33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Robot Applied Stance Loading Increases Hindlimb Muscle Mass and Stepping Kinetics in a Rat Model of Spinal Cord Injury

Jeff A. Nessler, *Member EMBS*, Moustafa Moustafa-Bayoumi, Dalziel Soto, Jessica E. Duhon, and Ryan Schmitt

Abstract—**Following spinal cord injury (SCI) reduced limb usage typically results in muscle atrophy. While robotic locomotor training has been shown to improve several aspects of stepping ability following SCI, little is known regarding the effects of automated training on the preservation of muscle function. The purpose of this study was to evaluate the effects of two robotic locomotor training algorithms on hindlimb strength and muscle mass in a rat model of SCI. Eighteen Sprague-Dawley rats received a mid-thoracic spinal cord transection at 5 days of age, and were randomly assigned to one of three groups: control (no training), standard robotic training, and robotic training with a downward force applied to the shank during the stance phase of gait. Training occurred 5 days/week for 5 min/day, and animals received 90% body weight support for all sessions. Following 4 weeks of training, vertical and propulsive ground reaction force during stepping and *en vitro* mass of two plantarflexor muscles were significantly increased for all of the trained animals when compared to the untrained control group. Post hoc analysis revealed that standard robotic training did not appear to increase ground reaction force and muscle mass to the same extent as the loaded condition. These results indicate that automated robotic training helps to preserve hindlimb muscle function in rats following SCI. Further, the addition of a plantarflexion stance load appears to promote greater increases in muscle mass and stepping kinetics.**

I. Introduction

Locomotor training is a commonly used therapeutic technique for the rehabilitation of gait following spinal cord injury (SCI). Because manual training provided by a therapist is very labor intensive, several robotic devices have been developed that can perform this type of therapy consistently and without fatigue [1, 2, 3]. Unfortunately, automated training algorithms with body weight support (BWS) and swing assist have been shown to result in training intensities that are suboptimal in humans, particularly with regard to muscle activation [4, 5, 6]. While previous investigators have focused on training algorithms to improve neurological outcomes following locomotor training, much remains unknown regarding the effects of automated training on muscle function. Because skeletal muscle plays a large role in the control of movement, decreases in muscle function could potentially influence or undermine gains in neurological function during rehabilitation. In addition, muscle atrophy may limit the duration and intensity of training activity tolerated by the patient. Therefore, it is important that locomotor training strategies promote not only neural plasticity, but the preservation of muscle function in patients following SCI.

A robot applied stance load represents one approach for augmenting the effects of automated training on lower extremity muscle function. Specifically, the application of load during the stance phase of gait might be an effective means to offset decreases in load-related afferent information and EMG activity that are typically observed when a portion of an individual's body weight is supported by an external device [7, 8]. While the within session effects of stance loading were described previously [9], the long term effects of this approach as a training technique have yet to be determined. However, previous study has demonstrated that exercise training is an effective means for preserving muscle function during periods of inactivity, often with as little as one week of training [10, 11]. Because these effects are use-dependent [12, 13], and because increased load results in greater lower limb muscle activity [7, 8], it is reasonable to suggest that stance load application over a relatively short training duration will lead to improved muscle function when compared to standard training techniques.

The purpose of this study was to investigate the effects of automated robotic training, with and without stance loading, on both hindlimb strength and muscle mass in a rat model of SCI. Rodent models are commonly used to study the recovery of locomotor function following SCI, and a system of robotic devices was previously developed for investigating locomotor training in these animals (Fig. 1) [3, 14, 15]. Results from this experiment will have implications for the use of robotic gait training devices in both the rat model of SCI and in human rehabilitation.

II. Methods

A. Animals and Surgery

All surgeries were performed under aseptic conditions, and all procedures were approved by the Institutional Animal Care and Use Committee at California State

Manuscript received March 26, 2011. This work was supported in part by the National Science Foundation under Grant 85668. Special thanks to the Office of Biomedical Research Training (OBRT) at California State University, San Marcos.

