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Spike-Timing-Dependent Plasticity in Lower-Limb Motoneurons after Human Spinal Cord Injury

M.A. Urbin, Recep A. Ozdemir, Toshiki Tazoe, and Monica A. Perez

Department of Neurological Surgery, The Miami Project to Cure Paralysis, University of Miami, Miami, FL; Bruce W. Carter Department of Veterans Affairs Medical Center
1201 NW 16th Street, Miami, FL 33125, USA

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Correspondence to:
Monica A. Perez, PhD.
Department of Neurological Surgery
The Miami Project to Cure Paralysis
University of Miami
Bruce W. Carter Department of Veterans Affairs Medical Center
Phone: (305) 243–7119
E-mail: perezmo@miami.edu

Abstract

Recovery of lower-limb function after spinal cord injury (SCI) likely depends on transmission in the corticospinal pathway. Here, we examined whether paired corticospinal-motoneuronal stimulation (PCMS) changes transmission at spinal synapses of lower-limb motoneurons in humans with chronic incomplete SCI and aged-matched controls. We used 200 pairs of stimuli where corticospinal volleys evoked by transcranial magnetic stimulation (TMS) over the leg representation of the motor cortex were timed to arrive at corticospinal-motoneuronal synapses of the tibialis anterior (TA) muscle 2 ms before antidromic potentials evoked in motoneurons by electrical stimulation of the common peroneal nerve (PCMS+) or when antidromic potentials arrived 15 or 28 ms before corticospinal volleys (PCMS-) on separate days. Motor evoked potentials (MEPs) elicited by TMS and electrical stimulation were measured in the TA muscle before and after each stimulation protocol. After PCMS+, the size of MEPs elicited by TMS and electrical stimulation increased for up to 30 min in control and SCI participants. Notably, this was accompanied by increases in TA electromyographic (EMG) activity and ankle dorsiflexion force in both groups, suggesting that this plasticity has functional implications. After PCMS-, MEPs elicited by TMS and electrical stimulation were suppressed if afferent input from the common peroneal nerve reduced TA MEP size during paired stimulation in both groups. In conclusion, PCMS elicits spike-timing-dependent changes at spinal synapses of lower-limb motoneurons in humans and has potential to improve lower-limb motor output following SCI.

New and Noteworthy

Approaches that aim to enhance corticospinal transmission to lower-limb muscles following spinal cord injury (SCI) are needed. We demonstrate that paired cortico-motoneuronal stimulation (PCMS) can enhance plasticity at spinal synapses of lower-limb motoneurons in humans with and without SCI. We propose that PCMS has potential for improving motor output in leg muscles in individuals with damage to the corticospinal tract.

Introduction

Transmission in the corticospinal tract projecting to lower-limb muscles can be impaired following spinal cord injury (SCI) (for review see Raineteau and Schwab 2001; Curt and Ellaway, 2012; Oudega and Perez 2012). Lesions of the corticospinal tract projecting to lower-limb muscles often result in inadequate lift of the hindlimb during locomotion in the rat (Metz and Whishaw 2002; Muir et al. 2007), cat (Jiang and Drew 1996), and monkey (Courtine et al. 2005; Capogrosso et al. 2016). In agreement, studies in humans showed that damage to corticospinal projections to lower-limb muscles is often accompanied by foot drop or an inability to lift the foot during locomotion (Hansen et al. 2005; Thomas and Gorassini 2005; Burridge et al. 2011). Indeed, a relationship was found between foot drop and impairments in corticospinal transmission in humans with SCI (Barthelemy et al. 2010).

Several neuromodulatory strategies have been used to improve the control of lower-limb muscles following SCI. For example, epidural electrical stimulation of the lumbar spinal cord, combined with motor training, improves adaptive locomotor outcomes and other related functions in people with SCI (Gerasimenko et al. 2007; Harkema et al. 2011; Angeli et al. 2014). Operant conditioning of spinal reflexes results in faster and more symmetrical locomotion

(Thompson et al. 2013) while high frequency repetitive transcranial magnetic stimulation (TMS) over the leg motor cortex have some effects on decreasing spasticity in lower-limb muscles (Kumru et al. 2010, 2013). Paired-associative stimulation have been used to target corticospinal projections to leg muscles at a cortical level in humans with SCI (Roy et al. 2010). Although it is likely that interactions between corticospinal drive and spinal motoneurons contribute to the aftereffects of these forms of plasticity, strategies that aim to target cortico-motoneuronal synapses in lower-limb motoneurons in humans with SCI remain untested.

In human upper-limb muscles, spinal synapses have been targeted noninvasively by using paired cortico-motoneuronal stimulation (PCMS), where corticospinal volleys evoked by TMS over the motor cortex are timed to arrive at cortico-motoneuronal synapses before or after antidromic potentials evoked in motoneurons by electrical stimulation of a peripheral nerve. PCMS increases corticospinal transmission and upper-limb motor output in controls (Taylor and Martin 2009; Fitzpatrick et al. 2016) and in humans with SCI (Bunday and Perez 2012). Evidence suggests that there is potential for inducing cortico-motoneuronal synaptic plasticity in lower-limb motoneurons in humans. Direct monosynaptic connections from the leg motor cortex to lower-limb motoneurons are likely present in both animals (Agnew et al. 1963; Jankowska et al. 1975) and humans (Brouwer and Ashby 1992; Nielsen and Petersen 1995), providing an opportunity for pairing presynaptic and postsynaptic inputs. Plasticity in spinal circuits involving lower-limb motoneurons is thought to contribute to improvements in voluntary motor output of leg muscles following motor training (Perez et al. 2005, 2007). Thus, we hypothesize that PCMS elicits spike-timing-dependent like-changes at spinal synapses of lower-limb motoneurons in humans and has potential to improve lower-limb motor output in humans with chronic anatomically incomplete SCI.

Materials and Methods

Subjects. Eighteen individuals with SCI (39.7±15.3 years, 3 female) and 23 control subjects (30.5±8.6 years, 6 female) participated in the study. All subjects gave informed written consent to experimental procedures, which were approved by the local ethics committee at the University of Miami. SCI subjects had chronic (≥ 1 year) injuries with a neurological level between C2 and T11, an intact or impaired, but not absent innervation in dermatome L4-L5 during light touch and pin prick stimulus using the International Standards for Neurological Classification of Spinal Cord Injury sensory scores (Table 1). Two of the 18 were categorized as ASIA A (complete injury) due to the lack of sacral sparing, despite residual voluntary ankle movement. The remaining 16 subjects were classified as incomplete ASIA C or D. All SCI subjects were able to exert maximal isometric voluntary contractions (MVC) into ankle dorsiflexion but to a lesser extent than controls (controls=0.72±0.41 mV, SCI=0.28±0.16 mV; $p<0.001$). Subjects participated in 2 main experiments in which we examined the effect of PCMS+ and PCMS- on MEPs elicited by TMS on different days in a randomized order (PCMS+: controls, n=18 and SCI, n=15; PCMS-: controls, n=13, and SCI, n=7). Some participants completed 3 additional experiments, where we examined the effect of PCMS+ and PCMS- on MEPs elicited by

electrical stimulation on different days in a randomized order (PCMS+: controls, n=6 and SCI, n=7; PCMS-: controls, n=5 and SCI, n=4) and the effect of peripheral nerve stimulation (PNS) over the common peroneal nerve on TA MEP size at ISIs of 20, 30, and 40 ms (controls, n=13).

Electromyographic (EMG) recordings. EMG was recorded from the right tibialis anterior (TA) muscle in control subjects and from the less affected leg in individuals with SCI through surface electrodes secured to the skin over the belly of each muscle (Ag–AgCl, 10 mm diameter). EMG signals were amplified, filtered (20–1000 Hz), and sampled at 2 kHz for off-line analysis (CED 1401 with Signal software, Cambridge Electronic Design, Cambridge, UK).

PCMS. During testing subjects were seated in an armchair with the tested foot placed on a custom platform with the ankle connected to a force transducer and restrained by straps (Fig. 1A). At the start of the experiment, subjects were instructed to perform 2 brief MVCs for 3-5 s into dorsiflexion, separated by ~30 s of rest. Subjects participated in two PMCS protocols in a randomized order in different sessions separated by at least 2-3 days. In each protocol, tested at rest, 200 pairs of stimuli were delivered every 10 s (~34 min, 0.1 Hz) where corticospinal volleys evoked by TMS over the leg motor cortex were timed to arrive at cortico-motoneuronal synapses of the TA muscle 2 ms before antidromic potentials evoked in motoneurons by PNS of the common peroneal nerve (PCMS+) or when antidromic potentials arrived 15 ms or 30 ms before corticospinal volleys (PCMS-) on separate days (Figs. 1B and C). The PCMS+ protocol was intended to strengthen corticospinal transmission and the PCMS- protocol was intended to weaken corticospinal transmission (Taylor and Martin 2009; Bunday and Perez 2012). Previous studies using PCMS in upper-limb muscles administered 50 or 100 pairs of stimuli (Bunday and Perez 2012; Fitzpatrick et al. 2016). Our preliminary data in SCI subjects showed that 200 pairs

of stimuli were more effective in inducing consistent changes in TA MEP size in SCI participants consistent with the view that more pairs of stimuli exert more reliable changes in corticospinal excitability (Fitzpatrick et al. 2016).

