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Potentiating Paired Corticospinal-Motoneuronal Plasticity after Spinal Cord Injury

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Abstract

Background: Paired corticospinal-motoneuronal stimulation (PCMS) increases corticospinal transmission in humans with chronic incomplete spinal cord injury (SCI).

Objective/Hypothesis: Here, we examine whether increases in the excitability of spinal motoneurons, by performing voluntary activity, could potentiate PCMS effects on corticospinal transmission.

Methods: During PCMS, we used 100 pairs of stimuli where corticospinal volleys evoked by transcranial magnetic stimulation (TMS) over the hand representation of the primary motor cortex were timed to arrive at corticospinal-motoneuronal synapses of the first dorsal interosseous (FDI) muscle ~1-2 ms before antidromic potentials were elicited in motoneurons by electrical stimulation of the ulnar nerve. PCMS was applied at rest ($PCMS_{rest}$) and during a small level of isometric index finger abduction ($PCMS_{active}$) on separate days. Motor evoked potentials (MEPs) elicited by TMS and electrical stimulation were measured in the FDI muscle before and after each protocol in humans with and without (controls) chronic cervical SCI.

Results: We found in control participants that MEPs elicited by TMS and electrical stimulation increased to a similar extent after both PCMS protocols for ~30 min. Whereas, in humans with SCI, MEPs elicited by TMS and electrical stimulation increased to a larger extent after $PCMS_{active}$ compared with $PCMS_{rest}$. Importantly, SCI participants who did not respond to $PCMS_{rest}$ responded after $PCMS_{active}$ and those who responded to both protocols showed larger increments in corticospinal transmission after $PCMS_{active}$.

Conclusions: Our findings suggest that muscle contraction during PCMS potentiates corticospinal transmission. PCMS applied during voluntary activity may represent a strategy to boost spinal plasticity after SCI.

Key words: Neuroplasticity, spinal cord injury, corticospinal-motoneuronal, spike timing-dependent plasticity, neurophysiology, corticospinal

Introduction

Human spinal cord injury (SCI) is mostly anatomically incomplete leaving a residual corticospinal pathway. Corticospinal responses elicited by noninvasive brain stimulation have delayed latencies and higher thresholds in humans with SCI compared with control participants (1-3). Anatomical studies suggest that this is related, at least in part, to surviving corticospinal axons undergoing demyelination and progressive atrophy (4-6). These factors might all contribute to the difficulty of improving voluntary motor output after SCI, either via clinical rehabilitation or stimulation interventions. We recently showed that pairing noninvasive stimulation of the primary motor cortex and a peripheral nerve, a protocol referred to as paired corticospinal-motoneuronal stimulation (PCMS), enhance corticospinal transmission in humans with chronic SCI (3, 7). The goal of our study was to determine whether we could potentiate PCMS effects on corticospinal transmission.

Different strategies have been used in humans to potentiate aftereffects of induced-plasticity by noninvasive neuromodulatory approaches. For example, paired associative stimulation over the primary motor cortex combined with transcranial direct current stimulation was shown to enhance synaptic plasticity (8). Combining transcutaneous electrical stimulation of the spinal cord with exercise (9) or pharmacological agents with repeated stimulation protocols (10, 11) was found to prolong and/or potentiate the efficacy of induced-plasticity protocols. A key question in our study is which strategy might be appropriate to boost PCMS aftereffect on corticospinal transmission. PCMS elicits spike-timing dependent changes at spinal synapses of

somatic motoneurons in control (12, 13) and SCI (3, 7) participants. Spike-timing dependent plasticity (STDP) is a process by which synaptic strength can be potentiated by repeated pairs of presynaptic action potentials arriving prior to post-synaptic depolarizing action potentials (14, 15). In animals, STDP-like plasticity is thought to engage long-term potentiation (LTP)-like mechanisms that depend on N-methyl-D-aspartate (NMDA) (14, 15). In humans, STDP-like plasticity (elicited by PCMS) can be blocked by the NMDA antagonist dextromethorphan (16). NMDA receptors in the spinal cord can affect on the output of motoneurons (17) and small levels of tonic voluntary activity facilitate NMDA-mediated plasticity in the human primary motor cortex (18). In addition, voluntary muscle contraction increases the recruitment of descending volleys in corticospinal neurons (19) and decreases the threshold of spinal motoneurons, which are both altered in humans with SCI (20, 21). Thus, voluntary contraction can represent a method for increasing descending volleys and decreasing the threshold of spinal motoneurons. We hypothesized that voluntary muscle contraction increases the efficacy of PCMS by enhancing transmission in the corticospinal pathway at the spinal level.

To examine our hypothesis, we used PCMS, where pairs of transcranial magnetic stimulation (TMS) were precisely timed to reach the cortico-motoneuronal synapses of the first dorsal interosseous (FDI) muscle ~1-2 ms before antidromic potentials elicited in motoneurons by electrical stimulation of the ulnar nerve at rest ($PCMS_{rest}$) or during small levels of isometric index finger abduction ($PCMS_{active}$) on separate days in a randomized order.

Materials and Methods

Participants. Seventeen individuals with SCI (mean age 47.5±12.3 years, 4 female; Table 1) and 14 age-matched controls (mean age 40.9±16.9 years, p=0.22, 6 female) participated in the study. All participants gave informed consent to experimental procedures, which were approved by the local ethics committee at the University of Pittsburgh and the University of Miami. Participants with SCI had a chronic (≥1 year), cervical injury (C3–C8), an intact (score=2) or impaired (score=1), but not absent, innervation in dermatome C6 during light touch and pin prick stimulus using the American Spinal Cord Injury Association (ASIA) sensory scores and residual hand motor function. Two individuals with SCI were categorized as ASIA A (complete injury) due to the lack of sacral sparing (22) despite being able to elicit voluntary force with hand muscle and the other 14 individuals were classified as incomplete ASIA C and D. Participants were able to exert maximal voluntary contraction (MVC, measured as the highest mean rectified EMG activity found in 1 s during the MVC burst) isometric contractions into index finger abduction [controls=0.5±.3 mV, SCI=0.2±0.1 mV, $p<0.001$]. Note that one participant with SCI and one control was excluded from the analysis since they did not complete all TMS testing procedures and another control and SCI participant completed only the testing involving electrical stimulation (ES). Therefore, we report results for MEPs elicited by TMS in 15 SCI participants and 12 control subjects.

Electromyographic (EMG) recordings. EMG was recorded from the FDI muscle of the right side in controls and from the less affected hand in individuals with SCI through surface electrodes secured to the skin over the belly of each muscle (Ag-AgCl, 10 mm diameter). The signals were

amplified, filtered (20–1000 Hz), and sampled at 2 kHz for offline analysis (CED 1401 with Signal software, Cambridge Electronic Design). Force was sampled at 200 Hz.