J.A. Nessler is with the Department of Kinesiology, California State University, San Marcos, CA 92096 USA (p: 760-750-7352; fax: 760-750-3190; e-mail: jnessler@csusm.edu).

M.B. Moustafa is with the Department of Kinesiology and Health Promotion at California State Polytechnic University, Pomona, CA 91768 (e-mail: mmoustafa@csupomona.edu).

D. Soto, J.E. Duhon, R. Schmitt are undergraduate students in the Department of Kinesiology at California State University, San Marcos (email: dalziel.s@hotmail.com, duhon001@cougars.csusm.edu, schmi082@cougars.csusm.edu).

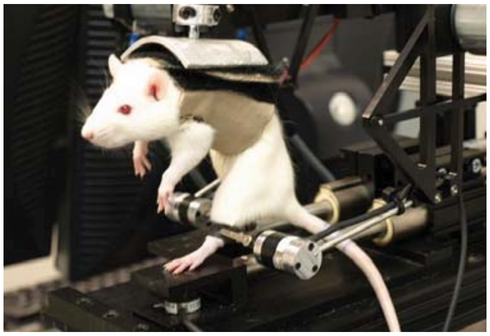

Fig 1. Spinal cord injured animal stepping in the instrumented robotic device. Load cells were placed at the interface between the robotic linkage and the animal's hindlimb, as well as beneath the sliding support platforms. Animals stepped bipedally in the device, and had approximately 90% of their body weight supported for the duration of the study.

University, San Marcos. At 5 days of age, eighteen female Sprague-Dawley rat pups were anesthetized via 2% isoflurane gas and received a mid-thoracic spinal cord transection. A collagen hemostatic sponge (Helistat®, Integra LifeSciences) was inserted between the severed ends of the cord to ensure separation. Following surgery, the pups were returned to the dam and were allowed to recover for 16 days. All rats were randomly assigned to one of three groups: a Control group (CONT) which received no locomotor training, a standard robot training group (SRT), and a load group (LOAD) which received robotic training with a downward force applied at the point of attachment (mid-shank) during the stance phase of gait.

B. Robotic Device and Locomotor Training

The rat stepper and control algorithms have been described previously [3, 9, 14]. Briefly, the rat stepper includes an adjustable BWS mechanism, two hindlimb manipulator robots, and either a motorized treadmill or reciprocating slide mechanism (used here) as the stepping surface. More recently, the rat stepper was instrumented with four force/torque sensors (Nano17, ATI Automation, 6 DOF, mass = 9g, diameter = 17mm, height = 15mm, force range: 0-12N, force resolution = $3.12\text{x}10^{-3}$ N) to facilitate the measurement of interaction forces during step training (Fig. 1). Animal-robot interaction forces were previously found to reflect locomotor ability in rats following SCI [16]. and may provide insight to muscle function by providing *en vivo* estimates of hindlimb muscle strength.

Animals wore a specialized vest that attached to the end of the BWS lever arm and allowed the rat to step bipedally without obstruction of the hindlimbs (Fig.1). A neoprene strip was wrapped around each shank (just above the ankle) and held in place by alligator clips which were attached to the hindlimb manipulator robots. Force/torque sensors were placed in series with the alligator clip and the end effector of the hindlimb manipulators, and the alligator clip was allowed to rotate about a mediolateral axis via revolute joint. Two independently actuated platforms were used in place of a standard treadmill, and were constrained to remain directly under the animal's respective hindpaw at all times. Two additional force sensors were placed under these platforms to measure vertical and horizontal (propulsive) GRF.