TMS. A double-cone coil (type number 9902-00) with a monophasic current waveform was used to deliver TMS (Magstim 200, Whitland, UK). We determined the optimal position for eliciting a motor evoked potential (MEP) in the TA muscle (hot spot) by moving the coil in small steps along the leg representation of the motor cortex. The hot spot was defined as the region where the largest MEP in the TA could be evoked with minimum intensity. The TMS coil was held with a custom coil holder, while the head was firmly secured to a headrest by straps. We used an intensity of 100% of the maximum stimulator output (MSO) for the TMS pulse in both groups during both protocols.

PNS. A constant current stimulator (200 μs pulse duration, model DS7AH, Digitimer, Welwyn Garden City, UK) was used to deliver PNS to the common peroneal nerve with the stimulating electrode positioned under the head of the fibula (anode and cathode 3 cm apart) at an intensity of 1.5 x above the threshold to elicit maximal compound action potentials (M-max) in the TA muscle in both groups during both protocols. During F-wave testing signals were filtered using a high passed filter of 100 Hz to identify the earliest F-wave latency for calculating conduction times while the low pass filter was maintained at 1000 Hz (Khan et al. 2012). The F-wave onset latency was defined as the time at which a signal was ~20 μV above the mean baseline measured 100 ms before the stimulus artifact and it was estimated in individual trials to identify the response with the shortest latency (Perez and Rothwell 2015).

TMS and PNS interstimulus interval (ISI). TMS and PNS targeted the right TA muscle in control subjects and the less affected leg in SCI participants. ISIs for each protocol were tailored to individual subjects based on conduction times calculated from latencies of MEPs, F-wave, and M-max (Fig. 2). MEP and F-wave latencies were recorded during isometric ~10% of MVC into dorsiflexion to determine the shortest and clearest response for our estimations. The onset latency was defined as the time when each response exceeded 2 SD of the mean rectified pre-stimulus activity (100 ms) in the averaged waveform. Peripheral conduction time (PCT) from the stimulating electrode overlying the CPN to the TA motor pool was calculated using the following equation:

$$(\text{F-wave latency} - \text{M-max latency}) * 0.5$$

Central conduction time (CCT) from the motor cortex to the TA motor pool was calculated using the following equation:

$$\text{MEP latency} - (\text{PCT} + \text{M-max latency})$$

We quantified the size of the F-wave and/or MEP elicited during paired stimulation throughout each protocol in both groups. Note that for analysis the 200 pairs of pulses were combined in 10 blocks of 20 pairs each. Repeated measure ANOVA showed no significant effect of TIME ($F_{(9, 315)}$=1.31, p=0.14), GROUP ($F_{(1, 35)}$=1.21, p=0.28), and in their interaction ($F_{(9, 315)}$= 0.89, p=0.53) on responses measured during PCMS+ (Figs. 3A and C). Similarly, repeated measure ANOVA showed no effect of TIME ($F_{(9, 144)}$=1.18, p=0.31), but GROUP ($F_{(2, 16)}$=4.76, p=0.02) nor in their interaction ($F_{(9, 144)}$=1.10, p=0.35) on responses measured during PCMS- (Figs. 3B and D). These results together indicate that the stimulation conditions were maintained constant across the 200 pairs of pulses. During PCMS+, due to the similar onset latency of the TA MEP and F-wave it is possible that both contributed to the response that was analyzed during

paired stimulation. During PCMS-, due to the interval between PNS and TMS, it is likely that the response present during paired stimulation was a MEP. During PCMS-, we observed in some control subjects that the MEP size was close to the MEP-max (79.5±7% of MEP-max, n=6) and in these individuals MEPs were not suppressed after PCMS-, therefore, we refer to them as non-responders (Non-responders: $control_{NR}$). In the rest of the control subjects, MEPs evoked during PCMS- were smaller than the MEP-max (32.2±3.8% of MEP-max, n=7; Figs. 3B and D) and in these individuals MEPs were suppressed after PCMS-, therefore, we refer to these subjects as responders (Responders: $control_{R}$). Evidence showed that PNS of the common peroneal nerve applied 20 to 40 ms before a TMS pulse suppressed the size of the TA MEP (Kasai et al. 1992; Roy and Gorassini 2008; Roy et al. 2010; Zewdie et al. 2014). During PCMS-, for volleys to arrive at the spinal motoneurons 15 ms before descending volleys, we used an ISI between PNS and TMS of ~20 ms. To determine the best interval for suppressing the TA MEP in $control_{NR}$ during PCMS-, we completed an additional control experiment in which we examined the effect of PNS of the common peroneal nerve 20, 30, and 40 ms before the TMS pulse.

MEPs elicited by TMS. The active motor threshold (AMT) was defined as the minimal stimulus intensity required to induce MEPs $\geq$200 μV peak-to-peak amplitude above the background EMG in 5/10 consecutive trials in the contracting muscle (controls=42±11% of MSO, SCI=59±9% of MSO, $p<0.001$). The MEP-max was defined at rest by increasing stimulus intensities in 5% steps of maximal device output until the MEP amplitude did not show additional increases (controls=2.5±2.5 mV, SCI=1.1±0.8 mV, $p=0.04$). Twenty MEPs evoked by TMS over the leg motor cortex were acquired using an intensity needed to produce an MEP of ~5-10% of M-max (controls=4.7±2.7 % of M-max, SCI=7.0±6.4 % of M-max, $p=0.2$) at rest and MEP peak-to-peak

amplitude was measured before (baseline), immediately after (0), and 10, 20, and 30 min after each protocol.

MEPs elicited by electrical stimulation. The corticospinal tract was stimulated using the D180 voltage stimulator (200 μs duration, expressing the intensity as a % of the stimulator output) passed between adhesive Ag-AgCl electrodes fixed to the scalp with the anode 2 cm lateral to the vertex and the cathode 2 cm anterior to the vertex (controls, n=5; SCI, n=4) to evoke motor responses in the TA. This stimulation likely activate axons of pyramidal tract neurons in the subcortical white matter (Nielsen et al. 1995). In addition, MEPs were elicited by passing high voltage electrical current (100 μs duration, Digitimer D180-260, 750 V) between two surface electrodes fixed over the thoracic spine (thoracic MEP; controls, n=5; SCI, n=4) with the cathode positioned between the spinal processes of T9 and T10 vertebrae and the anode ~10 cm above. For both types of stimulation, the intensity was set to elicit an MEP in the TA muscle of ~5-10% of the M-max (controls=8.2±2.1 % of M-max and SCI=10.1±5.9 % of M-max, p=0.3). The latency of MEPs elicited by spinal cord electrical stimulation (controls=18.55±2.35ms, SCI=20.56±1.26ms) were shorter than the latency of MEPs evoked by cortical stimulation (controls=29.06±2.34 ms, SCI=37.10±4.08 ms). Five to 10 MEPs elicited by cortical and thoracic electrical stimulation were tested at rest before (baseline), immediately after (0), and 30 min after each protocol. After PCMS+, we found a similar increased in the size of MEPs elicited by electrical stimulation of the leg motor cortex (controls=154.9±57.7%, SCI=192.2±10.6.1%) and the thoracic spinal cord (controls=142.2±34.8%, SCI=159.9±37.4%). Moreover, after PCMS-, MEPs elicited by electrical stimulation of the leg motor cortex (controls=80.8±10.3%, SCI=89.7±13.9%) and the thoracic spinal cord (controls=71.9±14.7%; SCI=66.2±19.0%) were

decreased below the baseline. Therefore, we grouped data together under MEPs elicited by electrical stimulation for statistical analysis for each muscle. If a subject completed both tests we used the results for MEPs elicited by cortical electrical stimulation for analysis.