Experimental setup. During testing, all participants were seated in an armchair with both arms relaxed and flexed at the elbow by 90° with the forearm pronated and the wrist and forearm restrained by straps. Participants participated in two PMCS protocols (i.e. $PCMS_{rest}$ and $PCMS_{active}$; Fig. 1A) in a randomized order separated by at least 2-3 days. For $PCMS_{rest}$, participants remained at rest for all electrophysiological measurements and during the stimulation protocol. For $PCMS_{active}$, measurements were taken at rest but PCMS was applied during 10% of MVC into index finger abduction in both groups (controls=12.1±2.9% of MVC, SCI=10.7±3.8% of MVC, p=0.15). The index finger was attached to a custom two-axis load cell (Honeywell), which measures finger abduction force. At the start of this experiment, participants performed 3 brief MVCs (3–5 s) with the index finger that were separated by 30 s. Maximal forces were used to set the targets for submaximal contractions during $PCMS_{active}$. Here, participants were instructed to remain at rest until they heard an auditory cue (given 2 s prior to the paired pulses), at which point they were asked to perform 10% of MVC into index finger abduction. Participants were instructed to hold the contraction until receiving the paired-pulses, after which they were asked to relax. Therefore, for each paired-pulse stimuli participants contracted for approximately 3-4 s and were relaxed for 7-6 s between contractions. Custom software (LabVIEW) was written to acquire signals from the load cell and to display visual feedback corresponding to rest and 10% of MVC in real time. Familiarization trials (without stimulation) were completed before both PCMS protocols to ensure that participants were able to complete the task. Participants were tested on the following electrophysiological measurements:

MEPs elicited by TMS, MEPs elicited by electrical stimulation of the primary motor cortex and/or the cervicomedullary junction, resting motor threshold (RMT), and maximal motor response (M-max). Measurements were tested before (baseline), immediately after (0 minutes) and 30 minutes after $PCMS_{rest}$ and $PCMS_{active}$.

PCMS. In each protocol, 100 pairs of stimuli were delivered every 10 s (~17 min, 0.1 Hz) where corticospinal volleys evoked by TMS over the hand representation of the primary motor cortex were timed to arrive at corticospinal-motoneuronal synapses of the FDI muscle ~1-2 ms before antidromic potentials evoked in motoneurons by peripheral nerve stimulation (PNS) of the ulnar nerve during $PCMS_{rest}$ and $PCMS_{active}$. Both PCMS protocols were intended to strengthen corticospinal transmission (3, 12).

TMS. Transcranial magnetic stimuli were delivered from a Magstim 200 stimulator (Magstim Company) through a figure-of-eight coil (loop diameter, 7 cm; type number SP15560) with a monophasic current waveform. TMS was delivered to the optimal scalp position for activation of the left or right FDI muscle. To identify the optimal scalp position for the FDI, the coil was held tangential to the scalp with the handle pointing backwards and 45° away from the midline. With this coil position, the induced current in the brain flowed in a posterior-anterior direction and probably produced D and early I wave activation of corticospinal neurons (23). The TMS coil was held to the head of the subject with coil holder (Manfrotto, NJ, USA), while the head was firmly secured to a headrest by straps to limit head movements. TMS stimuli were delivered at an intensity of 100% of the maximum stimulator output (3, 7) in both groups during both protocols.

PNS. Supra-maximum electrical stimulation was delivered to the ulnar nerve at the wrist (200 μs pulse duration, model DS7AH, Digitimer, Welwyn Garden City, UK). The anode and cathode were 3 cm apart and 1 cm in diameter with the cathode positioned proximally. The stimuli were delivered at an intensity of 120% (controls=64.3±21.0 mA and SCI=39.6±14.7 mV, p=0.003) of the M-max (controls=22.9±4.1 mV and SCI=11.9±5.3 mV, p<0.001).

TMS and PNS interstimulus interval (ISI). The ISI between TMS and PNS was set to allow descending volleys elicited by TMS to arrive at the presynaptic terminal of corticospinal neurons ~1-2 ms before antidromic PNS volleys in the motoneurons reached the dendrites during $PCMS_{rest}$ (controls=1.6±0.4 ms and SCI=1.7±0.5 ms, p=0.68) and $PCMS_{active}$ (controls=1.5±0.1 ms and SCI=1.6±0.4 ms, p=0.31, Table 2; Fig. 1). The methods by which the TMS and PNS volleys were timed to arrive at the spinal cord have been described previously (3). Briefly, the ISI was customized using the relative onset latencies of EMG responses to stimulation at different levels of the corticospinal pathway in each subject. FDI MEPs were elicited by stimulation of the primary motor cortex during 10% of MVC. Cervical roots (C-roots) were stimulated by TMS between the seventh cervical (C7) and first thoracic (T1) spinous processes (24, 25). The conduction time from the primary motor cortex to the synapse was calculated by adding to the latency from TMS of the cervical roots (C-root) to 1.5 ms [estimated time of synaptic transmission plus conduction to the nerve root at the vertebral foramina; 26, 27] and subtracting from the MEP latency [MEP – (C-root + 1.5)], as in our previous study (3). However, we want to note that F-wave latency may represent a more direct estimation of the conduction time from motoneurons to the muscle and needs to be considered for future studies. The conduction time from ulnar nerve stimulation to the spinal motoneurons

was calculated by subtracting the M-max latency from the C-root latency and adding 0.5 ms, the estimated time of antidromic conduction time from the vertebral foramina to the dendrites [(C-root – M-max)+ 0.5); 24]. The formula shown in Table 2 was used to calculate the ISI between TMS and PNS pulses, whereby PNS was given prior to TMS due to the increased PNS conduction time compared to central conduction time (CCT). MEP onset latencies during small levels of voluntary contraction (controls: p=0.12 and SCI: p=0.54), C-root (controls: p=0.32 and SCI: p=0.42), and M-max (controls: p=0.39 and SCI: p=0.33) were recorded at each session and remained similar across sessions in both groups.

MEPs elicited by TMS. RMT was defined as the minimal stimulus intensity required to induce MEPs greater than 50 μV peak-to-peak amplitude in at least 5/10 consecutive trials in the relaxed muscle [28; controls=48.8±7.1% of maximum stimulator output and SCI=64.3±17.2% of maximum stimulator output, p=0.003]. TMS intensity was set at 120% of RMT (controls=123.3±5.6% of RMT and SCI=119.4±7.3%, p=0.12). The same intensity was used after the paired stimulation. TMS pulses were delivered at 4 s intervals (0.25 Hz). Thirty MEPs were averaged for each time point before and after each protocol and peak-to-peak MEP amplitude was measured (controls, n=12; SCI, n=15).

MEPs elicited by Electrical Stimulation (ES). MEPs were elicited by using ES at the cervicomedullary junction and the primary motor cortex to make inferences about subcortical contributions to MEP size (29, 30). These two procedures were used because some participants preferred one or the other type of stimulation due to different comfort levels. In one group, MEPs were elicited by stimulating the corticospinal tract at the cervicomedullary junction by using high-voltage electrical current (100 μs duration, Digitimer DS7AH) passed between adhesive