The device was programmed to provide reciprocating, robot-assisted hindlimb extension, and to allow free movement of the hindlimb when swing was generated by the animal. Swing was detected in real-time by monitoring the position error of the hindlimb manipulator robot as it pulled the animal's hindlimb into extension along a desired trajectory. Any forward or upward movement of the animal caused the robot to deviate from this trajectory, and position errors above a pre-determined threshold were identified as steps. Successful generation of a step in one limb led immediately to extension of the contralateral limb in an attempt to facilitate continuous locomotor activity. Animals in the SRT group were trained using this algorithm. Animals in the LOAD group were also trained using this algorithm, but with the addition of a downward force applied during stance. This robot-applied force was programmed to be approximately 0.3N for each hindlimb (or 30% body weight on average), as this load increased ground reaction forces during stepping to levels typically seen with 60% BWS in the same injury model (unpublished data). Both groups received approximately 90% BWS for all training sessions. Training began at 21 days of age, and was applied for 5 minutes a day, 5 days a week, for 4 weeks.

C. Data Collection and Analysis

On the fifth session of each week, steps and GRF were recorded during two, one-minute stepping trials with no stance load applied. The first trial was performed with 90% BWS. The second was performed with 60% BWS to faciliate evaluation of stepping kinetics under conditions of greater load. On the fifth session of weeks 2 and 4, animals from the control group were also evaluated in a similar manner. GRF values were obtained by first identifying steps taken by each animal using an automated step detection algorithm described previously [14], identifying the peak vertical and horizontal GRF measured during the stance phase of each step, and then calculating the average of these peaks for each one-minute trial. GRF data from the left and right limbs were then averaged. Though data were also recorded from sensors attached to the hindlimb manipulators, only GRF data were included in the current analysis. All GRF data were normalized to animal body weight, as the adolescent rats experienced significant development over the course of the experiment. GRF projection angle was also calculated to assess any changes in direction of GRF over time (Fig. 2). At the end of the 4 week training period, all rats were euthanized and their hindlimb muscles were dissected to assess differences in mass.

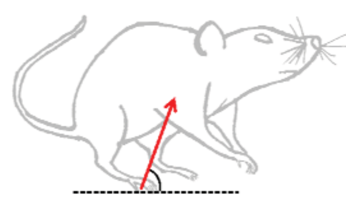

Fig 2. Calculation of ground reaction force projection angle.

Total steps, vertical and horizontal GRF, and GRF direction were analyzed via 2 way ANOVA with repeated measures (3 groups by 2 time points – weeks 2 and 4 of training). Because sample sizes were relatively low, muscle masses for *gastrocnemius*, *soleus*, *tibialis anterior*, and *extensor digitorum longus* were analyzed via non-parametric statistical tests (Kruskall-Wallis followed up with individual Mann-Whitney U tests).

III. Results

A. Time

The main effect for time was significant for number of steps taken in a one minute period, vertical and horizontal GRF, and GRF projection angle (Figs. 3-5). However, while number of steps and GRF projection angle increased significantly across time (Fig. 4), normalized vertical and horizontal GRF demonstrated an overall decreasing trend for the same period (with the exception of vertical GRF at 90% BWS, Figs. 3&4). On average, animal body mass increased from 52.1±8.7g to 127.9±29.2g over the 4 week training period.

B. Training Group Differences

On average, control animals performed significantly fewer steps than either of the trained groups (p=0.001). At week 4, vertical GRF was significantly greater for the LOAD and SRT groups relative to the CONT group for both levels of BWS (p=0.012 at 90% and p=0.025 at 60%, Figs. 3&4). In addition, significant differences were found between training groups for horizontal (propulsive) GRF at week 4 (p=.049 at 90% and p=.035 at 60%, Figs 3&4). No differences were found between groups for GRF projection angle (p=0.673, Fig 5). Comparison of *en vitro* muscle mass revealed increases in all 4 muscles for both SRT and LOAD groups when compared to the control group, though not all of these increases were statistically significant (Fig 6). Overall, the LOAD training paradigm resulted in significant increases in plantarflexor muscle mass when compared to the control group (p=0.006 and p=0.025 for the *gastrocnemius* and *soleus*, respectively), but not when compared to the SRT group (p=0.394 & p=0.296). Dorsiflexor muscle mass was similar for both the SRT and LOAD groups.