Voluntary Motor Output. Ankle dorsiflexion force and TA EMG activity were measured during short, ballistic, isometric contractions into dorsiflexion using a custom LabView program (controls, n=7; SCI, n=8). One cursor showed the target force (10% MVC) and another cursor showed the force exerted by the subject. Subjects were instructed to dorsiflex the ankle to move the actual force to the target force as fast as possible without making corrections for errors in force production. After familiarization, four sets of 20 contractions were performed with partially stimulated visual feedback of the cursor. Here, subjects controlled the movement of the cursor up to 8% of MVC and the remaining 2% of MVC was simulated by LabView. During the simulation period the speed of the cursor was maintained constant (1 video frame: 15.6ms duration, range=0.61 to 0.67% MVC/ms), while the end point of the cursor was randomly varied within ±0.5% standard deviation of the target (10% of MVC). This strategy was used to avoid online real feedback of the force exerted to help to maintain subjects unaware of the possible effects of the stimulation (Bunday and Perez 2012). Force and EMG during each contraction were measured during a 250 ms window (125 ms before and 125 ms after the peak force and EMG values). Twenty contractions were performed before, immediately after (0), and 10, 20 and 30 min after PCMS+.

Data analysis. Normal distribution was examined using the Shapiro-Wilk's test and Mauchly's test was examine to test sphericity. Data were log transformed when data was not normally

distributed. Greenhouse-Geisser correction statistics were used to reveal significant F values when sphericity could not be assumed. Repeated-measures analysis of variance (ANOVA) was performed to examine the effect of TIME (Baseline, 0, 10, 20, 30 min) and GROUP (control, SCI) on MEP size for PCMS+. Repeated-measures ANOVA was also performed to determine the effect of TIME and GROUP [$control_R$, $control_{NR}$, SCI] on MEP size for PCMS-. Bonferonni *post-hoc* tests were used to test for significant comparisons. The same test was performed to examine the effect of GROUP and TIME (Baseline, 0, 30 min) on MEPs elicited by electrical stimulation for PCMS+ and PCMS- and GROUP and BLOCK (1 to 20 blocks) on responses elicited during paired stimulation. Independent samples t-tests were used to examine differences in MEP and F-wave latencies, conduction times, MEP-max, AMT and MVC between groups. The significance level was set at $P<0.05$ and group data are presented as mean±SD in the text.

Results

MEPs elicited by TMS

Raw TA MEPs traces from a representative control and SCI participant are shown in Fig. 4A. Note that MEP size increased compared with baseline in both participants for 30 min after PCMS+. Repeated-measures ANOVA showed an effect of TIME ($F_{(2.2, 61.42)}=8.9$, $p<0.001$) but not GROUP ($F_{(1, 61.42)}=0.5$, $p=0.50$) nor their interaction ($F_{(2.2, 61.42)}=1.4$, $p=0.25$) on TA MEP amplitude. *Post-hoc* tests showed that MEP amplitude increased immediately after (controls=169±82.7%, SCI=163.3±18.3%; $p=0.03$), 10 (controls=157.2±57.2%, SCI=176.1±95.3%; $p=0.01$), 20 (controls=144±59 %, SCI=131.7±152%; $p<0.01$), and 30

(controls=149.7±57.7%, SCI=214.27±118.4%; $p<0.01$) min after PCMS+ compared with baseline in both groups (Fig. 4B). Note that MEPs were facilitated in the majority of control (17/18) and SCI (14/15) subjects.

Figure 5A illustrates raw MEPs traces from two representative control subjects [$control_R$, $control_{NR}$, see details in methods) and from one SCI participant before and after PCMS-. Here, note that MEP size decreased compared to baseline in the $control_R$ and SCI subject for 30 min after PCMS-, whereas, MEPs were facilitated in the $control_{NR}$ participant for 30 min after PCMS-. Repeated measures ANOVA showed no effect of TIME ($F_{(2.1, 35.64)}=1.48$, $p=0.24$) but GROUP ($F_{(2, 17)}=32.7$, $p=0.001$) and in their interaction ($F_{(4.19, 41.05)}=7.1$, $p=0.001$) was found on TA MEP amplitude. *Post-hoc* tests indicated that MEPs were suppressed immediately after ($control_R$=70.1±27.1%, $p=0.003$; SCI=73±20.2%, $p=0.002$), 10 ($control_R$=62.3±15.5%, $p<0.001$; SCI=55.9±23.7%, $p<0.001$), 20 ($control_R$=74.5±19.8%, $p=0.007$; SCI=60.5±26.6%, $p<0.001$), and 30 ($control_R$=80.7±39.9%, $p=0.01$; SCI=75.4±21.2%, $p=0.01$) min after PCMS- compared with baseline. In the $control_{NR}$ group, MEPs were facilitated immediately after ($p=0.03$), 10 ($p=0.01$), 20 ($p<0.01$), and 30 ($p<0.01$) min after PCMS- (Fig. 5B).

We completed additional experiments to examine the time at which PNS of the common peroneal nerve suppressed the size of the TA MEP (see details in methods). Repeated measured ANOVA showed an effect of ISI ($F_{3,33}=68.8$, $p<0.001$), not GROUP ($F_{1, 11}=2.64$, $p=0.13$) but in their interaction ($F_{3,33}=6.3$, $p=0.002$) on TA MEP size. TA MEPs were suppressed at all ISIs in $control_R$ ($p<0.001$) but only at the 30 and 40 ms in $control_{NR}$ ($p<0.001$). Therefore, in two of the $control_{NR}$ we completed PCMS- using an interval between PNS and TMS of 30 ms. Based on their central and peripheral conduction times, we estimated that in these two subjects antidromic potentials arrived in spinal motoneurons 28 ms before corticospinal volleys. Using this adjusted

interval, MEPs were suppressed immediately after (81.3±27.1%), 10 (72.1±13.4%), 20 (73.8±12.2%), and 30 (67.5±3.9%) min after PCMS- compared to baseline.

MEPs elicited by electrical stimulation

Raw MEP traces recorded from the TA muscle after electrical stimulation of the motor cortex from a control and SCI participant are shown in Figs. 6A and C. Note that MEPs were facilitated after PCMS+ and suppressed after PCMS- in both participants. Repeated-measures ANOVA showed an effect of TIME ($F_{(2, 18)}$=11.27, p<0.001) but not GROUP ($F_{(1, 9)}$=0.2, p=0.67) nor their interaction ($F_{(2, 18)}$=1.4, p=0.27) on TA MEPs in PCMS+. *Post-hoc* tests showed that MEP size increased immediately after (controls=167.08±17.39%, SCI=138.86±13.15%; p=0.001) and 30 min (controls=146.40±25.38%, ¾; SCI=169.35±19.18%, 7/7; p=0.005; Fig. 5B) after PCMS+ compared with baseline in most participants.

Repeated measures ANOVA also showed an effect of TIME ($F_{(2, 14)}$=12.71, p=0.001), but not GROUP ($F_{(1, 7)}$=0.1, p=0.97) nor their interaction ($F_{(2, 14)}$=1.5, p=0.26) on TA MEPs in PCMS-. *Post-hoc* tests indicated that MEPs were suppressed immediately after (controls=88.14±6.88%, SCI=79.15±4.15%; p=0.001) and 30 min (controls=84.56±7.69%, SCI=84.16±4.65%; p=0.001; Fig. 6D) after PCMS- in most control (4/5) and SCI (4/4) subjects.

Voluntary Motor Output

Raw EMG and force recordings are shown in Fig. 7A for an SCI participant. Repeated-measures ANOVA showed an effect of TIME ($F_{4, 52}$=14.0, p<0.001; $F_{2.51, 30.1}$=10.8, p<0.001, respectively) but not GROUP ($F_{(1,13)}$=1.8, p=0.20, $F_{(1,12)}$=2.2, p=0.16, respectively) nor their interaction ($F_{(4,52)}$=1.6, p=0.20, $F_{(2.51,30.1)}$=0.6, p=0.6, respectively) on dorsiflexion force. *Post-*

hoc tests showed that force increased above baseline immediately after (control=120.8±11%, SCI=117.1±18.7%, p=0.003), 10 (control=138.7±28.2%, SCI=116±13.2%; p=0.001), 20 (controls=132.3±19.3%, SCI=128.6±34.1%, p=0.002), and 30 (controls=142.4±31.3%, SCI=122.5±20.5%; p=0.001) min after PCMS+ in both groups (Fig. 7B). Similarly, EMG increased above baseline immediately after (control=129.9±25.6%, SCI=130.9±27.6%; p=0.004), 10 (control=145.9±43.3%, SCI=133.1±41.4%; p=0.01), 20 (controls=132.6±28.1%, SCI=148.8±53.6%, p=0.01), and 30 (controls=144.2±44.3%, SCI=148.7±50.3%, p=0.01) min after PCMS+ in both groups (Fig. 7C). No correlation was found between changes in TA MEP size and EMG (controls: r=-0.47, p=0.06; SCI: r=0.44, p=0.11) and force (controls: r=0.20, p=0.62; SCI: r=0.52, p=0.18) after the PCM+ protocol in controls and SCI participants.