Ag-AgCl electrodes fixed to the skin behind the mastoid process (controls, n=5 and SCI, n=3). The stimulation intensity was set to elicit an MEP of ~3% of the M-max at rest in the FDI muscle (controls: $PCMS_{rest}$=2.39±2.3, $PCMS_{active}$=2.8±1.5 % of the M-max; p=0.57; SCI: $PCMS_{rest}$=4.3±0.8%, $PCMS_{active}$=4.3±0.4% of the M-max; p=0.5). We monitored the stimulation to ensure that it was below the intensity required to activate the peripheral nerve directly by increasing the intensity and observing a decrease in latency. A decrease in latency of ~2 ms indicates that the response reflects a mixture of pre and post-synaptically activated motoneurons (29). The latency of cervicomedullary MEPs was shorter than the MEPs elicited by TMS in both groups (controls: MEPs elicited by TMS=23.3±0.4 ms, MEPs elicited by ES=18.6±1.6 ms; p=0.002; SCI: MEPs elicited by TMS=25.1±1.0 ms, MEPs elicited by ES=20.0±2.8 ms). MEPs were also elicited by ES at the level of the primary motor cortex using a high-voltage current (200 μs duration, Digitimer DS7AH) passed between brass electrodes (9 mm) fixed to the scalp with electrode conductive gel (SCI, n=5). Here, the cathode was located at the vertex and the anode 7 cm laterally. The stimulation intensity used before the paired stimulation was set to elicit an MEP of ~3% of the M-max at rest in the FDI muscle ($PCMS_{rest}$=2.3±1.1% of the M-max; $PCMS_{active}$=3.0±1.7% of the M-max; p=0.2, p=0.8, respectively). The latency of MEPs elicited by ES of the primary motor cortex were shorter than the MEPs elicited by TMS (MEPs elicited by TMS =26.5±1.5 ms, MEPs elicited by ES=24.3±1.5 ms; p=0.01). Since MEPs elicited by ES at both levels increased after both PCMS protocols, we grouped the data under MEPs elicited by ES using for statistical analysis a total of 5 control subjects and 8 SCI participants. Ten to 20 MEPs elicited by ES were averaged before and at each time point after PCMS at 4 s intervals. The same intensity was used after the paired stimulation.

Data analysis. Normal distribution was tested by the Shapiro-Wilk's test and homogeneity of variances by the Mauchly's test of sphericity. When sphericity could not be assumed, the Greenhouse- Geisser correction statistic was used. Repeated-measures analysis of variance (ANOVA) was performed to determine the effect of the GROUP (controls and SCI), PCMS ($PCMS_{rest}$ and $PCMS_{active}$), TIME (Baseline, 0, 30 min) on the size of MEPs elicited by TMS and ES, and mean rectified background EMG. Bonferroni *post hoc* tests were used to test for significant comparisons. Independent t-tests were used to compare central and peripheral conduction times, ISIs, age, M-max, force MVC, RMT, test stimulation intensities (TMS and electrical stimulation). T-test corrected values were used when equal variances were not assumed. Paired t-tests (two-tailed) were used to compare the size of MEPs elicited by electrical stimulation across PCMS protocols and MEP latencies. Significance was set at $p<0.05$. Group data are presented as the means±SD in the text. Pearson correlation analysis was used as needed.

Results

Responses elicited during $PCMS_{rest}$ and $PCMS_{active}$

Figures 2A-B shows responses elicited during the paired-stimulation from representative participants. Due to the similarity in latency between the F-wave and the FDI MEP, this response could represent the summation of MEPs elicited by TMS and F-waves elicited by PNS (black traces). For comparison, we also show F-waves elicited by PNS (grey traces). Note the larger size of responses during $PCMS_{active}$ compared with $PCMS_{rest}$ in both participants (SCI=1.2±0.8 mV, controls=5.0±2.6 mV; SCI=0.5±0.3 mV, controls=1.2±0.5 mV, respectively). Also, note that responses were larger in the control compared with the SCI participant (note scale

difference). Responses obtained during each of the 100 pairs of pulses were combined in 10 blocks of 10 pairs each for analysis.

A repeated measures ANOVA showed no effect of TIME ($F_{(3.0, 78.9)}$=1.0, p=0.44), an effect of GROUP ($F_{(1, 26)}$=20.2, p<0.001) but not in their interaction ($F_{(3.0, 78.9)}$=0.9, p=0.43) on responses measured during $PCMS_{rest}$ (Fig. 2C-D). Similarly, a repeated measures ANOVA showed no effect of TIME ($F_{3.2, 93.1)}$=1.7, p=0.17), an effect of GROUP ($F_{(1, 26)}$=35.1, p<0.001) but not in their interaction ($F_{(3.2, 93.1)}$=2.3, p=0.08) on responses measured during $PCMS_{active}$ (Fig. 2C-D). These results together indicate that the stimulation conditions were maintained constant across the 100 pairs of pulses in each protocol across groups. During $PCMS_{active}$, mean background EMG activity measured prior to the stimulation artefact was similar across groups (controls=12.1±2.9% of MVC, SCI=10.7±3.8% of MVC, p=0.15).

MEPs elicited by TMS

Figures 3A-B illustrates MEPs traces in a representative control and SCI participant before and after both protocols. Note that while the control participant shows a similar increase in MEP size immediately and 30 minutes after both PMC protocols, the SCI participant shows a greater increase in MEP size after $PCMS_{active}$ compared with $PCMS_{rest}$.

A three-way repeated measures ANOVA showed an effect of TIME ($F_{(1.6,39.0)}$=24.8, p<0.001) but not PCMS ($F_{(1,25)}$=1.2, p=0.29) or GROUP ($F_{(1,25)}$=1.6, p=0.23) on MEP size. The ANOVA also revealed a PCMS x GROUP interaction ($F_{(1,25)}$=6.4, p=0.02). *Post hoc* tests showed a difference in the magnitude of MEP facilitation between controls and SCI participants after $PCMS_{rest}$ (p=0.01) but not after $PCMS_{active}$ (p=0.52). Also, MEP facilitation after paired-stimulation was different between $PCMS_{rest}$ and $PCMS_{active}$ in SCI (p=0.01) but not in controls

(p=0.35) participants. We found a TIME x PCMS x GROUP interaction ($F_{(2,50)}$=4.6, p=0.02), showing that the groups performed differently as a function of time and PCMS protocol. In controls, MEPs were larger compared with the baseline ($PCMS_{rest}$: 1.7±0.6 mV; $PCMS_{active:}$ 1.6±0.9 mV) at both 0 ($PCMS_{rest}$: 2.5±0.9 mV; $PCMS_{active:}$ 2.2±1.2 mV, p=0.005; Fig. 3C) and 30 ($PCMS_{rest}$: 2.4±0.7 mV; $PCMS_{active:}$ 2.2±1.0 mV, p=0.006; Fig. 3C) minutes after $PCMS_{rest}$ and $PCMS_{active}$. In SCI participants, MEPs were larger compared with the baseline (0.5±0.5 mV) after 30 (0.6±0.6 mV, p=0.01; Fig. 3D) but not at 0 (0.5±0.5 mV, p=0.2; Fig. 3D) minutes after $PCMS_{rest}$. However, MEPs were larger compared with the baseline (0.5±0.4 mV) both at 0 (0.7±0.8 mV, p=0.003; Fig. 3D) and 30 (0.7±0.8 mV, p=0.001; Fig. 3D) minutes after after $PCMS_{active}$. These results demonstrate that voluntary contraction during PCMS enhances the facilitatory effects of the PCMS protocol on corticospinal excitability in SCI participants, while no differences were found in control participants.

MEPs elicited by ES

In order to test the effect of PCMS at a subcortical level, we measured MEPs elicited by electrical stimulation at the cervicomedullary junction and over the primary motor cortex. Since both these methods stimulate corticospinal axons directly we pooled the data (see methods). Figures 4A-B shows MEPs in a representative control and SCI participant before and after both protocols. Note that the control participants show a similar increase in MEP size after $PCMS_{active}$ and $PCMS_{rest}$. However, the SCI participant shows a greater increase in MEP size after $PCMS_{active}$ compared with $PCMS_{rest}$.