C. Interaction Effects

The interaction of training group by time was not found to be statistically significant for any of the variables studied here. However, there was a general trend for the control group to exhibit greater decreases in GRF over time when compared to the trained animals (SRT and LOAD).

IV. Discussion

Following neonatal spinal cord injury, automated, robotic training resulted in improvements in hindlimb muscle strength (inferred via changes in GRF) and muscle mass. Plantarflexor muscle mass was further increased with the addition of a robot applied stance load during step training. These data suggest that the additional load applied at the shank may stimulate a greater amount of motor recovery by increasing the load experienced by the plantarflexor muscles. This idea may have implications for body weight supported locomotor training as well as the design of robotic training devices and automated control algorithms.

These results are consistent with previous work that reported improvements in muscle function with exercise training [10, 11]. In particular, spinal cord injured rats who received one week of locomotor training were previously reported to exhibit 38% greater peak *soleus* tetanic force and 23% larger *soleus* cross sectional area when compared to untrained animals [10]. In the present data, trained animals demonstrated 53% and 74% greater vertical and propulsive GRF (respectively) relative to control animals at week 4. In addition, SRT animals exhibited dorsiflexor and plantarflexor muscle masses that were 27% and 37% greater (respectively) than those of the untrained control animals. In the LOAD group, plantarflexor muscle mass was increased by 50% relative to control animals (Fig. 6).

Little is known regarding changes in bipedal GRF with locomotor training in rat models of SCI, but the current data provide some insight for the injury model used here. Both horizontal and vertical ground reaction forces decreased over time for all training groups when normalized to animal body weight. This is most likely reflective of training in an adolescent model, whereby decreases in strength to mass ratio occurred as the injured animal developed. Disproportionate increases in mass and inertia may confound standard outcome measures of stepping ability and should therefore be considered in future analyses of locomotor function in this model. In particular, decreases in strength to mass ratio continued through the 3rd week of training (42 days of age). Trained animals began to increase their normalized GRF during the fourth week (Figs 3&4).

Additional research in an adult injury model, whose development has stabilized, will likely add clarification to these data. Further, the effects of robotic training on GRF and muscle mass should be evaluated for training durations

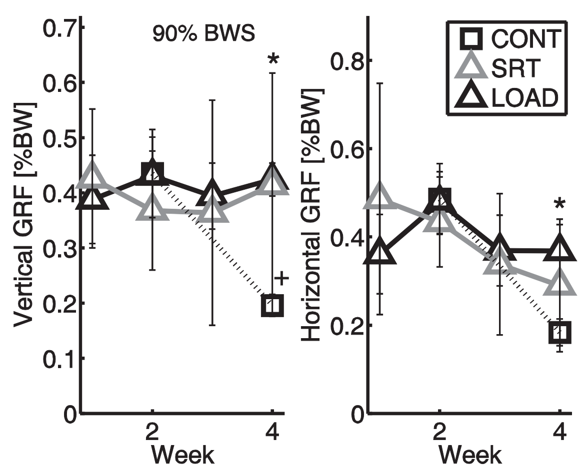

Fig 3. Vertical (left) and horizontal (right) ground reaction forces measured during locomotor activity in the rat stepper for 3 training groups at 90% BWS. All forces are normalized to animal body weight. CONT: control, SRT: standard robotic training, LOAD: robot applied stance load. * denotes significant decrease in GRF for the CONT group relative to both the SRT and LOAD groups. +denotes significant decrease across time. Bars represent median absolute deviation.