Discussion

Our novel findings demonstrate that 200 pairs of stimuli designed to produce spike-timing-dependent plasticity at cortico-motoneuronal synapses of the TA motoneuronal pool increases corticospinal transmission and voluntary motor output in lower-limb muscles in humans with and without SCI. We found that the size of MEPs elicited by TMS and by electrical stimulation increased when TMS-induced presynaptic volleys arrived 2 ms before antidromic volleys induced by PNS at cortico-motoneuronal synapses of the TA muscle (PCMS+). In contrast, the size of MEPs evoked by TMS and electrical stimulation was reduced when antidromic volleys arrived at the spinal cord 15 or 28 ms before presynaptic volleys (PCMS-). Dorsiflexion force and TA EMG activity increased after PCMS+ in both groups and SCI participants showed enhanced dorsiflexion angle and TA EMG activity during the early swing phase of locomotion. We propose that PCMS has potential to improve leg motor output in individuals with deficits in corticospinal transmission.

Spike-timing-dependent changes in lower-limb motoneurons

PCMS elicits spike-timing-dependent changes at spinal synapses of upper-limb motoneurons in control (Taylor and Martin 2009; Fitzpatrick et al. 2016) and SCI (Bunday and Perez 2012) subjects. In the lower limb, paired-associative stimulation have been used to target corticospinal projections to leg muscles at a cortical level in humans with SCI (Roy et al. 2010) and controls (Mrachacz-Kersting et al. 2007; Cortes et al. 2011). Other have used repeated trains of PNS combined with TMS timing the volleys to arrive simultaneously at lower limb motoneurons to induce LTP-like plasticity in control subjects (Shulga et al. 2015). Here, for the first time, we demonstrate that spike-timing-dependent changes can be extended to at spinal synapses of lower-limb muscles. We targeted the TA muscle because it plays an important role in the control of the foot trajectory during the swing phase of the gait cycle (Winter and Bishop, 1992) and because foot drop is seen in individuals with cortical and spinal cord damage (Burridge et al. 2001; Westhout et al. 2007). We found that the size of TA MEPs elicited by TMS over the leg motor cortex increased after PCMS+ for 30 min in control and SCI participants. We followed up the effects of PCMS+ in a subgroup of individuals and found that MEP size returned to baseline ~60 min after the paired stimulation. PCMS relies on the ability of pairing presynaptic and postsynaptic inputs, therefore, muscles with direct monosynaptic connections to spinal motoneurons are good candidates for inducing these effects. In agreement, evidence showed that direct monosynaptic connections from the leg motor cortex to TA motoneurons are likely present in both animals (Agnew et al. 1963; Jankowska et al. 1975) and humans (Brouwer and Ashby 1992; Nielsen and Petersen 1995). Our result also showed that TA MEPs elicited by electrical stimulation of the leg motor cortex increased after PCMS+ for 30 min in both groups. MEPs elicited by electrical stimulation of the motor cortex with the anode 2 cm lateral to the vertex and the cathode 2 cm anterior to the vertex have shorter latencies than

MEPs evoked by TMS and vertex anodal stimulation (Nielsen et al. 1995). Recordings from the epidural space also indicate that a single pulse of electrical stimulation, in the same location as described above, provides a way to ensure D wave activation of corticospinal axons from the leg area (Di Lazzaro et al. 2001), supporting the view that lateral anodal stimulation penetrated deeper into the brain or that it activated corticospinal cells directly. Thus, increases in the size of TA MEPs elicited by TMS and electrical stimulation of the leg motor cortex and the thoracic spine suggests that changes after PCMS+ had a spinal origin. Although it is possible that this plasticity is not limited to direct cortico-motoneuronal synapses but also involves spinal interneurons this possibility needs to be tested in future studies.

One of the important features of spike-timing-dependent plasticity is that these changes need to be reversible (Bi and Poo 1998; Dan and Poo 2004). We propose that our effects are related to spike-timing-dependent plasticity since when we reversed the order to arrival of volleys at the spinal cord, then antidromic volleys arrived at the spinal cord before presynaptic volleys, we found that TA MEPs elicited by TMS were suppressed after PCMS- for 30 min in both groups. Note that when antidromic volleys arrived at the spinal cord 15 ms before TMS-induced presynaptic volleys we observed two distinct responses in control subjects. In one group, the size of MEPs elicited during paired stimulation was reduced and in these subjects PCMS- reduced corticospinal excitability. Whereas, in a second group, the size of MEPs elicited during paired stimulation when applying PCMS- was similar as MEP-max and in these subjects paired stimulation was ineffective at reducing corticospinal excitability. Evidence showed that electrical stimulation of homonymous and heteronymous nerves at the knee and/or at the ankle as well as stimulation of the skin innervated by the deep peroneal nerve, which contains the cutaneous branch of the common peroneal nerve, suppresses the TA MEP size 20 to 40 ms before a TMS

pulse over the leg motor cortex (Kasai et al. 1992; Roy and Gorassini 2008; Roy et al. 2010; Zewdie et al. 2014). This is consistent with the results from our control experiment showing that a conditioning pulse to the common peroneal nerve, given 20 to 40 ms before TMS, decreased TA MEP size. Importantly, in individuals in whom a conditioning pulse to the common peroneal nerve reduced the size of TA MEPs at 20 ms, PCMS- was effective in suppressing corticospinal excitability. However, in those individuals in whom a conditioning pulse to the common peroneal nerve reduced the size of TA MEPs at 30 and 40 ms, PCMS- was only effective when the ISI was adjusted to match this time. Thus, it is clear from our results that the effectiveness of PCMS- was linked to the suppressive effects of the conditioning pulse to the common peroneal nerve on TA MEP size. The fact that in some subjects common peroneal nerve stimulation suppressed the TA MEP at an early or later ISI is consistent with the range of effects previously reported from stimulation of homonymous and heteronymous nerves in the leg (Roy and Gorassini 2008; Roy et al. 2010). To examine possible contributions from homonymous vs. heteronymous connections to TA motoneurons (Simonetta-Moreau et al. 1999) future experiments could determine the effect of this plasticity by stimulating the common peroneal nerve and the deep peroneal nerve. The mechanisms involved in the TA MEP facilitation observed in control subjects not responding to PCMS- also need to be determined.

A next important question is which neural mechanisms might have contributed to the MEP suppression after PCMS-. A possibility is that subcortical pathways contributed to this effect. This is supported by our finding showing that TA MEPs elicited by cortical electrical stimulation of the leg area of the motor cortex were suppressed after PCMS- for up to 30 min in both groups. Since we used suprathreshold stimulus intensity for PNS it is possible that circuits not only involving the homonymous common peroneal nerve but also heteromonymous nerves

449 and/or distant skin segments contributed to this effect. The short latency for this effect suggests
450 reflex contributions through spinal pathways, such as high threshold cutaneous afferents (Kasai
451 et al. 1992). In animals, Ia and Ib inhibitory interneurons projecting to lower-limb motoneurons
452 are excited by high threshold cutaneous afferent fibers (Hultborn 1972; Lundberg et al. 1975)
453 and these interneurons receive corticospinal inputs (Jankowska et al. 1975). In humans,
454 stimulation of cutaneous afferents at similar conditioning latencies also suppresses a
455 monosynaptic reflex response in the TA muscle (Delwaide et al. 1981; Delwaide and Crenna
456 1984). Pairs of TMS and PNS inputs inhibit the H-reflex in a calf muscle to the same degree as
457 PNS pulses delivered at an ISI between 20 and 40 ms, suggesting that spinal postsynaptic
458 mechanisms can also contribute to these effects (Poon et al. 2008). Another possibility is that
459 Renshaw cells contributed to this effect. In humans, at the ISIs tested in the study there is ample
460 time to activate Renshaw cells mediating recurrent inhibition to spinal motoneurons (Katz and
461 Pierrot-Deseilligny 1999). Future studies are needed to examine the contribution from group Ia
462 and skin cutaneous afferents, Renshaw cells and other sensory pathways to this plasticity.
463 Sensory information from a peripheral nerve close to the knee can reach the motor cortex ~40 ms
464 after the stimulation and a conditioning pulse to a heteronymous nerve facilitates TA MEP size
465 at a cortical level at longer ISIs than those used in this study (Roy and Gorassini 2008),
466 therefore, that it is less likely that cortical mechanisms contributed to our effects. Although most
467 evidence suggest a spinal origin for our findings the precise spinal mechanisms remain to be
468 tested.
469
470 *Functional considerations*

Several neuromodulatory strategies have been used to improve the control of lower-limb muscles following SCI. For example, epidural electrical stimulation of the lumbar spinal cord has been combined with motor training to improve adaptive locomotor and other related functions in people with SCI (Gerasimenko et al. 2007; Harkema et al. 2011; Angeli et al. 2014). Operant conditioning of spinal reflexes results in faster and more symmetrical locomotion (Thompson et al. 2013) while high frequency repetitive TMS over the leg motor cortex have some effects on decreasing spasticity in lower-limb muscles (Kumru et al. 2010, 2013). In addition, it has been proposed that repeated noninvasive stimulation targeting more direct and indirect corticospinal volleys to spinal motoneurons might influence spinal plasticity in lower-limb muscles (Cortes et al. 2011; Leukel et al. 2012). It is likely that interactions between corticospinal drive and motoneurons contribute to the aftereffects of all these forms of plasticity.

