Operant Conditioning to Increase Ankle Control or Decrease Reflex Excitability Improves Reflex Modulation and Walking Function in Chronic Spinal Cord Injury

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ABSTRACT

Ankle clonus is common after spinal cord injury (SCI) and is attributed to loss of supraspinally mediated inhibition of soleus stretch reflexes and maladaptive reorganization of spinal reflex pathways. The maladaptive reorganization underlying ankle clonus is associated with other abnormalities such as coactivation and reciprocal facilitation of tibialis anterior (TA) and soleus (SOL), which contribute to impaired walking ability in individuals with motor-incomplete SCI. Operant conditioning can increase muscle activation and decrease stretch reflexes in individuals with SCI. We compared two operant conditioning-based interventions in individuals with ankle clonus and impaired walking ability due to SCI. Training included either TA EMG activation (TA↑) to enhance supraspinal drive or SOL H-reflex suppression (SOL↓) to modulate reflex pathways at the spinal cord level. We measured clonus duration, plantar flexor (PF) reflex threshold angle, timed toe tapping, dorsiflexion (DF) active range of motion (ROM), lower extremity motor scores (LEMS), walking foot clearance, speed and distance, SOL H-reflex amplitude modulation as an index of reciprocal inhibition, presynaptic inhibition, and low-frequency depression, and SOL/TA clonus coactivation. TA↑ decreased PF reflex threshold angle (-4.33°) and DF active ROM angle (-4.32°), and increased LEMS of DF (+0.8 points), training leg (+2.2 points), and non-training leg (+0.8 points), and increased walking foot clearance (+4.8 mm) and distance (+12.09 m). SOL↓ decreased SOL/TA coactivation ratio (-0.21) and increased non-training leg LEMS (+1.8 points), walking speed (+0.02 m/s) and distance (+6.25 m). We found increased voluntary control associated with TA↑ outcomes and decreased reflex excitability associated with SOL↓ outcomes.

Key words: ankle clonus, soleus stretch reflex, reciprocal inhibition, presynaptic inhibition, antagonist coactivation, plantar flexor spasticity
INTRODUCTION

In persons with spinal cord injury (SCI) (see Appendix 1 for complete list of abbreviations), walking function is impaired by reduced ability to produce voluntary muscle contraction and by hyperactive spinal reflex activity (hyperreflexia). In those with motor-incomplete SCI, the impaired voluntary muscle contraction arises primarily from corticospinal tract disruption, but reduced corticomotor excitability, maladaptive cortical changes (Beekhuizen and Field-Fote 2005, Thomas and Gorassini 2005) and limited motor unit rate modulation (Zijdewind and Thomas 2003) also contribute to this impairment. During normal human walking, control of the tibialis anterior (TA) muscle is strongly related to corticospinal input (Capaday et al. 1999; Schubert et al. 1997). Evidence demonstrates that TA corticospinal excitability is enhanced with dorsiflexion imagery during imagined walking in non-disabled individuals (Bakker et al. 2008), functional electrical stimulation after chronic central nervous system lesions (Thompson et al. 2009), and locomotor training in persons with chronic motor-incomplete SCI (Thomas and Gorassini 2005).

Beyond the impairment of voluntary control, SCI also results in loss of modulation of involuntary reflex circuits. Clonus is a common manifestation of stretch reflex hyperexcitability (Decq 2003). Clonus manifests as involuntary 5-7 Hz joint oscillations (Wallace et al. 2005), and commonly occurs at the ankle in individuals with motor-incomplete SCI, and other forms of central nervous system pathology. Clonic soleus (SOL) activity due to inadequate modulation of the SOL stretch reflex may impede walking progression (Yang et al. 1991), compromise independent walking (Beres-Jones et al. 2003), and restrict quality of life in individuals with motor-incomplete SCI.
Reflex pathophysiology contributes to ankle clonus after SCI (Katz and Rymer 1989). Disrupted supraspinal input and maladaptive reorganization of spinal reflex pathways give rise to impaired modulation of peripheral sensory feedback (Field-Fote 2004; Hultborn 2003) and aberrant reflex responses (Gracies 2005); exaggerated soleus stretch reflexes emerge (Davies et al. 1995; Dietz 1997) and contribute to clonus in plantar flexors. After SCI, impaired SOL disynaptic reciprocal Ia inhibition (RI) manifests as SOL coactivation during TA contractions (Boorman et al. 1996), SOL reciprocal facilitation after TA activation (a reversal of the normal response) (Crone and Nielsen 1994; Crone et al. 2003; Morita et al. 2001), and antagonist response to SOL or TA tendon tapping (Xia and Rymer 2005). In individuals with motor-incomplete SCI, decreased SOL PI contributes to soleus stretch reflex hyperexcitability (Dietz 2001; Faist et al. 1994).

Increased SOL motoneuron excitability, reduced post-activation depression of repeated stretch activations, and antagonist coactivation may promote clonus. Elevated SOL H/M ratio, found in individuals with clonus (Koelman et al. 1993), has been associated with increased plantar flexor (PF) reflex threshold angle (the angle at clonus onset) (Manella and Field-Fote 2010) and number of clonic oscillations (Manella and Field-Fote 2009). SOL H-reflex low-frequency post-activation depression (LFD) (Lloyd and Wilson 1957; Pierrot-Deseilligny and Burke 2005) is impaired in persons with chronic SCI (Grey et al. 2008; Schindler-Ivens and Shields 2000), allowing the SOL to be easily activated with small amounts of sensory input. In spastic hemiparesis, dorsiflexor force is inversely related to plantar flexor spasticity and EMG SOL/TA cocontraction ratio (Levin and Hui-Chan 1994), suggesting a relationship between the amount of voluntary TA control and SOL hyperexcitability. After motor-incomplete SCI, SOL/TA coactivation during clonus has been reported (Manella and Field-Fote 2009). Therefore
a myriad of factors, including SOL reflex hyperexcitability, diminished SOL LFD, decreased voluntary dorsiflexor activation, and SOL/TA coactivation appear to influence clonic activity. Control of both voluntary movement and reflex activity are known to be modifiable with operant conditioning training, wherein reward is contingent upon performance of a targeted motor task. Operant conditioning training induces neuroplasticity in supraspinal and spinal reflex pathways. Such training increases voluntary EMG responses (Brucker and Bulaeva 1996; Seymour and Bassler 1977; Stein et al. 1990) and firing rates of spared motor units (Stein et al. 1990) after motor-incomplete SCI, and decreases stretch reflex excitability in individuals with and without spasticity (Evatt et al. 1989; Segal 1997; Wolf and Segal 1996). Successful SOL H-reflex suppression in animals (Wolpaw and Herchenroder 1990; Wolpaw and Chen 2001; Wolpaw and Chen 2006) and humans (Thompson et al. 2009, 2008, 2006) provides clear evidence of activity-dependent plasticity in spinal circuitry induced by operant conditioning training.

Based on the foregoing evidence that both impaired voluntary dorsiflexor control and plantar flexor hyperexcitability contribute to functional deficits and spasticity in persons with spastic hypertonia, we designed two operant conditioning-based interventions intended to improve ankle motor control in individuals with motor-incomplete SCI. The interventions were: 1) TA EMG activation (TA↑) to enhance supraspinal drive to spinal circuits and 2) SOL H-reflex suppression during voluntary DF (SOL↓) to modulate reflex pathways at the spinal cord level. TA↑ was intended to improve ability to voluntarily drive corticospinal activation of spared TA motor neurons and activate inhibitory interneurons (Iles and Pisini 1992) comprising SOL RI and PI pathways. We hypothesized TA↑ would be associated with increased DF active ROM and DF strength, foot clearance in swing phase, and walking speed and distance. SOL↓
was intended to suppress SOL H-reflex amplitude responses during DF. We hypothesized
SOL↓ would be associated with enhanced SOL H-reflex LFD, decreased SOL/TA clonus
coadactivation, decreased clonus duration, decreased PF reflex threshold angle, and increased toe
tapping ability. We compared TA↑ and SOL↓ interventions and assessed 15 outcomes: 1) two
measures of ankle clonus (clonus duration, PF reflex threshold angle); 2) two measures of ankle
motor control (toe tap test, DF active ROM); 3) four measures of lower extremity strength (DF
strength, PF strength, [training and non-training leg]); 4) three walking-related measures (foot
clearance, speed, and distance); and 5) four neurophysiologic measures (SOL H-reflex RI, PI,
and LFD, and SOL/TA clonus coactivation ratio). Secondarily, we also explored relationships
among changes in outcome measures to determine if neurophysiological mechanisms are
associated with changes in training, clinical, or walking measures.