A three-way repeated measures ANOVA on ranks showed an effect of TIME ($F_{(2,22)}$=28.4, p<0.001), but not PCMS ($F_{(1,11)}$=2.3, p=0.2) or GROUP ($F_{(1,11)}$=0.0, p=0.9) on MEP

size. Importantly, we found a PCMS x GROUP interaction ($F_{(1,11)}$=6.5, p=0.03), where *post hoc* tests revealed this was due to a different between $PCMS_{rest}$ and $PCMS_{active}$ in SCI (p=0.01; Fig. 4D) but not in controls (p=0.5; Fig. 4C) participants. In controls, MEPs increased at 0 ($PCMS_{rest}$: p=0.02; $PCMS_{active}$: p=0.04; Fig. 4C) and 30 ($PCMS_{rest}$: p=0.02; $PCMS_{active}$: p=0.04; Fig. 4C) minutes after stimulation compared with baseline. Similarly, in SCI participants, MEPs increased at 0 ($PCMS_{rest}$: p=0.01; $PCMS_{active}$: p=0.03; Fig. 4D) and 30 ($PCMS_{rest}$: p=0.03; $PCMS_{active}$: p=0.04; Fig. 4D) minutes after stimulation compared with baseline. These findings indicate that $PCMS_{active}$ increases the effects of the paired-stimulation at a subcortical level after SCI, yet increases in subcortical excitability remain similar in controls irrespective of the protocol used.

Based on our previous results (3), we divided individuals with SCI into those who responded (MEP facilitation >110%; 9/15 participants; 3) and did not respond (MEP facilitation <110%; 6/15 participants) after $PCMS_{rest}$. Figure 5 shows the proportion of responders vs. non-responders after $PCMS_{rest}$ and $PCMS_{active}$ and the average MEP size (across 0 and 30 minutes) after $PCMS_{rest}$ and $PCMS_{active}$ in each subgroup. We found that non-responders showed that MEPs increased only after $PCMS_{active}$ (121.4±24.7% of baseline MEP; p=0.05 Fig. 5B) and this MEP change was significantly larger compared to $PCMS_{rest}$ (93.6±10.0% of baseline MEP; p=0.02; Fig. 5B). SCI responders increased MEP size after both $PCMS_{rest}$ (126.2±18.5% of baseline MEP; p=0.003; Fig. 5A) and $PCMS_{active}$ (179.0±64.3% of baseline MEP; p=0.006; Fig. 5D), indeed increases in MEP size were larger after $PCMS_{active}$ compared with $PCMS_{rest}$ (p=0.04; Fig. 5D). These results suggest that while voluntary contraction can improve the aftereffects of PCMS on corticospinal excitability after SCI, this might be more critical for SCI patients that do not respond to PCMS at rest.

Discussion

Our findings demonstrate that the effects of PCMS on spinal synaptic plasticity can be potentiated by applying PCMS during voluntary activity in humans with SCI. We found, in control participants, that the size of MEPs elicited by TMS and electrical stimulation increased when PCMS was applied at rest or during voluntary activity for ~17 min. Whereas, in humans with SCI, MEPs elicited by TMS and electrical stimulation increased to a larger extent when PCMS was applied during voluntary activity compared with rest. Note that SCI participants who did not respond to PCMS at rest responded to voluntary activity and those participants who responded to both protocols showed larger increments in corticospinal transmission when PCMS was applied during voluntary activity. Therefore, we propose that PCMS applied during a small level of voluntary activity may represent a strategy to boost spinal plasticity after SCI.

Differential effects of PCMS applied at rest and during voluntary activity after SCI

Our results agree with previous findings showing that PCMS applied at rest increases corticospinal transmission in upper-limb muscles in control (12, 13) and SCI (3) participants. Here, for the first time, we applied PCMS during small levels of tonic voluntary activity and found that PCMS was more effective in enhancing corticospinal transmission when applied during voluntary activity compared with rest in humans with chronic cervical incomplete SCI. This is consistent with previous results showing that NMDA-like plasticity in the human primary motor cortex can be enhanced by performing voluntary activity (18). PCMS is thought to engage long-term potentiation (LTP)-like mechanisms that depend on NMDA receptor activity (14, 15). In humans, PCMS effects on corticospinal transmission can be blocked by the NMDA antagonist dextromethorphan (16). NMDA receptors in the spinal cord can affect the output of motoneurons

(17) and activation of NMDA receptors can facilitate neurons that contribute to the generation of muscle activity after SCI (31, 32). The recruitment of descending volleys (i.e. D-waves and I-waves) tested by TMS is decreased in humans with SCI at rest (20) and during voluntary activity (21) compared with control participants. In addition, spinal motoneurons of individuals with chronic SCI are activated by prolonged depolarization compared with control participants (33). Thus, performing PCMS during tonic voluntary activity might enhance the number of descending volleys (compared with rest; 19) and decrease the threshold of spinal motoneurons contributing to a better stage for this plasticity. This is consistent with what we observed during the paired stimulation. Here, the response following the M-max during PCMS could be due to a summation of an MEP and an F-wave, since the latency of these responses is similar (~25 ms). Interestingly, the size of the response at rest was approximately 1 mV in controls, which is substantially less than MEP-max (~6 mV), suggesting that the MEP is less likely to be the only factor contributing to this response. Collision between the antidromic potentials and the MEP and refractoriness could also contribute to the size of the response following the M-max during PCMS. This is particularly relevant for $PCMS_{active}$, where a collision between voluntary orthodromic and antidromic impulses may allow some motor axons to transmit an H-reflex to the muscle. Although the size of the response following the M-max during paired stimulation is larger in controls and SCI subjects during $PCMS_{active}$ compared with $PCMS_{rest}$, selective effects were observed only in SCI participants. We previously suggested that the measured response reflects in part the contribution from an F-wave (3) since TMS facilitates spinal motoneuron excitability when applied at a similar inter-stimulus interval before PNS (34, 35). Thus, our results support the idea that a decrease in the threshold of spinal motoneurons results in a larger activation by descending input (19).

We also found that the size of MEPs elicited by ES at the cervicomedullary junction and the primary motor cortex increased after PCMS applied at rest and during voluntary activity. Electrical stimulation of the primary motor cortex likely activates axons of pyramidal tract neurons in the subcortical white matter at the axon initial segment at low intensity while at high intensities, as used in our study, I-waves are also observed (30). However, MEPs elicited by stimulation of the corticospinal tract at the cervicomedullary junction likely reflect changes occurring at corticospinal-motoneuronal synapses (29, 36). Since we used both types of stimulation, a possible interpretation of our results is that changes in MEPs elicited by electrical stimulation after PCMS have a subcortical origin, which is consistent with previous studies (3, 7, 12).