Although we did not record directly from synaptic connections between corticospinal and spinal motoneurons, our protocol which is based on response latencies, targeted for the first time cortico-motoneuronal synapses in lower-limb motoneurons in humans with SCI. We found that paired stimuli precisely timed to arrive at the presynaptic terminal before postsynaptic depolarization results in improvements in voluntary motor output in dorsiflexor muscles in humans with chronic incomplete SCI. These observations support the results by Barthelemy and collaborators (2010) by showing that strengthening transmission in the corticospinal pathways improves TA EMG activity and ankle dorsiflexion during the early swing phase of the locomotor cycle in humans with SCI. Our results also support the view that targeted neuroplasticity might represent an avenue to enhance motor output in lower-limb muscles following SCI (Thompson and Wolpaw 2015). Our results, therefore, might be relevant for a number of motor disorders characterized by impaired corticospinal transmission.

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688 **Figure legends**

689 **Figure 1. Experimental setup. A,** Diagram showing the position of subjects during testing as
690 well as the transcranial magnetic stimulation (TMS) coil, electromyographic (EMG) recording in
691 the tibialis anterior (TA) muscle, and electrical stimulation of the common peroneal nerve. **B,**
692 Diagram showing paired cortico-motoneuronal stimulation (PCMS) where corticospinal volleys
693 evoked by TMS over the leg representation of the motor cortex were timed to arrive at cortico-
694 motoneuronal synapses of the tibialis anterior (TA) muscle 2 ms before antidromic potentials
695 evoked in motoneurons by electrical stimulation of the common peroneal nerve (PCMS+) or
696 when antidromic potentials arrived 15 or 28 ms before corticospinal volleys (PCMS-). We
697 combined the data from subjects in whom antidromic potentials arrived at spinal motoneurons 15
698 and 28 ms before corticospinal volleys because they showed similar suppression of MEP size
699 after PCMS-. **C,** Timeline of the experimental protocol. Following baseline testing, 200 pairs of
700 stimuli were applied over the leg representation of the motor cortex for ~34 minutes. Recordings
701 were taken immediately after (0), and 10, 20, and 30 min after the stimulation as shown by the
702 open blocks.

Figure 2. Response latencies. A, Raw traces showing a motor evoked potential (MEP), an F-wave, and the maximal motor response (M-max) for representative subjects recorded from the TA muscle. **B,** MEP, F-wave and M-max latencies were used to calculate central and peripheral conduction time used to estimate time the arrival of pre- and post-synaptic volleys at the cortico-motoneuronal synapse in control and SCI subjects (Mean±SD).

Figure 3. Responses during paired stimulation. Raw traces from the TA muscle during paired stimulation in the PCMS+ (**A**) and PCMS- (**B**) protocol for representative subjects. Note that in the PCMS+ protocol, the M-max is followed by an F-wave likely combined with a MEP from cortical stimulation. Note that during paired stimulation a large MEP was present in a control subject that was a non-responder ($control_{NR}$**)** and a smaller MEP is present in a control subject that was a responder ($control_{R}$) to the PCMS- protocol. In responders to PCMS-, we found the MEPs were suppressed after paired stimulation. Data is also shown in a SCI participant. Note that in B, the M-max has multiple peaks because it included a TMS stimulus artifact. During PCMS+ and PCMS-, TMS was used over the leg motor cortex and PNS was given to the common peroneal nerve. Graphs show group data (**C-D**). The abscissa shows the number of pairs of stimuli during each protocol (a total of 200 pairs of stimuli). At each point, the average of 20 responses is shown. The ordinate shows the size of the conditioned response expressed as % of the M-max in control (black squares, n=18) and SCI (green circles, n=15) participants during PCMS+ (**C**) and in $control_{R}$ (black squares, n=7), $control_{NR}$ (black triangles, n=6) and SCI (blue circles, n=7) participants during PCMS- (**D**). Note that due to the similarities between the onset latency of the TA MEP and the TA F-wave it is likely that during the PCMS+ protocol both responses were combined and this is what is reported in the analysis. Whereas, in the PCMS-

protocol the response reported it is likely to reflect the MEP size. Error bars indicate SEs. *P<0.05.

Figure 4. MEPs elicited by TMS over the leg motor cortex before and after PCMS+. A, Raw MEP traces from the TA muscle in representative participants at rest before and after PCMS-. Waveforms represent the average of 20 trials. **B,** Graph shows box-plots group data (controls, n=18 and SCI=, n=15). The abscissa shows the time at which measurements were taken (baseline, immediately after (0), and 10, 20, and 30 min after the stimulation). The ordinate shows the size of the MEP expressed as % of the MEP at baseline in control (black circles) and SCI (green circles) participants. The horizontal broken line shows the MEP size at baseline. Error bars indicate SEs. *P<0.05.

Figure 5. MEPs elicited by TMS over the leg motor cortex before and after PCMS-. A, Raw MEP traces from the TA muscle in a representative control subject that was a responder ($control_R$, black traces), a control subject that was a non-responder ($control_{NR}$, gray traces) to PCMS-, and in a SCI (blue traces) participant at rest before and after PCMS-. Waveforms represent the average of 20 trials. **B,** Graph shows group data ($control_R$, n=7, $control_{NR}$, n=6, and SCI, n=7). The abscissa shows the time at which measurements were taken (baseline, immediately after (0), and 10, 20, and 30 min after the stimulation). The ordinate shows the size of the MEP expressed as % of the MEP at baseline in $control_R$ (black circles), $control_{NR}$ (black triangles) and SCI (blue circles) participants. The horizontal broken line shows the MEP size at baseline. Error bars indicate SEs. *P<0.05.

Figure 6. MEPs elicited by cortical electrical stimulation before and after PCMS+ and PCMS-. Raw MEP traces from the TA muscle elicited by cortical electrical stimulation of the leg motor cortex in a representative control (**A**) and SCI (**C**) participant at rest before and after PCMS+ (upper traces) and PCMS- (lower traces). Waveforms represent the average of 5-10 trials. **B,** Graph shows group data (PCMS+: controls, n=6 and SCI, n=7; PCMS-: controls, n=5 and SCI, n=4). The abscissa shows the time at which measurements were taken (baseline, immediately after (0), and 30 min after the stimulation). The ordinate shows the size of the MEP expressed as % of the MEP at baseline in control (black bars) and SCI (green bars for PCMS+ and blue bars for PCMS-) participants. Data from individual subjects is shown in controls and SCI subjects (open circles). The horizontal broken line shows the MEP size at baseline. Error bars indicate SEs. *P<0.05.

Figure 7. Voluntary motor output. A, Force (upper) and EMG (lower) raw traces from the TA muscle measured during brief, fast, ankle isometric voluntary contractions in the dorsiflexion direction before (baseline) and after (0, 10, 20, and 30 min) PCMS+ from a representative SCI subject. Waveforms represent the average of 20 force and EMG traces. Before PCMS+, 20 ankle isometric voluntary contractions were measured on 4 different times with periods of rest in between to establish the baseline. The average of these 4 measurements was used to estimate the baseline. Force and EMG during were measured 125 ms before and 125 ms after the peak force and EMG values. Group data (controls, n=7; SCI, n=8) shows force (**B**) and mean rectified EMG activity (**C**) in both groups. The abscissa shows the time at which measurements were taken [baseline (dotted line), immediately after (0), and 30 min after PCMS+]. The ordinate shows the mean force and EMG expressed as % of the baseline in control (black bars) and SCI (white bars

773 for PCMS+ and PCMS-) participants. The horizontal broken line shows the mean force and

774 EMG activity at baseline. Bonferonni *post-hoc* tests were used to test for significant

775 comparisons. Error bars indicate the SE. *P<0.05.