METHODS

Research participants

Of sixteen volunteers recruited from The Miami Project to Cure Paralysis research
subject registry; twelve signed written consent and were enrolled in the study (Figure 1).
Participants were 16 to 75 years old, with stable motor-incomplete SCI (> 1 year), lesion level of
T12 or above, and ability to walk 6 meters with devices or assistance as needed. All participants
exhibited an American Spinal Injury Association (ASIA) Impairment Scale D (AIS D) category
of SCI (ASIA 2002; Marino et al. 2003), and in at least one lower extremity had a palpable TA
contraction, a SOL H-reflex response, and ankle clonus elicited by manual plantar flexor stretch.
Participants were without other conditions impairing walking function, or cognitive deficits that
would impede adherence to the study protocol. Participants were stratified according to toe tap
score (toe taps in 10 seconds) into one of two categories indicating extent of voluntary ankle
control: low control (< 15 taps) and high control (≥ 15 taps) (Figure 1), with each training session lasting approximately 2 hours including set-up time. Following stratification, participants were randomly assigned to the TA↑ group (n = 6, mean age = 44.2, 6 men) or the SOL↓ group (n = 6, mean age = 45.2, 4 men) (Table 1). We trained the ankle with most severe clonus as assessed by clonus duration after manual plantar flexor stretch. Training was conducted three times weekly for five weeks for a total of 15 sessions (3 baseline and 12 training sessions) (Figure 1). Testing was performed the week before and the week after training. All twelve participants completed the study (mean age = 44.6 years, 10 men). The study was conducted in accordance with the standards of the Declaration of Helsinki and was approved by the University of Miami Human Subjects Research Office.

Clinical Measures of Ankle Clonus, Motor Control, and Walking

Ankle clonus

The Ankle Clonus Drop Test (Manella and Field-Fote 2009) provides reliable ankle clonus measures that correlate with the Spinal Cord Assessment Tool for Spastic reflexes clonus score (Benz et al. 2005) and SOL H/M ratio (Manella and Field-Fote 2009). Briefly, with the individual sitting, the foot was placed on a 10-cm high platform, the leg was lifted to a height of 10 cm above the platform and released; impact of the forefoot with the platform edge provided a SOL stretch to elicit clonus (Figure 2). Kinematic ankle angles and SOL and TA EMG activity were recorded for three trials. Clonus duration was measured from first PF deflection after platform impact to last PF deflection of at least 1 degree greater than the rest angle. The PF reflex threshold angle (Manella and Field-Fote 2010) was identified kinematically as first PF
deflection after platform impact. Mean clonus duration and PF reflex threshold angle were calculated.

Ankle motor control

Timed toe tapping (Knights and Moule 1967) was performed seated with the ball of the foot positioned on a pressure sensitive footswitch embedded in a platform. Dorsiflexion activated the footswitch that triggered data acquisition (Spike 2 v7, Cambridge Electronic Design, Cambridge, England). With the heel maintaining platform contact, the participant performed voluntary concentric and eccentric dorsiflexor contractions as quickly as possible for 10 seconds for four trials with 60-second rest periods between trials. Mean number of taps achieved in the best 3 of 4 trials was calculated.

Voluntary DF active ROM was recorded using an 8-camera Peak Motus 8.5 motion analysis system (Vicon, Centennial, CO, USA). Reflective markers were placed laterally on the 5th metatarsal head, malleolus, heel, and knee. In sitting, hip and knee were flexed to 90° and 30°, respectively, with the lower leg supported allowing unrestricted ankle motion. Three trials of maximal voluntary dorsiflexion and 5 seconds of relaxation were performed; mean maximum dorsiflexion angle was calculated.

Bilateral lower extremity strength was assessed using ASIA lower extremity motor scores (LEMS) (ASIA 2002; Marino et al. 2003). With the subject supine, hip flexors, knee extensors, dorsiflexors (DF), great toe extensor, and plantar flexors (PF) were graded from 0 (no palpable contraction) to 5 (maximal resistance against gravity); LEMS for DF, PF, training leg and non-training leg were recorded.
Walking function

Foot clearance and speed were analyzed using an 8-camera Peak Motus 8.5 motion analysis system (Vicon, Centennial, CO, USA); data were sampled at 60 Hz, smoothed (zero-lag Butterworth filter) and low-pass filtered (6 Hz). Twenty-one reflective markers were placed bilaterally at lateral 5th metatarsal head, malleolus, heel, and knee, and greater trochanter, anterior superior iliac spine, posterior wrist, lateral elbow and shoulder; and at C7, T10 and the sacrum. Participants, wearing a safety harness, walked with assistance and/or devices as needed at their fastest comfortable speed over a 6-meter walkway for three trials. Foot clearance, defined as maximal vertical excursion of the 5th metatarsal marker, was quantified for three swing phases for each trial. Walking speed was calculated for the central 4 meters of each trial.

Mean foot clearance and speed were computed. Distance traversed during a 2-minute period was determined by participants walking, with assistance and/or devices as needed, at fastest comfortable speed around an 80-ft (24.39 m) track.

Neurophysiologic Tests

SOL H-reflexes (peak-to-peak amplitude, mV) in response to RI, PI, and LFD conditioning stimuli were recorded using surface electrodes (interelectode distance, 36 mm) positioned as described by Rainoldi et al. (2004). SOL electrodes were placed at mid-line, 10 cm proximal to the calcaneus. Instrumentation included a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Hertfordshire, England), AC amplifier (Grass P511, Grass Technologies, West Warwick, RI, USA), and analog-to-digital converter (CED 1401, Cambridge Electronic Design, Cambridge, England). Inter-stimulus intervals (ISIs) were controlled with an electrical stimulator (Grass S88, Grass Technologies, West Warwick, RI, USA). A peripheral nerve stimulator (MiniStim MS-IVA, Life-Tech Inc, Stafford, TX, USA) was used to locate the
posterior tibial nerve (PTN) in the popliteal fossa, common peroneal nerve (CPN) distal to the
fibular head, and femoral nerve (FN) in the femoral triangle. Cathodes were placed at the nerve
site where the largest muscle contraction was evoked with lowest stimulus intensity; anodes were
placed on the patella medially for PTN, laterally for CPN, and superiorly for FN stimulation.
Stimulus intensity (mA) was maintained between 10 – 30% Mmax (Crone et al. 1990).

**Reciprocal Ia inhibition test**

For testing RI of the SOL H-reflex (Crone et al. 1987), TA electrodes were placed on the
muscle belly, lateral to tibial crest and at mid-point between tibial tuberosity and inter-malleolar
line. Twenty interleaved test (PTN) and conditioned (CPN-PTN, 2 ms ISI) reflexes were evoked
at 0.10 Hz. TI testing was repeated using CPN-PTN conditioning stimuli at 3 ms ISI. The
conditioned reflex amplitude was normalized to the test amplitude and expressed as percent (%)
of control reflex; for each ISI interval the mean RI% was calculated and smallest mean RI% was
selected.

**Presynaptic inhibition test**

PI of the SOL H-reflex was assessed using heteronymous SOL H-reflex facilitation (Faist
et al. 1994). Electrodes were placed on quadriceps of the training leg at mid-line on the muscle
belly, 15 cm proximal to the patella. A 0.5 ms PTN test pulse was administered prior to a 0.1 ms
FN conditioning pulse. FN stimulus intensity was 4X motor threshold. The ISI at onset of SOL
H-reflex facilitation was identified and increased by 0.4 ms. Twenty interleaved test and
conditioned reflexes were evoked at 0.10 Hz. The difference between test and conditioned reflex
amplitudes was expressed as a percent of Mmax. Values ranged from 2 – 18%, consistent with
Faist et al. (1994). The largest PI% was selected for analysis.
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254 **Low-Frequency Depression test**

255 SOL H-reflexes were evoked using paired-pulse PTN stimuli with ISI of 1 second. Ten
256 trials with 10-second rests between trials were recorded. The amplitude of the conditioned
257 (second) response was expressed as % of the control (first) reflex amplitude (LFD%); mean
258 LFD% was calculated (Field-Fote et al. 2006).

259 **Coactivation during clonus**

260 SOL and TA EMG activity were recorded during the ankle clonus test (described above).
261 The coactivation ratio of SOL and TA EMG bursts (± 2SD mean resting EMG) during a 2-
262 second window of clonus was calculated using customized software (MATLAB, The
263 MathWorks, Inc., Natick, MA, USA) (Manella and Field-Fote 2009). Mean SOL/TA clonus
264 coactivation ratio for three trials was calculated.