An interesting finding in SCI participants was that both PCMS applied at rest and during voluntary activity increased MEP size, but the changes were more pronounced when PCMS was applied during voluntary activity. Since we observed in SCI participants that MEPs elicited by electrical stimulation were larger after PCMS was applied during voluntary activity compared with rest, we propose that this change also has a subcortical origin. Multiple descending volleys are evoked during TMS and voluntary contraction. PCMS protocols have targeted the initial descending volleys arriving at motoneurons because estimations are based on the latency of MEPs elicited during a small level of voluntary contraction (3, 12), but the timing of both early and later volleys might be important to potentiate and depress some corticospinal synapses. Thus, changes in the number and size of descending volleys elicited by voluntary contraction (19) might also contribute to the larger increases in MEPs elicited by electrical stimulation when PMCS was applied during contraction. It is possible that in control participants this plasticity was saturated (i.e., ceiling effects). For example, during 100 pairs of stimuli, the TMS intensity

was set at 100% of maximum stimulator output, which matched an effective intensity previously used in SCI participants (3). This agrees with evidence showing that high-intensity TMS increases the number and amplitude of descending volleys (including direct (D) waves and I-waves; 19). However, Taylor and Martin (2009) showed that in controls much lower intensities and few pulses were required to produce the similar PCMS effects in corticospinal transmission to those found in our study (approximately 68% of maximum stimulator output, 50 pairs of pulses). Thus, another possibility is that if we had used lower TMS intensities, which preferentially produce smaller descending volleys, we may have seen PCMS potentiation with the addition of muscle contraction. Indeed, a weak voluntary contraction during 50 pairs of time-dependent stimuli that targeted the sensorimotor cortex showed significantly increased corticospinal output compared to when the same protocol was applied at rest (18).

In the current study, control participants revealed an increase in FDI MEPs of approximately 40% after PCMS at rest, which is comparable to changes previously reported in the same (3) and in a more proximal muscle (12). We found here that 60% of individuals with SCI showed increases in MEP size 30 min after PCMS was applied at rest. This is consistent with our previous results where we found that 89% of individuals with SCI showed increases in MEP size (3). The lower number of responders in the present study compared with our previous results might be related to the population of SCI participants tested in our study. SCI participants tested in the present study had reduced MEP-max, a reduced M-max and weaker finger MVCs compared with participants tested before (3). Despite these factors, MEPs elicited by TMS and electrical stimulation did increase up to 20% for 30 minutes in this particular group of individuals with SCI. Thus, in this more impaired group of SCI participants, PCMS at rest can evoke corticospinal plasticity.

Functional considerations

Our results support previous findings that after SCI corticospinal drive is impaired, as evidenced by delayed MEP latencies, high stimulation thresholds, decreased maximum MEPs amplitudes, and impaired corticospinal recruitment (1, 2). Specifically, delayed MEP latency and increased thresholds suggest that there is a slowing of corticospinal neurons, likely due to demyelination and progressive axon atrophy (4-6). All of these factors are affected by SCI and might contribute to the efficacy of stimulation strategies after SCI including PCMS. Several neuromodulatory stimulation strategies have been used to improve the control of upper-limb muscles following SCI. For example, epidural electrical stimulation of the cervical spinal cord (38), pairs of TMS pulses targeting late I-waves (39), and high-frequency repetitive TMS over the hand primary motor cortex (40) with and without exercise. We and others have shown that the spinal cord is a critical site for the restoration of function after SCI (3, 7, 41, 42). We expand these results by showing that these effects can be potentiated by performing this protocol in conjunction with a voluntary contraction. Here, voluntary contraction could represent a method for increasing the size and number of descending volleys and decreasing the threshold of spinal motoneurons. Our new data suggests that it might be particularly important for SCI participants who have significantly reduced corticospinal drive.

Acknowledgments. We thank the spinal cord injury individuals for their participation in this study. Funding: This work was supported by funding from the National Institute of Neurological Disorders and Stroke–National Institutes of Health (NINDS R01 NS090622, NINDS R01 NS076589) and the Department of Veterans Affairs (Veteran Affairs RR&D Merit Review I01RX002474, Veterans Affairs RR&D Merit Review I01RX001807).

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Figure legends

Figure 1. PCMS stimulation protocols. Illustration of the PCMS protocol tested at rest ($PCMS_{rest}$; A) and during 10% of isometric maximal voluntary contraction (MVC) into index finger abduction ($PCMS_{active}$; B). In both PCMS protocols, corticospinal neurons were activated at the cortical level using transcranial magnetic stimulation (TMS) delivered over the hand representation of the primary motor cortex and spinal motoneurons were activated antidromically by peripheral nerve stimulation (PNS) delivered to the ulnar nerve at the wrist. The inter-stimulus interval (ISI) between the paired-pulses was designed to allow descending volleys to arrive at the presynaptic terminals of corticospinal neurons (1st, red arrow) 1-2 ms before antidromic volleys in the motoneurons reached the dendrites (2nd, black arrow). During $PCMS_{rest}$ participants remain at rest shown by the quiet background electromyographic (EMG) activity (A). During $PCMS_{active}$ participants performed 10% of MVC into index finger abduction in response to an auditory cue given 2 s prior to the pairs of stimuli (B). Here, participants were instructed to hold the contraction until receiving the paired-pulses, after which they were asked to relax. For each paired-pulse stimuli, participants contracted for approximately 3-4 s and were relaxed for 7-6 s between contractions.

Figure 2. Responses during $PCMS_{rest}$ and $PCMS_{active}$. EMG recordings from the first dorsal interosseous (FDI) muscle showing a representative average of the maximal motor response (M-max) and a subsequent response during each paired-pulse stimulation protocol (black traces) and

during isolated PNS stimulation (grey traces) in a control (A) and an SCI (B) participant. Due to the similarity in latency between the F-wave and the FDI motor evoked potential (MEP), this response could be due to the summation of MEPs elicited by TMS and F-waves elicited by PNS. The graphs show the group data in controls (C, n=14, open and closed triangles) and SCI (D, n=17, open and closed circles) participants. The ordinate shows the size of the response in millivolts and the abscissa shows the paired-pulses tested (a total of 100 paired pulses). At each point, the average of ten responses is shown. Note the difference in the amplitude scale in traces and graphs. Error bars indicate the SE. *$p<0.05$.