Figure 1

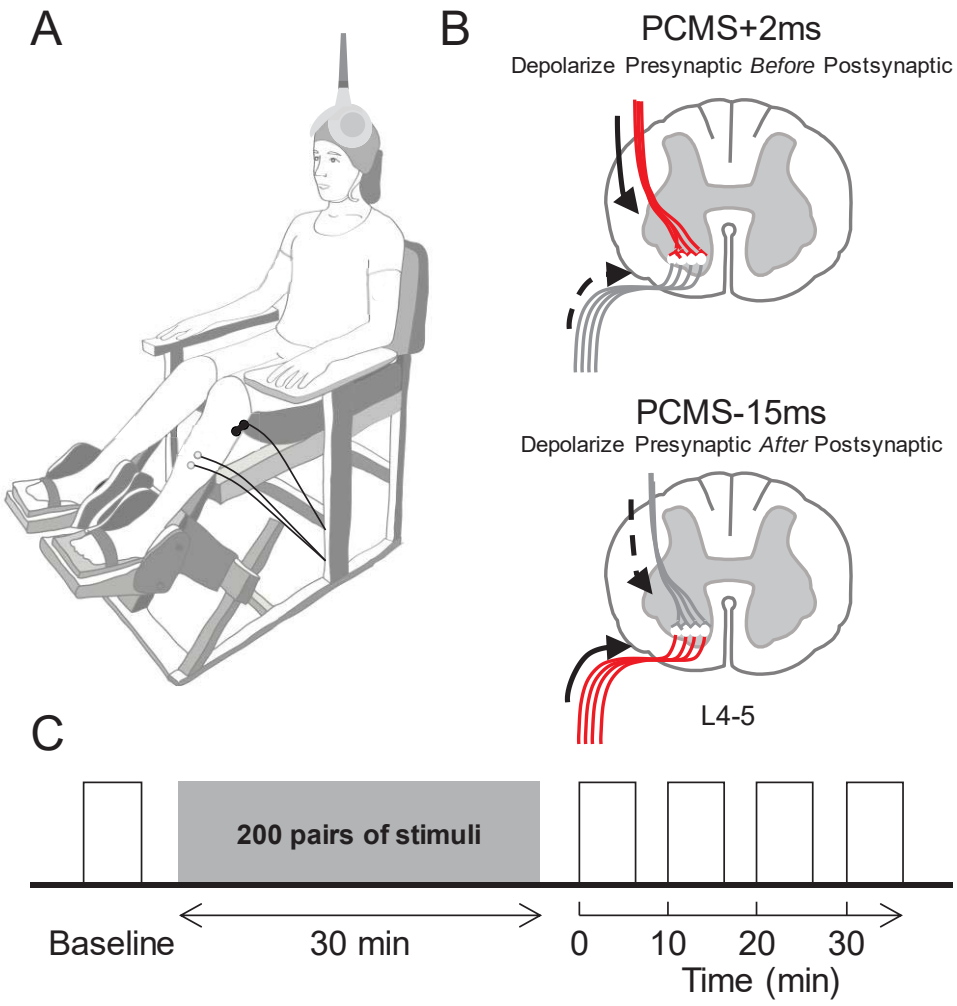
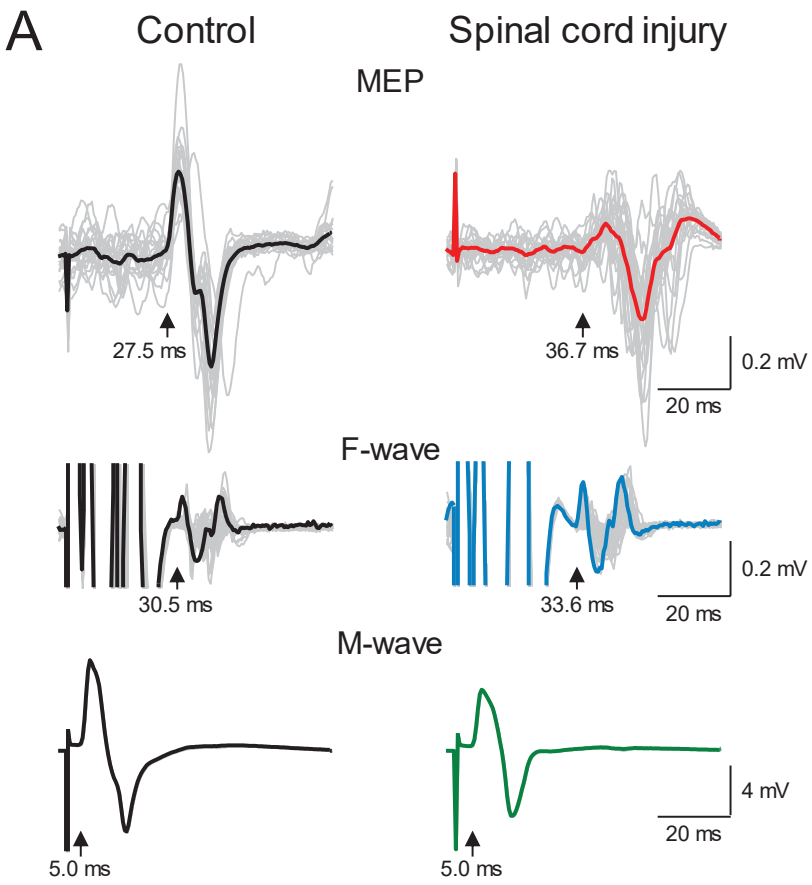

A
B
PCMS+2ms
Depolarize Presynaptic *Before* Postsynaptic
PCMS-15ms
Depolarize Presynaptic *After* Postsynaptic
L4-5
C
200 pairs of stimuli
Baseline
30 min
0
10
20
30
Time (min)

Figure 2



B

Time (ms)	Control	Spinal Cord Injury	P values
Response Latencies			
M-wave (ms)	4.6 ± 0.6	4.9 ± 0.6	p = 0.23
F-wave (ms)	33.7 ± 2.4	35.9 ± 4.6	p = 0.11
MEP (ms)	30.0 ± 2.5	37.9 ± 4.3	p < 0.001
Conduction Times			
Central Conduction (ms)	10.8 ± 1.4	17.5 ± 4.9	p < 0.001
Peripheral Conduction (ms)	14.5 ± 1.2	15.5 ± 2.2	p = 0.14

Figure 3

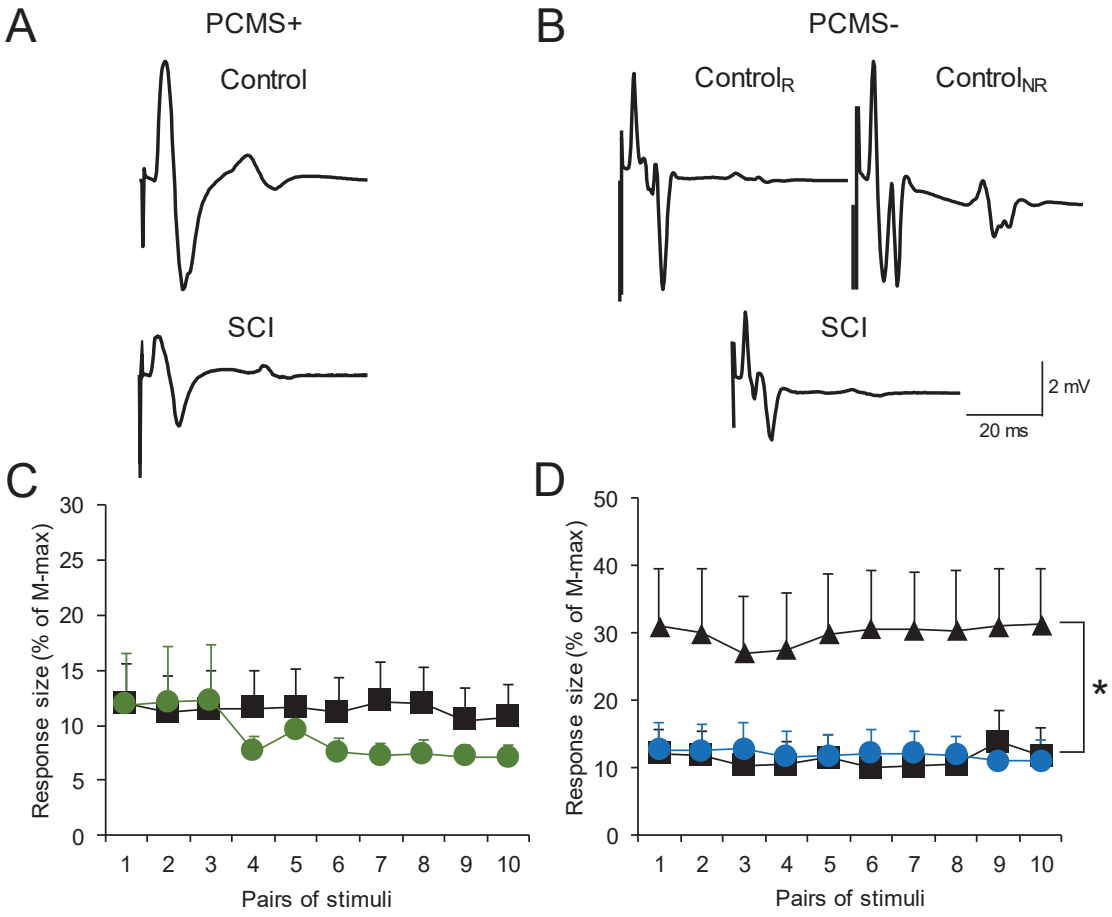

A
PCMS+
Control
SCI
B
PCMS-
Control$_R$
Control$_{NR}$
SCI
2 mV
20 ms
C
Response size (% of M-max)
Pairs of stimuli
D
Response size (% of M-max)
Pairs of stimuli
*