265 **Training Procedures**

266 Surface recording electrodes were placed after standard skin preparation. For both
267 interventions, the participant was seated with approximately 100° hip flexion, 70° knee flexion
268 and 120° ankle plantar flexion; the training foot was positioned on a footswitch imbedded in a
269 platform. A shoe was worn if footswitch activation was achieved with it on; if not then a sock
270 and shower slipper were used. An auditory signal cued the participant to perform DF at 0.10 Hz
271 for 30 repetitions; 10 bouts of 30 repetitions were performed with 60-second rest intervals
272 between each bout. During baseline testing no visual or auditory feedback was provided.
273 During training EMG biofeedback and a visual tally of the number of repetitions that met or
274 exceeded target during each bout were displayed (methods for determining target TA↑ and
275 SOL↓ EMG are described below in the respective sections). Immediate verbal reward of “good
job” or similar accolade was given and faded to summary feedback upon 80% success rate (number of repetitions exceeding target/total repetitions) (Figure 1).

TA\uparrow

TA electrodes were placed as described previously. EMG signals were amplified (x2k) (Grass amplifier, Grass Technologies, West Warwick, RI, USA), notch filtered (60 Hz), bandpass filtered (10 Hz - 1000 Hz), sampled at 1k Hz, full-wave rectified, and smoothed (RMS, 8 ms moving average filter). The footswitch was activated by dorsiflexion, and triggered a TTL input to an analog-to-digital converter (CED 1401, Cambridge Electronic Design, Cambridge, England). For each participant, at session start, three 3-second TA maximal voluntary contractions (MVCs) were performed with 60-second rest intervals. Mean MVC peak amplitude was computed and %MVC amplitude target for the session was identified. TA EMG biofeedback using customized software (Spike 2, v7, Cambridge Electronic Design, Cambridge, England) was displayed on a split screen (Figure 3A). On the right, the %MVC amplitude target was displayed (horizontal line), a vertical bar moved up or down reflecting increasing or decreasing TA EMG activity. Instructions were to dorsiflex the foot and move the TA EMG bar above the target line; when the target was exceeded the screen flashed green. On the left side of the screen, reward, EMG, and footswitch activations were displayed; a gold icon appeared when TA EMG activity exceeded target. For training session 1, the mean %MVC amplitude of the prior baseline session was used as the training target. For training sessions 2 – 12, each %MVC amplitude target was based on prior session performance; for session success rates of at least 50%, the target was increased 10% for the next session.
Footswitch activation, EMG recording and signal amplification equipment and parameters were as described above for TA. During training, SOL H-reflexes during brief voluntary DF contractions were recorded as described by Crone et al. (1987), Crone and Nielson (1989), Morin and Pierrot-Deseilligny (1977), and Pierrot-Deseilligny and Burke (2005). Electrodes were placed as described earlier for PTN stimulation and SOL H-reflex recording and the foot was positioned on the platform over the embedded footswitch. The footswitch was connected to a stimulator (Grass S88, Grass Technologies, West Warwick, RI, USA) that provided a 50-ms delay between footswitch activation and SOL H-reflex stimulation. This delay reflected the time course of depression of SOL H-reflex by voluntary DF, which increases greatly from 50 – 100 ms following movement onset in non-disabled individuals (Crone et al. 1987). For each participant, at session start, SOL H-reflex and M-wave recruitment curves at rest and during voluntary DF were obtained by incrementally increasing the stimulus intensity by 2 mA; H-reflex and M-wave maximum amplitudes were recorded. PTN stimulus intensity was maintained at 20% of M-wave maximum amplitude during DF throughout each session.

SOL H-reflex biofeedback using customized software (Signal, Cambridge Electronic Design, Cambridge, England) was presented on a split screen (Figure 3B). On the right, the SOL H-reflex amplitude (mV) target was displayed (horizontal line). A black square, representing the peak-to-peak amplitude of the SOL H-reflex response, appeared above or below the target line for responses greater or smaller than the target, respectively. Instructions were to dorsiflex (which triggered SOL H-reflex stimulation) and generate a black square (reflex amplitude) below the target line; the screen flashed green for responses smaller than target amplitude. On the left side of the screen, the SOL H-reflex signal was displayed (Figure 3B). For each training session,
the first bout was a baseline bout (no feedback) in which the median SOL H-reflex amplitude
target was calculated to determine the training target for the second bout; for subsequent training
bouts the median SOL H-reflex target was calculated from the previous training bout.
Participants were encouraged to employ any strategy to accomplish the task while remaining
seated with the foot on the platform and footswitch.

Data analysis

Non-parametric statistical tests were conducted using SPSS Statistics 18 software (SPSS, Inc., Chicago, IL, USA) because the assumption of normal distribution required for use of
parametric test was not met due to small sample size. Mann-Whitney U or Chi-square statistics
were used to compare demographic and pre-intervention clinical characteristics for each
intervention group. Spearman correlation coefficients were used to determine pre-intervention
relationships between outcome measures.

Training dosage was compared between TA↑ and SOL↓ groups using Mann-Whitney U-
test. For each group, the magnitude of change in the training measure was examined with one-
tailed Wilcoxon signed ranks test. For the SOL↓ group, only SOL H-reflexes that were elicited
with stimulus intensities between 10 and 30% M-max were included in data analysis, to ensure
equivalence of stimuli across trials. Effect size was assessed with standardized response mean
(SRM) (Liang et al. 1990), which divides mean pre-post intervention change by standard
deviation of the change. SRM interpretation, based on Cohen’s criteria, was 0.20, 0.50 and 0.80
for small, moderate, and large effects, respectively (Liang et al. 1990). For TA↑, mean %MVC
amplitude was compared between three baseline sessions and the final three training sessions.
For SOL↓, stability of SOL Mmax across sessions was assessed using repeated measures
ANOVA; SOL Mmax values were obtained from the recruitment curve for each session and were found to be stable (p = 0.24). Mean SOL H-reflex amplitude during dorsiflexion was calculated for the last three training sessions (conditioned SOL $H_{DF}$) and was normalized to the mean SOL $H_{DF}$ for three baseline sessions and expressed as percent (%) of baseline (Thompson et al. 2009). In four participants, the mean SOL $H_{DF}$ value for baseline session 3 was identified as an outlier (> 2 SD of mean of baseline sessions 1 and 2) and was removed from analysis.

Change was determined by subtracting the pretest from the posttest measure. One-tailed Mann-Whitney U-tests were used to compare magnitude of change, between TA↑ and SOL↓ groups for each outcome measure. Within each group, some outcome measures exhibited change that appeared to be clinically relevant; therefore, for each group separately, we analyzed change in outcome measures using one-tailed Wilcoxon signed ranks tests, calculated SRM (Liang et al. 1990) for each outcome, and explored relationships among outcome changes with one-tailed Spearman correlation coefficients.

RESULTS

Pre-intervention characteristics

The TA↑ and SOL↓ groups were similar in pre-intervention demographic, clinical, and neurophysiologic measures. However, for clinical measures, the TA↑ group had greater DF active ROM (96.9° vs. 112.4°, p = 0.04) and non-training leg strength (22.5 vs. 19.2 LEMS, p = 0.04) (Table 1). Stratification of participants by toe tap score resulted in equal distribution of participants between the two groups based on level of ankle motor control; each group had four individuals with low control (<15 taps) and two with high control (≥ 15 taps).

Change in training measures and effect size (standardized response mean)
Training dosage (repetitions) was similar between TA↑ and SOL↓ groups (3100 vs. 2740, p = 0.21). For TA↑, TA%MVC amplitude increased between baseline (43% ± 17) and final training sessions (91% ± 18, p = 0.01) (Table 2) and a large training effect was observed (SRM = 5.19). For SOL↓, the conditioned SOL H_{DF} % resulted in a decrease from baseline that approached statistical significance (-16.17 % ± 26.22, p = 0.09) (Table 2) and a moderate training effect was observed (SRM = -0.62). SOL H_{DF}-reflex responses during training sessions 2 and 11 for an example participant are illustrated in Figure 4.

Change in clinical measures and effect size (standardized response mean)

Change in PF reflex threshold angle was the only clinical measure for which there was a between-groups difference that approached statistical significance (Table 2). This angle decreased toward more dorsiflexion for TA↑ (-4.33°) and did not change significantly for SOL↓ (+3.15°) (Table 2). Within-group analysis of data from the TA↑ group identified five clinical measures that achieved statistical significance and had a large effect size. These outcomes were: decreased PF reflex threshold angle (-4.33°) and DF active ROM angle (-4.32°), and increased dorsiflexion LEMS (+0.8 points), walking foot clearance (+4.8 mm), and walking distance (+12.09 m) (Table 2). In addition, an increase in LEMS for training leg (+2.2 points) and non-training leg (+0.8 points) approached statistical significance and demonstrated a large effect size (Table 2). Within-group analysis of data from the SOL↓ group identified one clinical measure, increased walking distance (+6.25 m), achieved statistical significance and had a large effect size (Table 2). In addition, two clinical measures approached statistical significance: increased non-training leg LEMS (+1.8 points), which demonstrated a large effect size, and increased walking speed (+0.02 m/s), which demonstrated a moderate effect size (Table 2). A power analysis of
the training and clinical outcomes that approached statistical significance revealed that our study was considerably underpowered to detect change in the following measures: SOL HDF% (31%), SOL and TA non-training LEMS (33%), TA non-training LEMS (20%), SOL walking speed (14%), and between-groups difference for PF reflex threshold angle (34%).