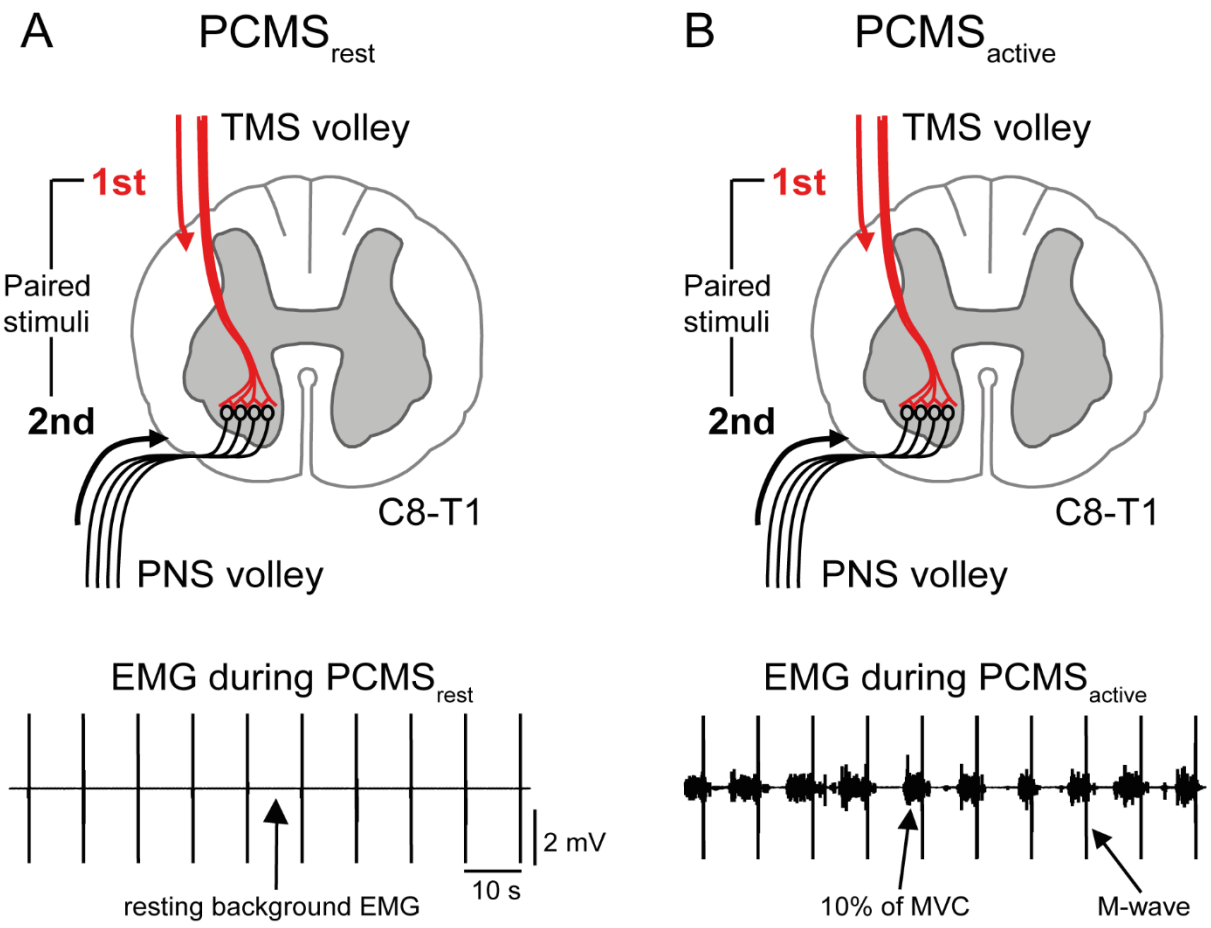
Figure 3. MEPs elicited by TMS. Raw MEP traces from the FDI muscle elicited by TMS in a representative control (A) and SCI (B) participant before and after $PCMS_{rest}$ and $PCMS_{active}$. Traces show the average of 30 MEPs. The grey horizontal bar shows the paired stimulation (paired-pulse stimuli; 100 paired pulses at 0.1 Hz for ~17 min). Graphs show the group data (C & D). The abscissa shows the time of measurements (0 and 30 min) and the ordinate shows the peak-to-peak amplitude of FDI MEPs as a % of the baseline MEP in controls (C; black bars, $PCMS_{rest}$; grey bars, $PCMS_{active}$; n=13) and in SCI participants (D; black bars, $PCMS_{rest}$; grey bars, $PCMS_{active}$; n=15). Averaged MEP size for each individual subject at each time point is also shown in each graph (open circles). Note that MEP size increased at all times after both PCMS protocols in control participants, but MEPs were larger after $PCMS_{active}$ compared with $PCMS_{rest}$ in SCI participants. Error bars indicate the SE *$p<0.05$.

Figure 4. MEPs elicited by electrical stimulation (ES). Raw MEP traces from the FDI muscle elicited by electrical stimulation in a representative control (A) and SCI (B) participant before

and after both PCMS protocols. The grey horizontal bar shows the paired stimulation (paired-pulse stimuli; 100 paired pulses at 0.1 Hz for ~17 min). Graphs show the group data (C & D). The abscissa shows the time of measurements (0 and 30 min) and the ordinate shows the peak-to-peak amplitude of FDI MEPs, elicited by electrical stimulation, as a % of the baseline MEP in controls (C; black bars, $PCMS_{rest}$ and grey bars, $PCMS_{active}$; n=5) and SCI (D; black bars, $PCMS_{rest}$ and grey bars, $PCMS_{active}$; n=8) participants. Averaged MEP size for each individual subject at each time point is also shown (open circles: MEPs elicited by ES over the cervicomedullary junction; open squares: MEPs elicited by ES over the primary motor cortex). Note that MEP size increased at all times after both PCMS protocols in controls, but MEPs were larger after $PCMS_{active}$ compared with $PCMS_{rest}$ in SCI participants. Error bars indicate the SE *$p<0.05$.

Figure 5. MEPs in responders and non-responders. SCI participants were grouped into responders and non-responders based on the amount of TMS MEP facilitation after $PCMS_{rest}$. A-B. Pie charts show the proportion of responders (n=9) and non-responders (n=6). C-D. Graphs show the subgroup data. The abscissa shows the paired-pulse protocol and the ordinate shows the average (across 0 and 30 minutes) FDI MEP size in responders (A; open bar: $PCMS_{rest}$; closed bar: $PCMS_{active}$) and in non-responders (B; open bar: $PCMS_{rest}$; closed bar: $PCMS_{active}$) as a % of baseline MEP. Note that in responders, the size of MEPs increased after $PCMS_{rest}$ and further increased after $PCMS_{active}$ whereas in non-responders MEP size increased only after $PCMS_{active}$. Error bars indicate the SE *$p<0.05$.

Figure 1


A
PCMS rest
TMS volley
1st
Paired stimuli
2nd
C8-T1
PNS volley
EMG during PCMS rest
2 mV
10 s
resting background EMG
B
PCMS active
TMS volley
1st
Paired stimuli
2nd
C8-T1
PNS volley
EMG during PCMS active
10% of MVC
M-wave

.