Figure 4

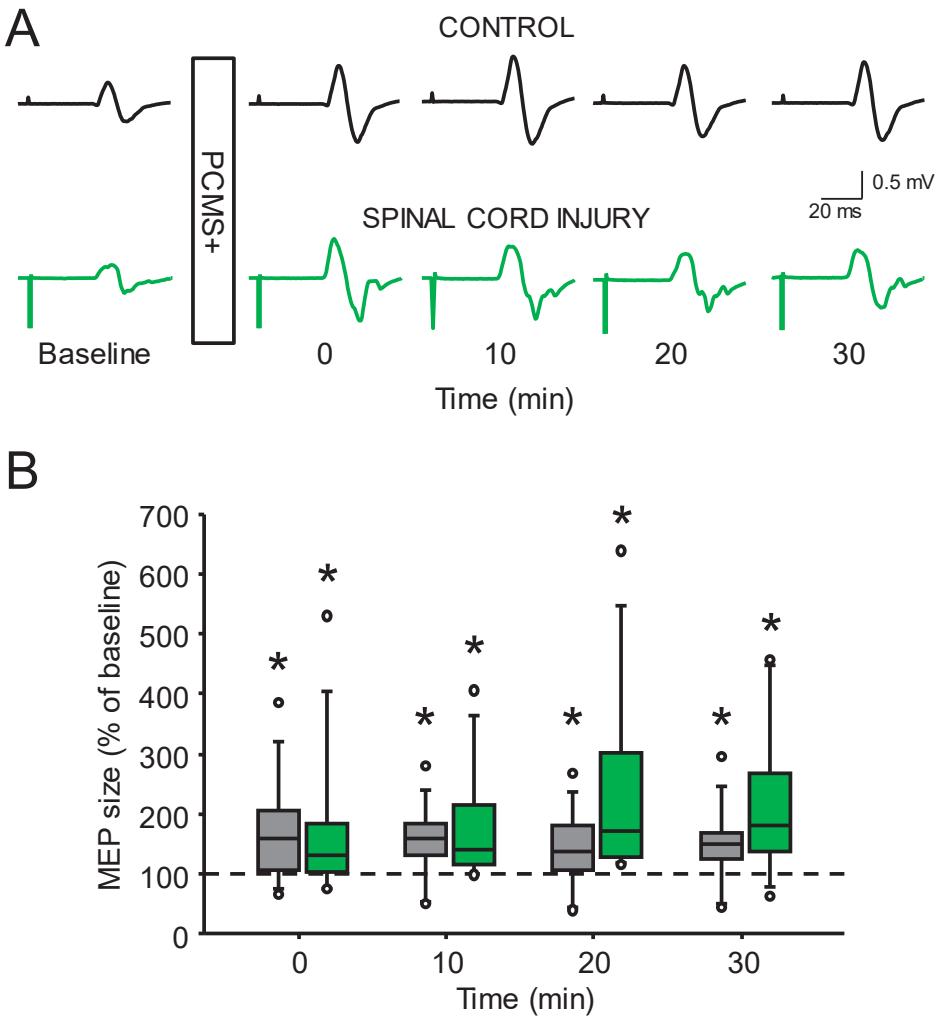

A
CONTROL
PCMS+
0.5 mV
20 ms
SPINAL CORD INJURY
Baseline
0
10
20
30
Time (min)
B
MEP size (% of baseline)
700
600
500
400
300
200
100
0
0
10
20
30
Time (min)

Figure 5

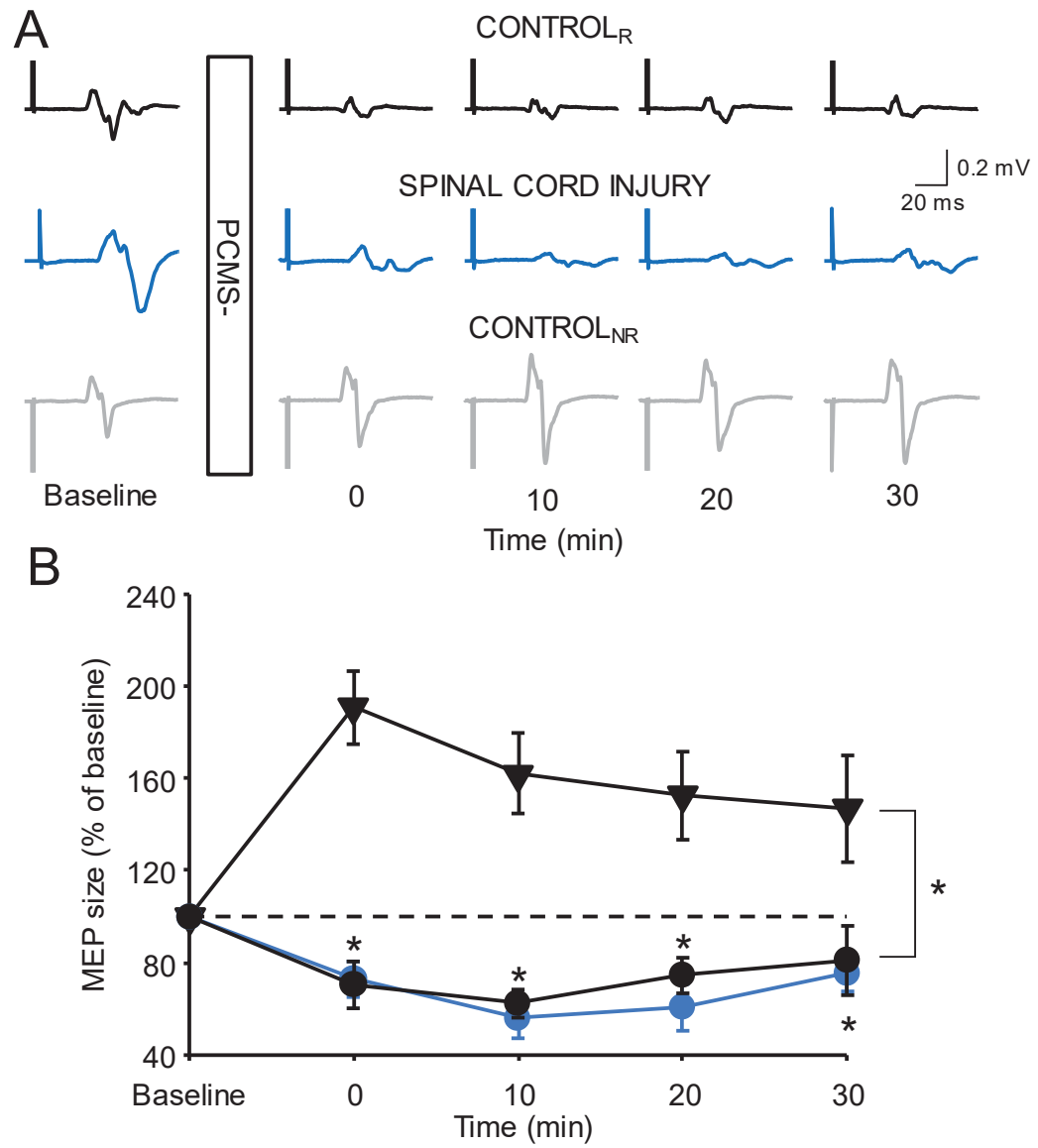

A
CONTROL$_R$
SPINAL CORD INJURY
CONTROL$_{NR}$
PCMS-
0.2 mV
20 ms
Baseline
0
10
20
30
Time (min)
B
MEP size (% of baseline)
240
200
160
120
80
40
Baseline
0
10
20
30
Time (min)
*

