms of motor function recovery. With this, EMG and other mechanisms may enhance and improve the assessment of rehabilitation potential for individuals with spinal cord injuries. Further statistical analysis and quantification of changes in the EMG is needed to determine the reliability of this
measurement. This may involve analysis of individual EMG bursts rather than averaged data, before and after SCI. Variation in step cycle timing after SCI may produce misleading averages; analysis of individual bursts for each step preserves the organization of those steps. Additional burst parameters may also be analyzed to provide objective measurements of consistency of recruitment patterns associated with the degree of recovery from SCI. Consistent locomotion training through treadmill walking can contribute to some recovery of activity of these muscle recruitment patterns and thus motor function ability. EMG analysis reveals differences in muscle recruitment, especially for muscles such as semitendinosus that have complex functions. Subsequent applications may involve additional muscles with complex function and a variety of motor tasks applied to the rat.

Because this was an exploratory study, some limitations are expected. In the present study, BBB was related to the changes in flexor onset latencies, as revealed by the results. With low BBB scores, an experimental treatment (i.e. walking) for SCI rats can already be judged as a failure; with higher BBB scores, the experimental SCI treatment can be called a success. However, the analysis of flexor onset latencies may offer information on the quality of walking that is not currently present in the BBB. Further studies are required to determine whether EMG analysis adds new information to the BBB score. Other possible adjustments in future studies could be the consistency in treadmill speeds. If control rats walked at the same slow speeds of SCI rats immediately after the injury, this could result in a more reliable comparison of the EMG latencies.
The potential for EMG analysis to contribute to the assessment of recovery from SCI deserves further study for the potential to contribute to rehabilitation services.

In conclusion, thoracic level SCI does evoke notable changes in muscle recruitment patterns in the selected muscles of the hindlimb in a walking rat. Certain parameters of EMG data, such as onset and offset latencies, also reveal changes. Flexor muscles onsets and the relationship between swing and stance duration are worth further investigation. With a reliable set of EMG measurements, this tool could allow analysis of details of recovery throughout an experiment, rather than necessary sacrifice of the animal to allow anatomical analysis of the spinal cord at multiple time points in a study. Expansion of the possible experiments to clinical trials with EMG may relate to various rehabilitation tactics involving SCI patients. The ultimate question to address is once EMG can contribute to assessment of SCI recovery, how we can translate this from the rat to a human model.
REFERENCE LIST