Figure 2

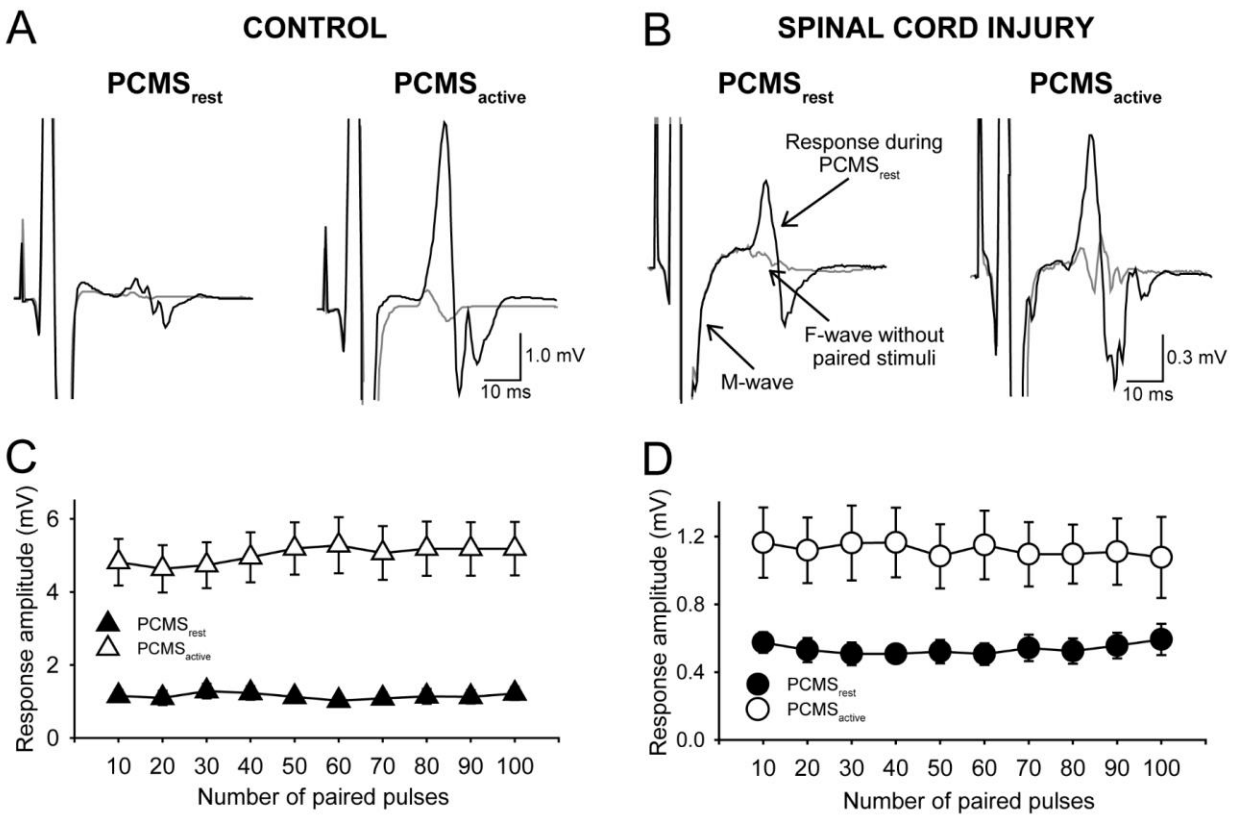

A
CONTROL
PCMS rest
PCMS active
1.0 mV
10 ms
B
SPINAL CORD INJURY
PCMS rest
PCMS active
Response during PCMS rest
F-wave without paired stimuli
M-wave
0.3 mV
10 ms
C
Response amplitude (mV)
PCMS rest
PCMS active
Number of paired pulses
D
Response amplitude (mV)
PCMS rest
PCMS active
Number of paired pulses

Figure 3

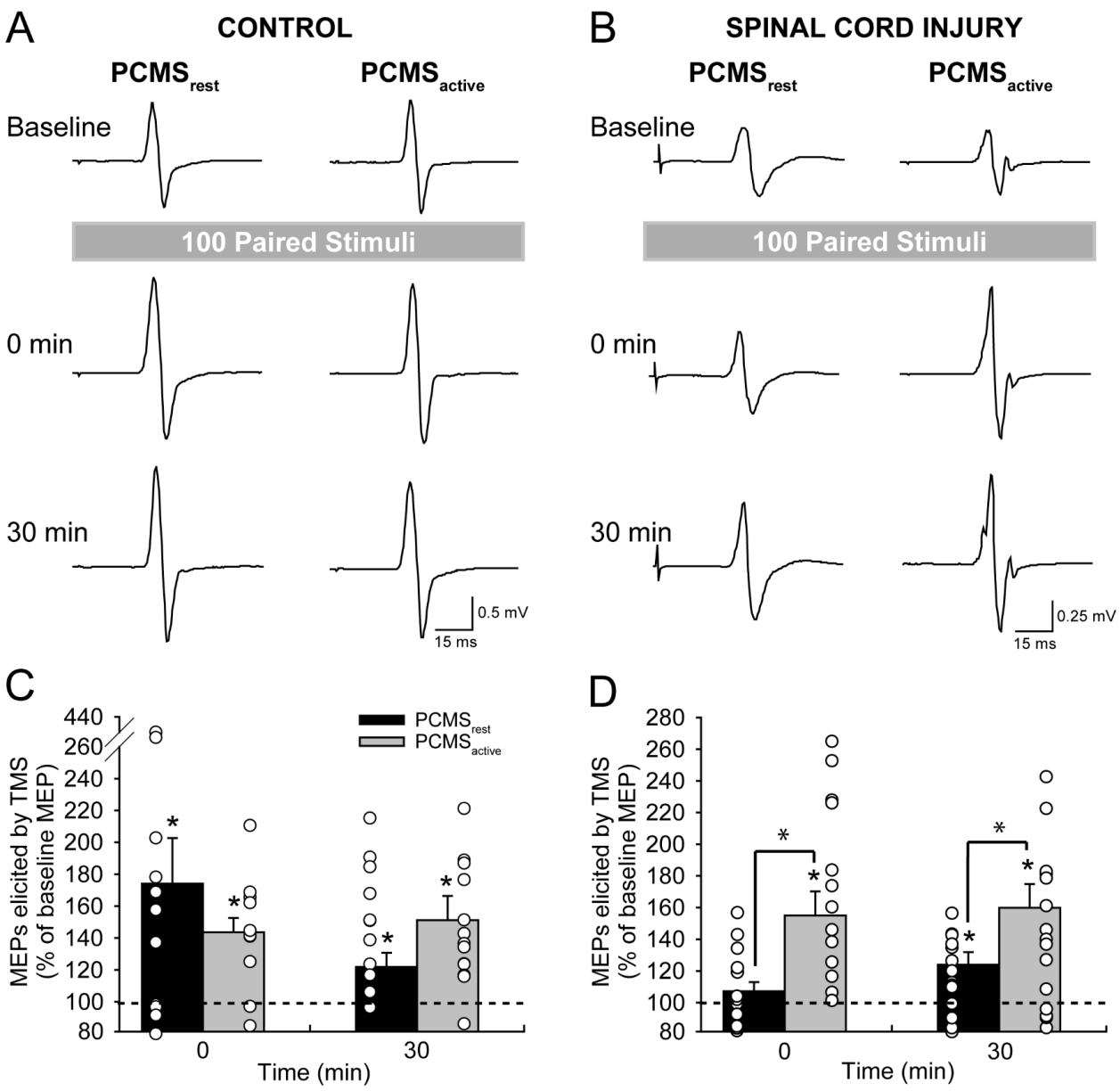

A
CONTROL
PCMS rest
PCMS active
Baseline
100 Paired Stimuli
0 min
30 min
0.5 mV
15 ms
B
SPINAL CORD INJURY
PCMS rest
PCMS active
Baseline
100 Paired Stimuli
0 min
30 min
0.25 mV
15 ms
C
PCMS rest
PCMS active
MEPs elicited by TMS (% of baseline MEP)
440
260
240
220
200
180
160
140
120
100
80
0
30
Time (min)
D
MEPs elicited by TMS (% of baseline MEP)
280
260
240
220
200
180
160
140
120
100
80
0
30
Time (min)