**Change in neurophysiologic measures and effect size (standardized response mean)**

The only neurophysiologic measure in which we observed a significant change was SOL/TA clonus coactivation ratio, which decreased (-0.21) for the SOL↓ group and exhibited a moderate effect size (Table 2).

**Relationship among training, clinical, and neurophysiologic outcomes**

**TA↑**

We had hypothesized TA↑, which was intended to activate SOL RI and PI pathways, would be associated with increased DF active ROM and strength, walking foot clearance, speed, and distance. We did not find a significant change in SOL RI, PI, or walking speed. However, increased walking distance was moderately correlated with increased DF (r = 0.66, p = 0.08), and training leg strength (r = 0.62, p = 0.10) (Figure 5).

**SOL↓**

We had hypothesized SOL↓ would be associated with increased SOL H-reflex LFD and decreased SOL/TA clonus coactivation that would be related to decreased clonus duration, decreased PF reflex threshold angle, and enhanced toe tap count. We did not find significant change in SOL LFD, clonus duration, PF reflex threshold angle, or toe tap count. However, decreased SOL/TA clonus coactivation ratio (-0.21, p = 0.02) was moderately correlated with increased walking speed and distance (both r = -0.60, p = 0.10) (Figure 6).
DISCUSSION

We investigated whether operant conditioning to either increase voluntary TA activation or decrease SOL H-reflexes improved ankle clonus, motor control, or walking function in individuals with AIS D SCI. We also explored relationships among change in training, clinical, and neurophysiologic outcomes. Our hypotheses that TA↑ would increase DF active ROM and strength, walking foot clearance, and walking distance were supported; however, improvement in walking speed was not attained. In addition, an unexpected outcome of decreased PF reflex threshold angle was observed. For SOL↓ our hypothesis of decreased SOL/TA coactivation was supported; however, decreased clonus duration and PF reflex threshold angle, and increased toe tap count were not observed. Unexpectedly, SOL↓ was associated with increased walking distance. Only one measure, PF reflex threshold angle, differed substantially between the two groups. Our findings provide evidence that both TA↑ and SOL↓ were associated with significant improvement in distance walked during a 2-minute period. Each training protocol may have modulated clinical and neurologic variables in unique ways.

Change in Training Measures

TA↑ was associated with an increase in TA contraction strength (%MVC amplitude) of approximately 48% (Figure 5), a finding consistent with prior reports of increased voluntary EMG responses after such training (Brucker and Bulaeva 1996; Seymour and Bassler 1977). SOL↓ was associated with a decrease in SOL H-reflex excitability (conditioned SOL H_{DF} %) of about 16% that approached statistical significance (Figure 6). This result concurs with Thompson et al. (2008) who reported a 10 – 15% reduction after down-conditioning of SOL H-reflex in standing for three individuals with SCI. Our mean training dosage was approximately
3000 repetitions (300 X 12 training sessions) for TA↑ and SOL↓ and was less than the dosage of 5400 repetitions used in other studies (Birkenmeier et al. 2010; Thompson et al. 2009). Our results are similar to Thompson et al. (2009) who used 225 repetitions per session and reported a significant decrease in the conditioned SOL H-reflex within sessions 7 – 12 that continued to decrease through session 24; change the investigators attributed to early phase task-dependent adaptation. Thompson et al. (2009) also identified a significant change in the control SOL H-reflex within sessions 19 – 24 that was attributed to a long-term change in spinal cord plasticity.

In our participants with spinal cord injury, we observed a decrease in conditioned SOL H-reflex that approached significance after 12 sessions; an additional six sessions (total of 5400 repetitions) may have improved the effectiveness of SOL↓ training. Further study is needed to assess the most effective dosage-response parameters.

**Effects of TA↑ and SOL↓ on Clinical Measures of Ankle Motor Control**

Change in PF reflex threshold angle was the only outcome that differed substantially between the two groups. TA↑ exhibited a decreased angle (i.e., towards more dorsiflexion, indicating a more lengthened plantar flexor position at which stretch of the soleus evoked a reflex contraction), signifying decreased responsiveness to muscle stretch and therefore decreased spasticity (Ness and Field-Fote 2009). TA↑ was effective in enhancing TA activation, as exhibited by increased DF active ROM and strength. In addition, increased DF strength was associated with increased walking distance (Figure 5). Functional magnetic resonance imaging studies suggest that 10° of voluntary ankle dorsiflexion is a useful measure of motor control of walking that in depends, in part, upon increased TA activation (Dobkin et al. 2004).

In non-disabled individuals, PI of SOL motor neurons increases 60 – 80 ms after onset of voluntary TA EMG activation, and the extent of inhibition is proportional to TA contraction.
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strength (Meunier and Morin 1989). Our findings for TA↑ of increased DF active ROM and
strength are congruent with a supraspinally-mediated enhancement of SOL PI. However, our
findings of no change in PI are in conflict with a mechanism of enhanced SOL PI. A possible
explanation is that SOL PI was tested at rest, which may not reflect modulation of the PI
pathway during active dorsiflexion.

TA↑ was associated with an increase in both training leg and non-training leg strength
that approached statistical significance (Figure 5), while SOL↓ was associated with an increase
in non-training leg strength that approached statistical significance (Figure 6). The finding of
increased strength in the non-training leg for both groups was unexpected. For TA↑ increased
DF strength and training leg strength were correlated with increased walking distance (Figure 5).
We surmise that TA↑ improved the ability to isolate and activate dorsiflexors in the weaker
training leg, which likely contributed to the ability to walk farther distances. Participants were
not restricted from walking outside of training sessions and reported the ability to walk farther
distances during their daily activities, which likely provided an opportunity for re-conditioning
of the non-training leg. Our finding of increased non-training leg strength is also congruent with
studies of contralateral strength training, wherein increased motor neuron output, increased
strength, and decreased H-reflex gain in the untrained homonymous muscle have been reported
(Dragert and Zehr 2012, Carroll et al 2006, Hortobagyi 2005). Wolf et al 1995 reported a
contralateral effect after down-training of biceps brachii stretch reflexes in human subjects
wherein contralateral responses were reduced to 80% of baseline. The authors suggested that
both intra-spinal mechanisms and bilateral descending systems may contribute to this
contralateral training effect.
Effects of TA↑ and SOL↓ on Walking Function

Both TA↑ and SOL↓ were associated with increased walking distances (Table 2) that exceeded the minimally important difference of 4 meters reported for the 2-minute walk test in individuals with motor-incomplete SCI (Field-Fote and Roach 2011). The cumulative effects of TA↑ (decreased PF reflex threshold angle and increased DF active ROM, DF and leg strength, foot clearance and walking distance) (Figure 5) are consistent with a mechanism of increased corticospinal activation. Upon initiation of voluntary DF, the corticospinal tract activates TA motor neurons in parallel with activation of interneurons mediating PI and RI of SOL motor neurons (Iles and Pisini 1992). Our findings are consistent with studies of locomotor training effects in chronic motor-incomplete SCI that report increased voluntary activation of TA and slope of TMS-elicited TA motor evoked potential recruitment curves; findings that provide evidence of a training-induced increase in corticospinal excitability (Thomas and Gorassini 2005).

For SOL↓, decreased SOL/TA coactivation was associated with increased walking distance and increased walking speed that approached statistical significance (Figure 6). However, the increase in speed did not exceed the minimally important difference of 0.05 m/s reported for individuals with SCI (Musselman 2007). The SOL↓ effects are consistent with improved SOL reflex modulation during walking. Both excitatory and inhibitory Ib interneuron pathways to ankle extensors are easily activated by the corticospinal tract during voluntary movement (Jankowska 1992), and appear to regulate stance-to-swing phase transition during locomotion (Pearson et al. 1992). The cumulative effects of SOL↓ may be attributable to a Ib neuroplastic effect of SOL↓ that may have contributed to improved walking function (Figure 6).
Effects of TA↑ and SOL↓ on Neurophysiologic Measures

The only substantial neurophysiologic effect, exhibited by SOL↓, was decreased SOL/TA clonus coactivation from 52% to 31% that was associated with the ability to walk faster and farther (Figure 6). These findings may suggest that reduced SOL/TA coactivation during clonus was associated with improved SOL↓ walking outcomes.