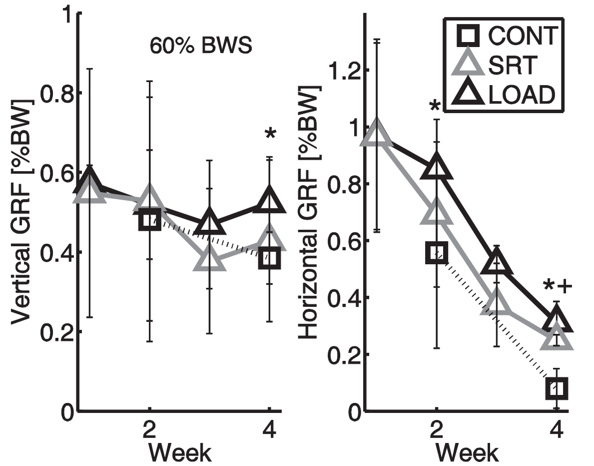

Fig 4. Vertical (left) and horizontal (right) ground reaction forces measured during locomotor activity in the rat stepper for 3 training groups at 60% BWS. All forces are normalized to animal body weight. * denotes significant decrease in GRF for the CONT group relative to both the SRT and LOAD groups. +denotes significant decrease across time. Bars represent median absolute deviation.

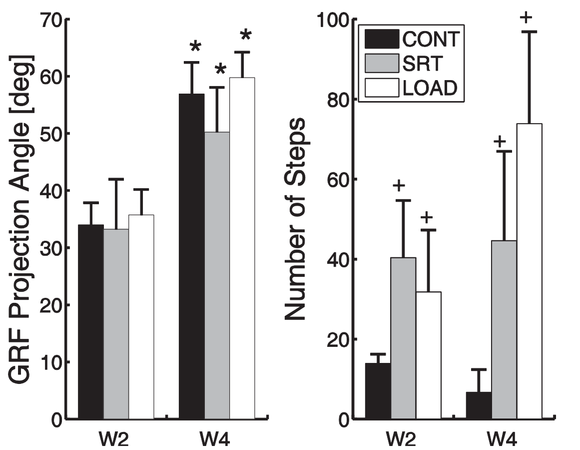

Fig 5. Average GRF projection angle (left) and number of steps taken (right) during a one minute stepping session for 3 training groups following 2 and 4 weeks of training. These data represent the average performance for the 90% and 60% BWS conditions. * denotes significant increase from week 2 to week 4, + denotes significant increase with respect to control group. Bars represent median absolute deviation.

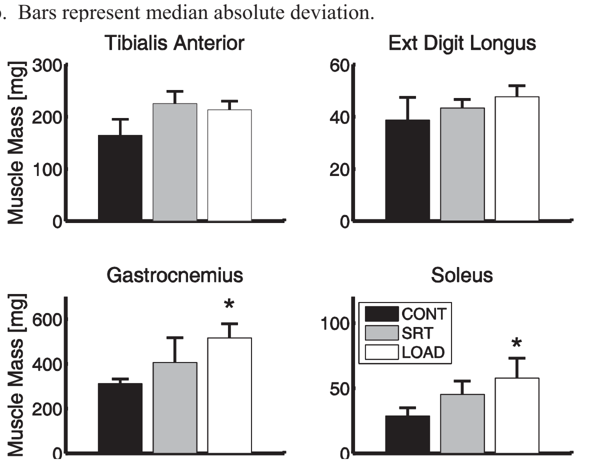

Fig 6. Average mass for two dorsiflexor (top) and two plantarflexor (bottom) muscles for 3 training groups. Muscle masses for two animals in the SRT group were excluded due to problems with the sample. * denotes significant increase with respect to the control condition. Bars represent median absolute deviation.

greater than 4 weeks, as hindlimb function did not appear to improve relative to control until the 4th week of training. Finally, future experiments on the effects of stance loading should involve molecular analyses of signaling pathways related to muscle hypertrophy. These measures will likely respond more quickly to individual bouts of training and can contribute to a mechanistic understanding of the effects of this training strategy on muscle function.

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