Figure 4

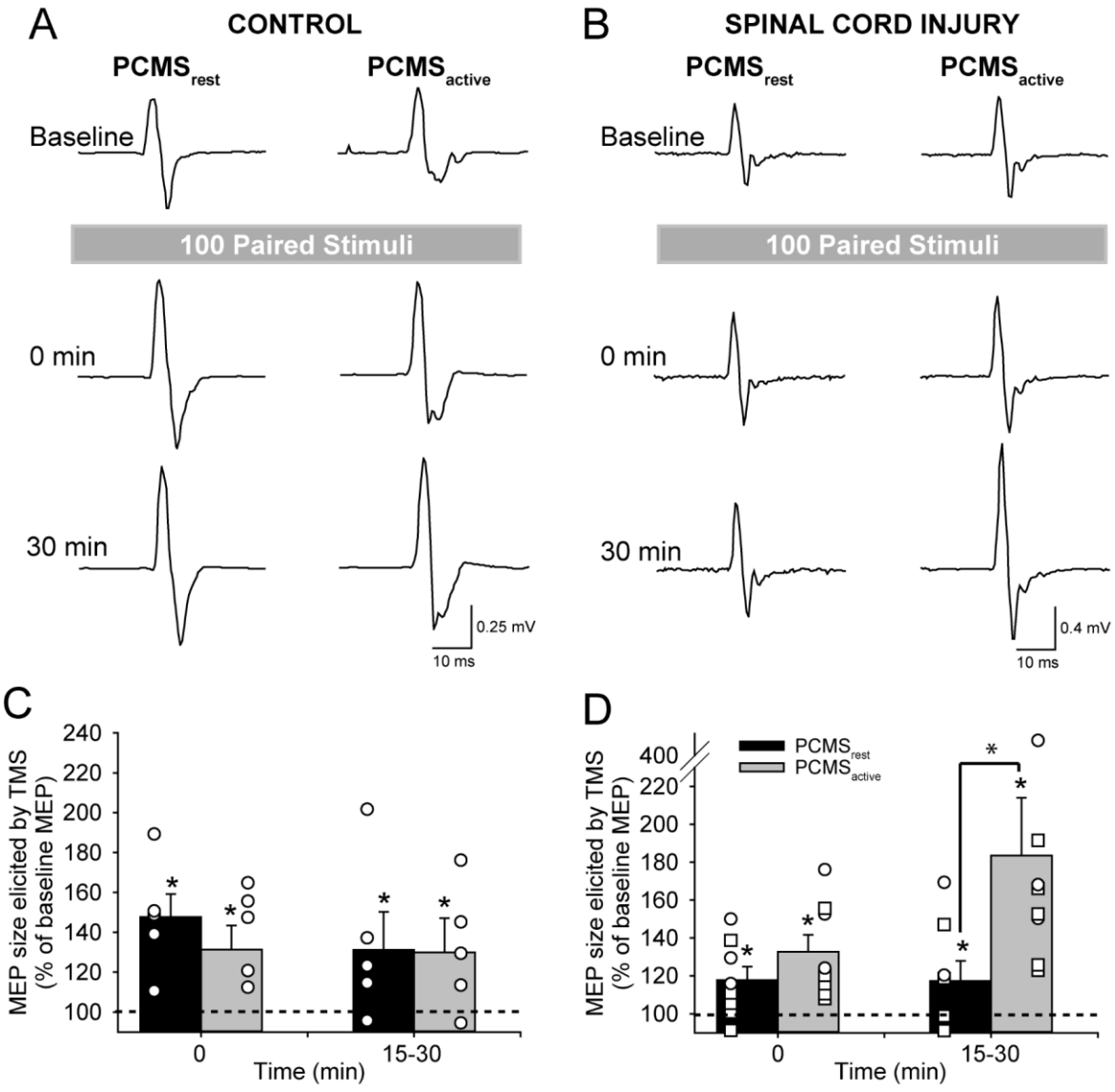
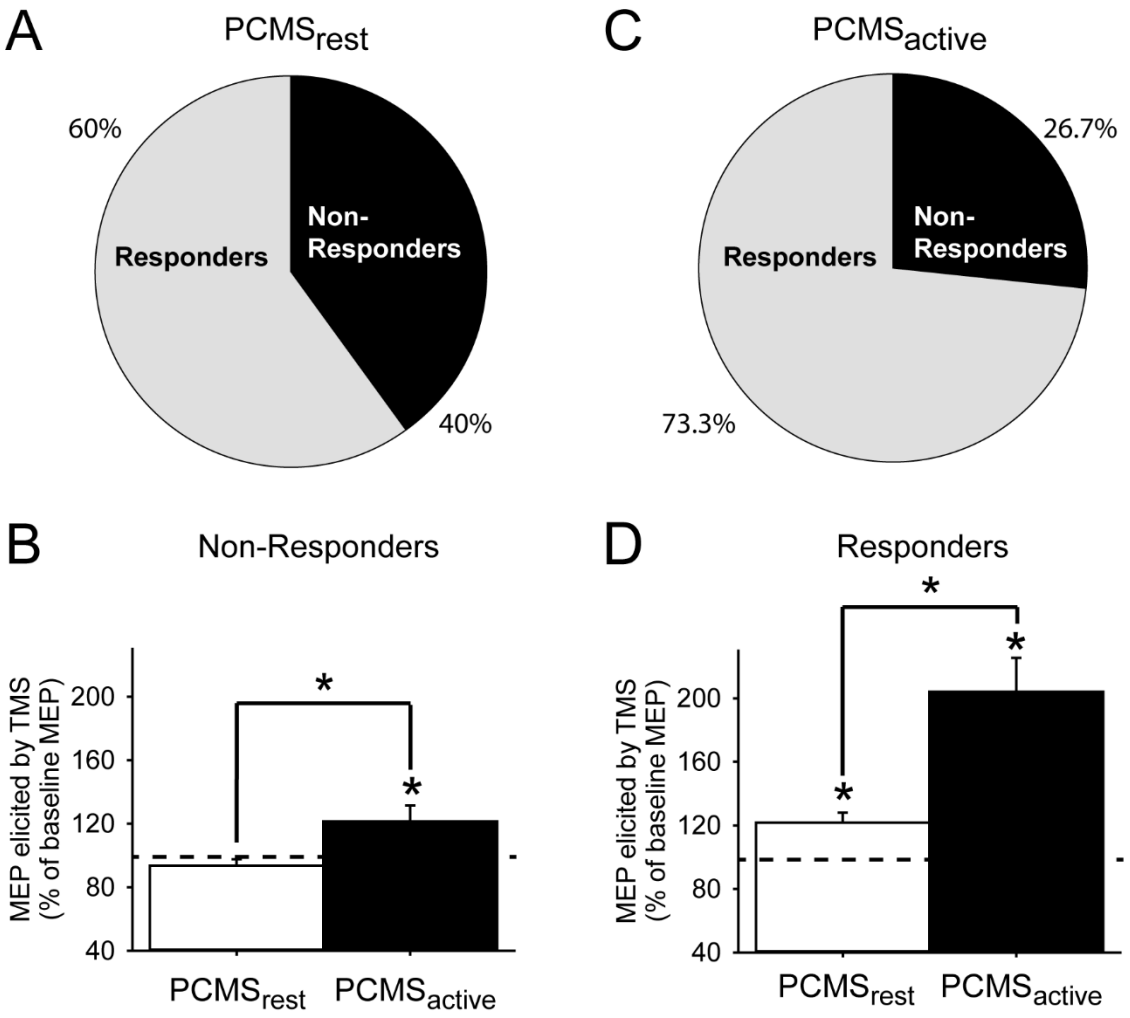

A
CONTROL
PCMS rest
PCMS active
Baseline
100 Paired Stimuli
0 min
30 min
0.25 mV
10 ms
B
SPINAL CORD INJURY
PCMS rest
PCMS active
Baseline
100 Paired Stimuli
0 min
30 min
0.4 mV
10 ms
C
MEP size elicited by TMS (% of baseline MEP)
240
220
200
180
160
140
120
100
0
15-30
Time (min)
D
PCMS rest
PCMS active
MEP size elicited by TMS (% of baseline MEP)
400
220
200
180
160
140
120
100
0
15-30
Time (min)

Figure 5


A
PCMSrest
60%
Responders
Non-
Responders
40%
C
PCMSactive
26.7%
Non-
Responders
Responders
73.3%
B
Non-Responders
MEP elicited by TMS
(% of baseline MEP)
200
160
120
80
40
*
*
PCMSrest
PCMSactive
D
Responders
MEP elicited by TMS
(% of baseline MEP)
200
160
120
80
40
*
*
PCMSrest
PCMSactive