Prior studies have described adverse antagonist coactivation and impaired SOL RI and PI after SCI (Boorman et al. 1996; Crone and Nielsen, 1994; Crone et al. 2003; Dietz 2001; Faist et al. 1994; Morita et al. 2001; Xia and Rymer 2005). We observed decreased SOL/TA coactivation after SOL↓ training; however, no significant changes in SOL RI or PI were identified. In individuals with PF hyperexcitability, Crone et al. (2003) reported reciprocal facilitation of plantar flexors after CPN stimulation (as opposed to reciprocal inhibition) and proposed that activation of disynaptic Ib pathways may contribute to adverse antagonist cocontraction. Our finding of a predominant pattern of SOL/TA coactivation during clonus is consistent with Xia and Rymer (2005) who reported altered agonist and antagonist activation after SCI as a consequence of reciprocal facilitation of TA during SOL tendon taps, as well as response latencies that are consistent with oligosynaptic short-latency pathways. Citing animal studies, these investigators proposed Ib afferent excitation as a possible source of reciprocal facilitation after SCI (Xia and Rymer 2005).

Recently it has been postulated that increased facilitation or suppressed inhibition of the antagonist muscle following perturbation of the agonist involves Ib force-sensitive pathways (Lewis et al. 2010). Yanagawa et al. (1991) observed that weak TA contractions increased Ib inhibition of SOL H-reflexes and concluded that Ib inhibition, which arrived prior to spinal-level TA-induced inhibitory effects on SOL Ia reflex, was corticospinally activated. We surmise that
decreased coactivation of SOL and TA during clonus following SOL↓ (which employed weak TA contractions) may be attributable to enhanced Ib inhibitory mechanisms.

**Conclusion and Functional Implications**

Two operant conditioning training programs, one to increase voluntary dorsiflexor motor control and the other to decrease plantar flexor stretch reflex excitability, were both associated with improved walking function in individuals with chronic motor-incomplete spinal cord injury. TA↑ decreased plantar flexor spasticity, increased ankle motor control and was associated with increased walking foot clearance and walking distance. Step initiation is mediated by supraspinal input to spinal interneurons that activate stepping (Gerasimenko et al. 2002) and the corticospinal tract is more closely linked with TA and leg flexor spinal motor circuits (Capaday et al. 1999; Schubert et al. 1997). Intensive, repetitive TA EMG activation during TA↑ may have unmasked dormant corticospinal pathways that preferentially increased recruitment of TA and leg flexor motor neurons, step initiation and walking function.

SOL↓ was associated with decreased SOL/TA coactivation during clonus and increased walking distance. Antagonist coactivation modulation has been attributed to Ib input to spinal interneurons (Feldman 1993, Morita et al. 2006, Yanagawa et al. 1991). It is possible that intensive training to inhibit SOL H-reflexes during weak voluntary TA contractions enhanced corticospinal activation of SOL Ib interneurons, and the combined effects of decreased SOL/TA clonus coactivation and increased SOL stretch reflex inhibition improved ankle motor control and walking function.

Our study was considerably underpowered to detect change in SOL HDF%, TA training LEMS, TA and SOL non-training LEMS, and SOL walking speed. However, change in these measures did approach statistical significance, and since the minimal clinically important
difference (MCID) for most of these measures is not known, it is possible that the changes observed may be clinically relevant. The MCID for walking speed is 0.05 m/s, the mean change of 0.4 m/s in the TA↑ approached this value. There were subjects in both groups that met the criteria for clinically meaningful improvement in walking speed. We included these outcomes in our discussion and interpretation of results to elucidate clinically relevant relationships among change in training and clinical measures.

LIMITATIONS

Our two-group pre-test post-test randomized design allowed us to compare two different interventions. Our study design lacked a no-treatment control group, which limits interpretation of whether either training approach was more beneficial than no intervention (Portney and Watkins 2000). However, given the chronicity of SCI in our participants, little change in motor function and reflex activity would be expected in the absence of an intervention. We did not monitor or control for background SOL EMG during SOL↓ training, therefore the effects of SOL EMG activity on modulation of the SOL H-reflex are not known, which may have limited our ability to fully assess the extent of inhibition. Additionally, the neurophysiologic tests for SOL RI, PI, and LFD were performed at rest. Performing these tests during dorsiflexion may have better elucidated modulation of these pathways during movement. Our conclusions are restricted to outcomes observed in a small sample of individuals with AIS D SCI. Further research is warranted to corroborate effects of TA↑ and SOL↓ in AIS D SCI and generalizability to individuals with other similar upper motor neuron pathology and functional limitations.

ACKNOWLEDGEMENTS

The authors thank Simon Gray for software design, Ian Hentall, PhD, for footswitch design, Meggie Safford, DPT, and Rachel Cowen, PhD, for data processing assistance, the
support of the Miami Project to Cure Paralysis, the contributions of Christine K. Thomas, PhD, and T. George Hornby, PT, PhD to manuscript development, and the time and effort volunteered by our research participants with spinal cord injury.
REFERENCES


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TA EMG and SOL H-reflex Training Improves Function in SCI

FIGURE LEGEND

Figure 1. Methodological design of study. RI, reciprocal inhibition; PI, presynaptic inhibition; LFD, low frequency depression; SOL/TA, soleus/tibialis anterior; PF, plantar flexor; DF, dorsiflexion; ROM, range of motion; LE, lower extremity; MVC, maximum voluntary contraction.

Figure 2. Schematic of ankle clonus drop test set-up and kinematic, kinetic, and EMG output. PF, plantar flexor; SOL, soleus; MG, medial gastrocnemius; TA, tibialis anterior.

Figure 3. Feedback displays and change in performance measures. A. TA↑ training display: left screen, TA EMG feedback and reward; right screen, TA%MVC amplitude target and response. B. SOL↓ training display: left screen SOL H-reflex stimulus, M-wave, and HDF-reflex; right screen, SOL HDF-reflex amplitude target and response. C. TA↑ graph of change in mean TA %MVC amplitude, (*) p = 0.02. D. SOL↓ graph of change in conditioned SOL HDF reflex (% baseline), (+) p = 0.09. Week 1= Baseline, Week 5 = last week of training. (Wilcoxon signed ranks one-tailed tests)

Figure 4. Example of effect of SOL↓ training on SOL HDF –reflex amplitude responses in one participant. A. Training session 2. B. Training session 11. Black line, SOL HDF –reflex responses; Grey line, M-wave responses evoked at 10-30% Mmax stimulus intensity. Baseline bout, 30 repetitions without feedback; Training bouts, 30 repetitions with feedback with 1-minute rests between bouts.

Figure 5. Effects of TA (↑) training. Change in outcome measures and change score correlations. Solid single headed arrow, p ≤ 0.05, change achieved significance; Dashed single headed arrow, 0.05 < p ≤ 0.10, change approached statistical significance; Dashed double headed arrow, moderate correlation, 0.5 ≤ r < 0.75, 0.05 < p ≤ 0.10; Spearman correlation coefficients, one-tailed test.

Figure 6. Effects of SOL (↓) training. Change in outcome measures and change score correlations. Solid single headed arrow, p ≤ 0.05, change achieved statistical significance; Dashed single headed arrow, 0.05 < p ≤ 0.10, change approached statistical significance; Dashed double headed arrow, moderate correlation, 0.5 ≤ r < 0.75, 0.05 < p ≤ 0.10; Spearman correlation coefficients, one-tailed test.
### Appendix 1. List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS</td>
<td>ASIA Impairment Scale</td>
</tr>
<tr>
<td>ASIA</td>
<td>American Spinal Injury Association</td>
</tr>
<tr>
<td>CPN</td>
<td>common peroneal nerve</td>
</tr>
<tr>
<td>DF</td>
<td>dorsiflexion</td>
</tr>
<tr>
<td>DFAROM</td>
<td>dorsiflexion active range of motion</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyography</td>
</tr>
<tr>
<td>FN</td>
<td>femoral nerve</td>
</tr>
<tr>
<td>H/M</td>
<td>H-reflex/M-wave ratio</td>
</tr>
<tr>
<td>H$_{DF}$</td>
<td>H-reflex during dorsiflexion</td>
</tr>
<tr>
<td>H$<em>{max</em>{DF}}$</td>
<td>H-reflex maximum amplitude during dorsiflexion</td>
</tr>
<tr>
<td>H$<em>{max</em>{rest}}$</td>
<td>H-reflex maximum amplitude at rest</td>
</tr>
<tr>
<td>ISI</td>
<td>inter-stimulus interval</td>
</tr>
<tr>
<td>LEMS</td>
<td>lower extremity motor score</td>
</tr>
<tr>
<td>LFD</td>
<td>low frequency depression</td>
</tr>
<tr>
<td>M$<em>{max</em>{DF}}$</td>
<td>M-wave maximum amplitude during dorsiflexion</td>
</tr>
<tr>
<td>M$<em>{max</em>{rest}}$</td>
<td>M-wave maximum amplitude at rest</td>
</tr>
<tr>
<td>MVC</td>
<td>maximum voluntary contraction</td>
</tr>
<tr>
<td>PF</td>
<td>plantar flexion</td>
</tr>
<tr>
<td>PFRTA</td>
<td>plantar flexion reflex threshold angle</td>
</tr>
<tr>
<td>PI</td>
<td>presynaptic inhibition</td>
</tr>
<tr>
<td>PTN</td>
<td>posterior tibial nerve</td>
</tr>
<tr>
<td>RI</td>
<td>reciprocal inhibition</td>
</tr>
</tbody>
</table>
TA EMG and SOL H-reflex Training Improves Function in SCI