Figure 6

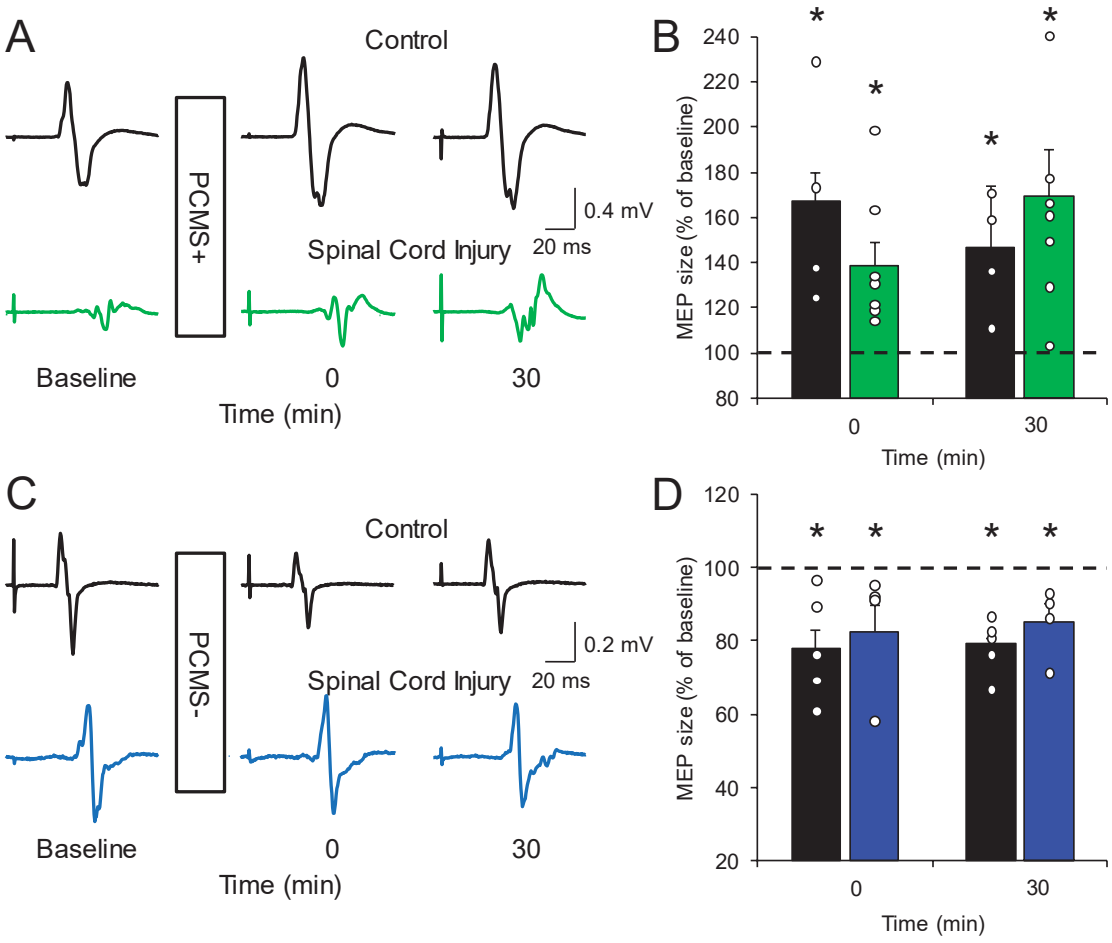

A
Control
PCMS+
0.4 mV
20 ms
Spinal Cord Injury
Baseline
0
30
Time (min)
B
MEP size (% of baseline)
240
220
200
180
160
140
120
100
80
0
30
Time (min)
C
Control
PCMS-
0.2 mV
20 ms
Spinal Cord Injury
Baseline
0
30
Time (min)
D
MEP size (% of baseline)
120
100
80
60
40
20
0
30
Time (min)

Figure 7

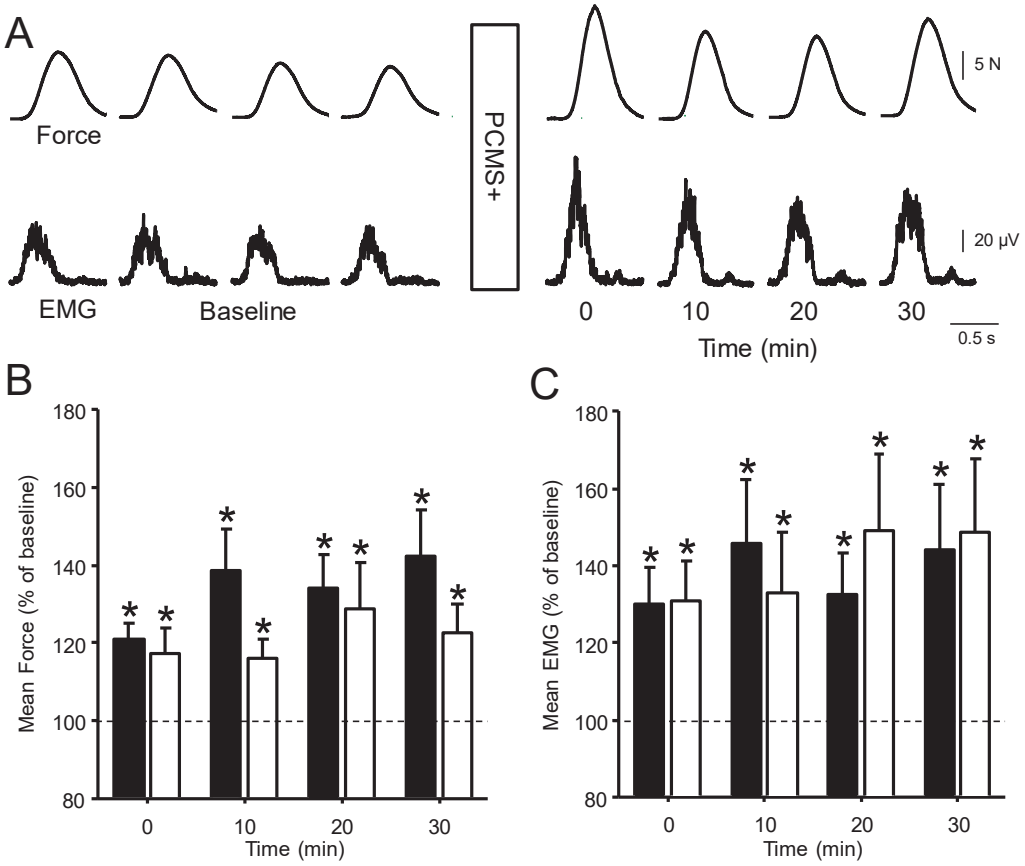

A
Force
EMG
Baseline
PCMS+
5 N
20 μV
0
10
20
30
Time (min)
0.5 s
B
Mean Force (% of baseline)
180
160
140
120
100
80
0
10
20
30
Time (min)
C
Mean EMG (% of baseline)
180
160
140
120
100
80
0
10
20
30
Time (min)

Table 1

Table 1. Spinal cord injury participants

Subject	Age (yrs)	Gender	Level	ASIA Score	Etiology	Time Post Injury (years)	TA MVC (mV)	Light Touch	Pin Prick	Spasm Frequency
1	31	F	T10	C	T	14	0.11	1	1	4
2	30	M	T9	D	T	9	0.19	1	0	4
3	42	M	C6	C	T	19	0.14	2	0	2
4	37	M	L3	A	T	13.2	0.55	2	2	0
5	35	M	C5	C	T	9.3	0.10	2	2	0
6	31	M	C2	D	T	7.9	0.25	2	2	2
7	58	M	T5	C	NT	19	0.11	1	1	4
8	44	M	T4	D	T	16.3	0.19	2	1	4
9	52	M	T11	D	T	19.3	0.34	1	1	0
10	49	M	C4	D	T	11.5	0.47	1	1	4
11	70	F	T6	C	NT	2.8	0.16	1	0	2
12	55	F	C2	D	T	2.9	0.55	1	0	2
13	66	M	C5	D	NT	5.5	0.34	1	0	4
14	36	M	C2	D	T	3.6	0.26	2	2	4
15	32	F	T11	D	T	1.3	0.41	1	2	1
16	44	M	C5	A	T	1	0.36	2	2	4
17	73	M	C2	A	T	7	0.16	0	0	2
18	64	M	C4	D	T	5.4	0.16	1	2	0

M = Male, F = Female, T = Traumatic, NT = Non-traumatic, TA = Tibialis Anterior, MVC = Maximum voluntary contraction, mV = Millivolts, Light Touch and Pin Prick; 1 = impaired, 2 = intact, Spasm Frequency Score: 0 = no spasms, 1 = one or fewer spasms per day, 2 = between 1 and 5 spasms per day, 3 = 5 to <10 spasms per day, and 4 = 10 or more spasms per day.