816 ROM – range of motion
817 SCI – spinal cord injury
818 SOL - soleus
819 SRM – standardized response mean
820 TA – tibialis anterior
821 TTL – Transistor-Transistor Logic
TA EMG and SOL H-reflex Training Improves Function in SCI

Appendix 2A. Pre-intervention relationships between neurophysiologic measures and clinical and walking measures.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Clonus duration</th>
<th>Plantar flexor Reflex Threshold Angle</th>
<th>Toe Tap Test</th>
<th>Dorsiflexion active ROM</th>
<th>Dorsiflexion strength</th>
<th>Plantar flexion strength</th>
<th>Training leg strength</th>
<th>Non-training leg strength</th>
<th>Foot Clearance</th>
<th>Distance</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI (%)</td>
<td>0.035 0.457</td>
<td>-0.280 0.189</td>
<td>-0.028 0.465</td>
<td>0.077 0.406</td>
<td>-0.085 0.396</td>
<td>0.486 0.054</td>
<td>0.169 0.299</td>
<td>0.139 0.333</td>
<td>-0.042 0.448</td>
<td>0.217</td>
<td>0.105</td>
</tr>
<tr>
<td>PI (%)</td>
<td>-0.287 0.183</td>
<td>0.014 0.483</td>
<td>0.392 0.104</td>
<td>-0.294 0.177</td>
<td>0.074 0.410</td>
<td>0.247 0.219</td>
<td>0.261 0.206</td>
<td>0.275 0.193</td>
<td>-0.294 0.448</td>
<td>0.448</td>
<td>0.573</td>
</tr>
<tr>
<td>LFD (%)</td>
<td>0.350 0.133</td>
<td>-0.343 0.138</td>
<td>-0.018 0.478</td>
<td>-0.049 0.440</td>
<td>-0.052 0.436</td>
<td>-0.020 0.475</td>
<td>-0.141 0.331</td>
<td>-0.018 0.478</td>
<td>0.140 0.161</td>
<td>0.140</td>
<td>0.077</td>
</tr>
<tr>
<td>SOL/TA Ratio</td>
<td>0.112 0.365</td>
<td>0.133 0.340</td>
<td>0.042 0.448</td>
<td>0.524 0.040</td>
<td>-0.333 0.145</td>
<td>0.056 0.432</td>
<td>-0.533 0.037</td>
<td>-0.543 0.034</td>
<td>-0.364 0.140</td>
<td>-0.140</td>
<td>-0.091</td>
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</table>

Appendix 2B. Pre-intervention relationships between gait and clinical measures.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Clonus duration</th>
<th>Plantar flexor Reflex Threshold Angle</th>
<th>Toe Tap Test</th>
<th>Dorsiflexion active ROM</th>
<th>Dorsiflexion strength</th>
<th>Plantar flexion strength</th>
<th>Training leg strength</th>
<th>Non-training leg strength</th>
<th>Foot Clearance</th>
<th>Distance</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI (%)</td>
<td>-0.259 0.208</td>
<td>-0.580 0.024</td>
<td>-0.409 0.093</td>
<td>-0.154 0.317</td>
<td>-0.026 0.468</td>
<td>-0.187 0.280</td>
<td>0.014 0.483</td>
<td>0.079 0.404</td>
<td>-0.294 0.177</td>
<td>0.177</td>
<td>0.177</td>
</tr>
<tr>
<td>PI (%)</td>
<td>-0.538 0.035</td>
<td>-0.091 0.389</td>
<td>0.166 0.303</td>
<td>-0.594 0.021</td>
<td>0.100 0.379</td>
<td>0.614 0.017</td>
<td>0.476 0.059</td>
<td>0.718 0.004</td>
<td>-0.294 0.177</td>
<td>0.177</td>
<td>0.177</td>
</tr>
<tr>
<td>Distance</td>
<td>-0.294 0.177</td>
<td>0.021 0.474</td>
<td>0.374 0.116</td>
<td>-0.524 0.040</td>
<td>0.344 0.137</td>
<td>0.702 0.005</td>
<td>0.646 0.012</td>
<td>0.711 0.005</td>
<td>-0.294 0.177</td>
<td>0.177</td>
<td>0.177</td>
</tr>
</tbody>
</table>

Spearman correlation coefficients (top number) and p-value (bottom number) within each cell; RI = reciprocal inhibition; PI = presynaptic inhibition; LFD = low frequency depression; SOL/TA = Soleus/Tibialis Anterior; ROM = range of motion.
## Appendix 3A. Relationship between changes in neurophysiologic and clinical, gait and performance measures for TA↑ group

<table>
<thead>
<tr>
<th>Measure</th>
<th>↓ Clonus duration</th>
<th>↓ Plantar flexor reflex threshold angle</th>
<th>↑ Toe Tap Test</th>
<th>↓ Dorsiflexion active ROM</th>
<th>↑ Dorsiflexion strength</th>
<th>↑ Plantar flexion strength</th>
<th>↑ Training leg strength</th>
<th>↑ Non-training leg strength</th>
<th>↑ Foot Clearance</th>
<th>↑ Distance</th>
<th>↑ Speed</th>
<th>↑ %MVC Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ RI (%)</td>
<td>-0.086</td>
<td>0.657</td>
<td>0.100</td>
<td>-0.393</td>
<td>-0.525</td>
<td>-0.883</td>
<td>-0.278</td>
<td>0.714</td>
<td>-0.429</td>
<td>-0.257</td>
<td>-0.486</td>
<td>0.164</td>
</tr>
<tr>
<td>↑ PI (%)</td>
<td>-0.086</td>
<td>0.436</td>
<td>0.129</td>
<td>-0.393</td>
<td>-0.216</td>
<td>0.265</td>
<td>0.802</td>
<td>-0.371</td>
<td>-0.486</td>
<td>0.429</td>
<td>0.657</td>
<td>0.078</td>
</tr>
<tr>
<td>↓ LFD (%)</td>
<td>-0.600</td>
<td>0.436</td>
<td>0.392</td>
<td>-0.655</td>
<td>-0.463</td>
<td>-0.618</td>
<td>0.309</td>
<td>-0.200</td>
<td>-0.714</td>
<td>0.600</td>
<td>0.086</td>
<td>0.436</td>
</tr>
<tr>
<td>↓ SOL/TA Ratio</td>
<td>-0.029</td>
<td>0.257</td>
<td>0.143</td>
<td>0.655</td>
<td>0.309</td>
<td>0.265</td>
<td>-0.772</td>
<td>-0.314</td>
<td>0.771</td>
<td>-0.200</td>
<td>-0.771</td>
<td>-0.036</td>
</tr>
</tbody>
</table>

Arrows indicate direction of change; Spearman correlation coefficients (top number) and p-value (bottom number) within each cell; RI = reciprocal inhibition; PI = presynaptic inhibition; LFD = low frequency depression; SOL/TA = Soleus/Tibialis Anterior; ROM = range of motion. Yellow = measures related to hypotheses; Blue: direction of correlation differed from results unclear relationship; Grey = moderate or strong correlations not related to original hypotheses.

## Appendix 3B. Relationship between changes in gait and clinical and performance measures for TA↑ group

<table>
<thead>
<tr>
<th>Measure</th>
<th>↓ Clonus duration</th>
<th>↓ Plantar flexor reflex threshold angle</th>
<th>↑ Toe Tap Test</th>
<th>↓ Dorsiflexion active ROM</th>
<th>↑ Dorsiflexion strength</th>
<th>↑ Plantar flexion strength</th>
<th>↑ Training leg strength</th>
<th>↑ Non-training leg strength</th>
<th>↑ Foot Clearance</th>
<th>↑ Distance</th>
<th>↑ Speed</th>
<th>↑ %MVC Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Foot Clearance</td>
<td>0.257</td>
<td>0.314</td>
<td>0.058</td>
<td>0.714</td>
<td>-0.131</td>
<td>-0.432</td>
<td>-0.441</td>
<td>-0.093</td>
<td>-0.093</td>
<td>0.029</td>
<td>0.479</td>
<td>0.046</td>
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<tr>
<td>↑ Speed</td>
<td>0.143</td>
<td>0.394</td>
<td>-0.486</td>
<td>0.290</td>
<td>-0.543</td>
<td>-0.655</td>
<td>-0.926</td>
<td>-0.265</td>
<td>0.463</td>
<td>0.178</td>
<td>-0.200</td>
<td>0.352</td>
</tr>
<tr>
<td>↑ Distance</td>
<td>0.314</td>
<td>0.272</td>
<td>0.143</td>
<td>0.275</td>
<td>0.655</td>
<td>0.617</td>
<td>0.618</td>
<td>0.463</td>
<td>0.178</td>
<td>0.352</td>
<td>-0.314</td>
<td>0.272</td>
</tr>
<tr>
<td>↑ %MVC Amplitude</td>
<td>0.143</td>
<td>0.394</td>
<td>-0.486</td>
<td>-0.086</td>
<td>-0.131</td>
<td>-0.185</td>
<td>0.353</td>
<td>0.679</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
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</table>
### Appendix 4A. Relationship between changes in neurophysiologic and clinical, gait and performance measures for SOL↓ group

<table>
<thead>
<tr>
<th>Measure</th>
<th>↓ Clonus duration</th>
<th>↑ Plantar flexor reflex threshold angle</th>
<th>↑ Toe Tap Test</th>
<th>↑ Dorsiflexion active ROM angle</th>
<th>↓ Dorsiflexion strength</th>
<th>↑ Plantar flexion strength</th>
<th>↑ Training leg strength</th>
<th>↑ Non-training leg strength</th>
<th>↑ Foot Clearance</th>
<th>↑ Distance</th>
<th>↑ Speed</th>
<th>↓ HDF % of baseline</th>
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</thead>
<tbody>
<tr>
<td>↑ RI (%)</td>
<td>-0.314 0.272</td>
<td>-0.714 0.055</td>
<td>0.290 0.289</td>
<td>-0.086 0.436</td>
<td>-0.169 0.354</td>
<td>0.372 0.234</td>
<td>0.232 0.329</td>
<td>0.464 0.177</td>
<td>-0.754 0.042</td>
<td>-0.200</td>
<td>-0.143 0.394</td>
<td>-0.029 0.479</td>
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<tr>
<td>↓ PI (%)</td>
<td>0.086 0.436</td>
<td>-0.314 0.272</td>
<td>0.754 0.042</td>
<td>-0.143 0.394</td>
<td>0.304 0.279</td>
<td>-0.270 0.100</td>
<td>0.609 0.042</td>
<td>0.174 0.371</td>
<td>-0.116 0.413</td>
<td>0.029</td>
<td>-0.143 0.394</td>
<td>0.394 0.394</td>
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<tr>
<td>↑ LFD (%)</td>
<td>0.667 0.074</td>
<td>0.203 0.350</td>
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<td>-0.051 0.461</td>
<td>0.206 0.348</td>
<td>0.162 0.380</td>
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<td>-0.232</td>
<td>-0.232 0.551</td>
<td>0.394 0.394</td>
</tr>
<tr>
<td>↓ SOL/TA Ratio</td>
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<td>0.714 0.478</td>
<td>0.055 0.017</td>
<td>-0.845 0.034</td>
<td>0.778 0.114</td>
<td>-0.580 0.177</td>
<td>0.464 0.231</td>
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<td>-0.600</td>
<td>-0.600 0.371</td>
<td>0.129 0.129</td>
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</table>

### Appendix 4B. Relationship between changes in gait and clinical and performance measures for SOL↓ group

<table>
<thead>
<tr>
<th>Measure</th>
<th>↓ Clonus duration</th>
<th>↑ Plantar flexor reflex threshold angle</th>
<th>↑ Toe Tap Test</th>
<th>↑ Dorsiflexion active ROM angle</th>
<th>↓ Dorsiflexion strength</th>
<th>↑ Plantar flexion strength</th>
<th>↑ Training leg strength</th>
<th>↑ Non-training leg strength</th>
<th>↑ Foot Clearance</th>
<th>↑ Distance</th>
<th>↑ Speed</th>
<th>↓ HDF % of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Foot Clearance</td>
<td>0.116 0.413</td>
<td>0.638 0.087</td>
<td>-0.059 0.456</td>
<td>-0.058 0.457</td>
<td>-0.017 0.487</td>
<td>-0.189 0.360</td>
<td>-0.471 0.173</td>
<td>-0.426 0.200</td>
<td>-0.928 0.004</td>
<td>0.257</td>
<td>0.311 0.0311</td>
<td>0.086 0.436</td>
</tr>
<tr>
<td>↑ Distance</td>
<td>-0.771 0.036</td>
<td>-0.257 0.311</td>
<td>-0.319 0.269</td>
<td>-0.886 0.009</td>
<td>0.778 0.034</td>
<td>-0.372 0.234</td>
<td>0.638 0.087</td>
<td>-0.116 0.413</td>
<td>-0.812 0.025</td>
<td>0.086</td>
<td>0.436 0.086</td>
<td>1.000 1.000</td>
</tr>
<tr>
<td>↑ Speed</td>
<td>-0.771 0.036</td>
<td>-0.429 0.198</td>
<td>0.029 0.478</td>
<td>-0.543 0.133</td>
<td>0.372 0.234</td>
<td>-0.788 0.034</td>
<td>0.290 0.289</td>
<td>-0.812 0.025</td>
<td>0.004 0.449</td>
<td>0.231</td>
<td>0.329 0.329</td>
<td>0.000 0.000</td>
</tr>
<tr>
<td>↓ HDF % of baseline</td>
<td>-0.029 0.479</td>
<td>-0.543 0.133</td>
<td>-0.058 0.457</td>
<td>-0.200 0.352</td>
<td>-0.034 0.475</td>
<td>0.068 0.449</td>
<td>0.377 0.231</td>
<td>0.232 0.329</td>
<td>1.000 1.000</td>
<td>0.231</td>
<td>0.329 0.329</td>
<td>0.000 0.000</td>
</tr>
</tbody>
</table>

Arrows indicate direction of change; Spearman correlation coefficients (top number) and p-value (bottom number) within each cell; RI, reciprocal inhibition; PI, presynaptic inhibition; LFD, low frequency depression; SOL/TA, Soleus/Tibialis Anterior; ROM, range of motion; HDF, SOL H-reflex during dorsiflexion
Participants (n=12)

Pre-training Tests

Clonus Drop Test
- Clonus duration
- PF reflex threshold angle
- SOL/TA Coactivation

Ankle Motor Control
- 10-s Toe Tap Test
- DF Active ROM

LE Motor Scores
- Dorsiflexors
- Plantar flexors
- Training leg
- Non-training leg

Walking
- 6-m walk
- Foot Clearance
- Speed
- 2-min Distance

SOL H-Reflex
- RI
- PI
- LFD

Randomization

Toe Tap Score

< 15
- Training Parameter
  - Increase % TA MVC Amplitude

≥ 15
- Training Parameter
  - Decrease SOL H-reflex amplitude during active dorsiflexion

15 Sessions
- 30 repetitions/bout
- 1 minute rest between bouts
- 10 bouts
- 300 total repetitions/session
- 2-hr, 3X/week

3 Baseline Sessions
- Dosage: 900 repetitions

12 Training Sessions
- Dosage: 3600 repetitions

Post-Training Tests
A. TA↑

- **Reward**: [Graph showing EMG activity with Footswitch]
- **%MVC Amp (RMS)**: [Graph showing %MVC amplitude response]
- **%MVC target**: [Graph showing %MVC target]

B. SOL↓

- **Stimulus**: [Graph showing M-wave and H-reflex]
- **H<sub>Df</sub>-reflex target (mV)**: [Graph showing H<sub>Df</sub>-reflex response]
- **H<sub>Df</sub>-reflex response**: [Graph showing Conditioned SOL H<sub>Df</sub> reflex (% baseline)]

C. TA↑

- **TA % MVC Amplitude**: [Graph showing TA % MVC amplitude over weeks]

D. SOL↓

- **Conditioned SOL H<sub>Df</sub> reflex (%) baseline**: [Graph showing Conditioned SOL H<sub>Df</sub> reflex (% baseline) over weeks]
A. Training Session 2

![Graph showing amplitude (mV) vs repetition for Training Session 2 with baseline and training bouts marked.]

B. Training Session 11

![Graph showing amplitude (mV) vs repetition for Training Session 11 with baseline and training bouts marked.]

Conditioned SOL H_{DF} reflex

Target Stimulus

↓ Conditioned SOL H_{DF} reflex

↓ Clonus
↓ SOL/TA Coactivation

↑ Walking Function

↑ Speed
↑ Distance

↑ Strength (LEMS)
↑ Non-training leg

SOL ↓

↑ Walking Function

↑ Speed
↑ Distance

SOL ↓

↑ Non-training leg

SOL/TD Coactivation
Table 1. Analysis of differences in participant baseline characteristics and pre-intervention outcome measures (mean ± SD) between TA↑ and SOL↓ groups

<table>
<thead>
<tr>
<th>Characteristics:</th>
<th>TA↑</th>
<th>SOL↓</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>44.2 ± 12.0</td>
<td>45.2 ± 12.8</td>
<td>1.00</td>
</tr>
<tr>
<td>Post-injury (yrs)</td>
<td>10.8 ± 10.0</td>
<td>10.8 ± 08.8</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>6 men</td>
<td>4 men, 2 women</td>
<td>0.12 (Χ²)</td>
</tr>
<tr>
<td>Injury Level</td>
<td>C7 (median)</td>
<td>C5 (median)</td>
<td>0.39 (Χ²)</td>
</tr>
<tr>
<td>AIS</td>
<td>D (all)</td>
<td>D (all)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Measures:</th>
<th>TA↑</th>
<th>SOL↓</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonus Duration (sec)</td>
<td>11.3 ± 14.5</td>
<td>12.4 ± 10.2</td>
<td>0.75</td>
</tr>
<tr>
<td>PFRTA (degrees)</td>
<td>83.7 ± 5.5</td>
<td>85.2 ± 7.2</td>
<td>0.75</td>
</tr>
<tr>
<td>Toe Tap Test Score</td>
<td>13.5 ± 10.9</td>
<td>11.5 ± 6.3</td>
<td>0.75</td>
</tr>
<tr>
<td>DF AROM (degrees)</td>
<td>96.9 ± 13.7</td>
<td>112.4 ± 11.8</td>
<td>0.05 *</td>
</tr>
<tr>
<td>DF LEMS</td>
<td>3.5 ± 1.1</td>
<td>3.8 ± 0.8</td>
<td>0.55</td>
</tr>
<tr>
<td>PF LEMS</td>
<td>2.5 ± 0.8</td>
<td>2.5 ± 0.6</td>
<td>0.78</td>
</tr>
<tr>
<td>Training leg LEMS</td>
<td>17.3 ± 3.4</td>
<td>16.2 ± 3.2</td>
<td>0.42</td>
</tr>
<tr>
<td>Non-training leg LEMS</td>
<td>22.5 ± 2.0</td>
<td>19.2 ± 2.8</td>
<td>0.04 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Walking Measures:</th>
<th>TA↑</th>
<th>SOL↓</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot Clearance (mm)</td>
<td>5.0 ± 2.0</td>
<td>5.1 ± 1.6</td>
<td>0.87</td>
</tr>
<tr>
<td>Speed (m/s)</td>
<td>0.29 ± 0.26</td>
<td>0.14 ± 0.08</td>
<td>0.15 §</td>
</tr>
<tr>
<td>Distance (m)</td>
<td>44.9 ± 49.3</td>
<td>21.3 ± 17.2</td>
<td>0.52 §</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurophysiologic Measures:</th>
<th>TA↑</th>
<th>SOL↓</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI SOL H-reflex (%)</td>
<td>98.9 ± 4.5</td>
<td>99.6 ± 1.1</td>
<td>0.87</td>
</tr>
<tr>
<td>PI SOL H-reflex (%)</td>
<td>7.8 ± 5.8</td>
<td>4.2 ± 1.6</td>
<td>0.20</td>
</tr>
<tr>
<td>LFD SOL H-reflex (%)</td>
<td>91.9 ± 16.5</td>
<td>95.0 ± 10.0</td>
<td>0.75</td>
</tr>
<tr>
<td>SOL/TA Ratio</td>
<td>0.49 ± 0.23</td>
<td>0.52 ± 0.39</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Mann-Whitney U-test for all analyses, except Chi-square (Χ²) test for nominal data; C, cervical; AIS, American Spinal Injury Association Impairment Scale (ASIA, 2002); RI, reciprocal inhibition as a % of control reflex; PI, presynaptic inhibition as a % of Mmax; LFD, low frequency depression as a % of control reflex; H/M, H-reflex/M-wave; SOL/TA, soleus/tibialis anterior; PFRTA, plantar flexor reflex threshold angle; DF, dorsiflexion; AROM, active range of motion; LEMS, lower extremity motor score; PF, plantar flexion; (*) significant difference (p ≤ 0.05); (§) large but not significant difference.
<table>
<thead>
<tr>
<th>Training Measures:</th>
<th>TA↑ Group</th>
<th>SOL↓ Group</th>
<th>Between-Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>SRM</td>
</tr>
<tr>
<td>TA %MVC Amplitude</td>
<td>48.19 ± 9.29</td>
<td>0.01 *</td>
<td>5.19</td>
</tr>
<tr>
<td>Conditioned SOL H_\text{DF} (% baseline)</td>
<td>-16.17 ± 26.22</td>
<td>0.09 +</td>
<td>-0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Measures:</th>
<th>TA↑ Group</th>
<th>SOL↓ Group</th>
<th>Between-Groups</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>SRM</td>
</tr>
<tr>
<td>Clonus duration (sec)</td>
<td>-7.38 ± 12.63</td>
<td>0.22</td>
<td>-0.58</td>
</tr>
<tr>
<td>PF reflex threshold angle (degrees)</td>
<td>-4.33 ± 2.23</td>
<td>0.02 *</td>
<td>-1.86</td>
</tr>
<tr>
<td>Toe Tap Test (score)</td>
<td>2.3 ± 5.3</td>
<td>0.16</td>
<td>0.43</td>
</tr>
<tr>
<td>DF active ROM angle (degrees)</td>
<td>-4.32 ± 4.53</td>
<td>0.05 *</td>
<td>-0.95</td>
</tr>
<tr>
<td>Dorsiflexion strength (LEMS)</td>
<td>0.8 ± 0.4</td>
<td>0.03 *</td>
<td>2.08</td>
</tr>
<tr>
<td>Plantar flexion strength (LEMS)</td>
<td>0.3 ± 0.8</td>
<td>0.19</td>
<td>0.41</td>
</tr>
<tr>
<td>Training leg strength (LEMS)</td>
<td>2.2 ± 1.9</td>
<td>0.06 +</td>
<td>1.16</td>
</tr>
<tr>
<td>Non-training leg strength (LEMS)</td>
<td>0.8 ± 0.8</td>
<td>0.06 +</td>
<td>1.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Walking Measures:</th>
<th>TA↑ Group</th>
<th>SOL↓ Group</th>
<th>Between-Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>SRM</td>
</tr>
<tr>
<td>Foot Clearance (mm)</td>
<td>4.8 ± 5.3</td>
<td>0.05 *</td>
<td>0.91</td>
</tr>
<tr>
<td>Speed (m/s)</td>
<td>0.04 ± 0.07</td>
<td>0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>Distance (m)</td>
<td>12.09 ± 9.03</td>
<td>0.02 *</td>
<td>1.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurophysiologic Measures:</th>
<th>TA↑ Group</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>SRM</td>
</tr>
<tr>
<td>RI SOL H-reflex (%)</td>
<td>0.1 ± 0.5</td>
<td>0.50</td>
<td>-0.02</td>
</tr>
<tr>
<td>PI SOL H-reflex (%)</td>
<td>1.0 ± 5.4</td>
<td>0.42</td>
<td>-0.18</td>
</tr>
<tr>
<td>LFD SOL H-reflex (%)</td>
<td>-3.1 ± 14.6</td>
<td>0.42</td>
<td>0.24</td>
</tr>
<tr>
<td>SOL/TA Clonus Coactivation Ratio</td>
<td>-0.13 ± 0.36</td>
<td>0.28</td>
<td>-0.36</td>
</tr>
</tbody>
</table>

TA, tibialis anterior; SOL, soleus; SRM, standardized response mean; MVC, maximum voluntary contraction; H\_\text{DF}, H-reflex during dorsiflexion; Mmax\_\text{DF}, maximum M-wave during dorsiflexion; RI, reciprocal inhibition as a % of control reflex; PI, presynaptic inhibition as a % of Mmax; LFD, low frequency depression as a % of control reflex; PF, plantar flexor; DF, dorsiflexion; ROM, range of motion; LEMS, lower extremity motor score; (*) = p ≤ 0.05, achieved statistical significance; (+) = p > 0.05 but ≤ 0.10, approached statistical significance; SRM interpretation of effect size = 0.20, 0.50, and 0.80 for small, moderate, and large effects, respectively.