



Published in final edited form as:

Exp Neurol. 2021 January ; 335: 113483. doi:10.1016/j.expneurol.2020.113483.

Acute Intermittent Hypoxia Boosts Spinal Plasticity in Humans with Tetraplegia

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Abstract

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Paired corticospinal-motoneuronal stimulation (PCMS) elicits spinal synaptic plasticity in humans with chronic spinal cord injury (SCI). Here, we examined whether PCMS-induced plasticity could be potentiated by acute intermittent hypoxia (AIH), a treatment also known to induce spinal synaptic plasticity in humans with chronic incomplete cervical SCI.

During PCMS, we used 180 pairs of stimuli where corticospinal volleys evoked by transcranial magnetic stimulation 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. During AIH, participants were exposed to brief alternating episodes of hypoxic inspired gas (1 min episodes of 9% O₂) and room air (1 min episodes of 20.9% O₂). We examined corticospinal function by measuring motor evoked potentials (MEPs) elicited by cortical and subcortical stimulation of corticospinal axons and voluntary motor output in the FDI muscle before and after 30 min of PCMS combined with AIH (PCMS+AIH) or sham AIH (PCMS+sham-AIH). The amplitude of MEPs evoked by magnetic and electrical stimulation increased after both protocols, but most after PCMS+AIH, consistent with the hypothesis that their combined effects arise from spinal plasticity. Both protocols increased electromyographic activity in the FDI muscle to a similar extent. Thus, PCMS effects on spinal synapses of hand motoneurons can be potentiated by AIH. The possibility of different thresholds for physiological vs behavioral gains needs to be considered during combinatorial treatments.

Keywords

Spinal cord injury; plasticity; acute intermittent hypoxia; paired stimulation

Introduction

Pairing noninvasive stimulation of the primary motor cortex and a peripheral nerve (paired corticospinal-motoneuronal stimulation; PCMS) enhances corticospinal synaptic transmission and voluntary motor output in humans with chronic SCI (Bunday and Perez, 2012; Bunday et al., 2018; Jo and Perez, 2020; Urbin et al., 2017). PCMS-induced plasticity in humans with chronic SCI is enhanced by applying stimulation during mild tonic voluntary activity (Bunday et al., 2018) or by combining stimulation with exercise training (Jo and Perez, 2020). Thus, PCMS may have maximal benefits in motor recovery when paired with other treatments, what it remains unclear which combinatorial treatments would optimize PCMS enhancement of corticospinal synaptic transmission.

The question that we addressed in the present study is how we can enhance the effect of PCMS to maximize recovery in humans with SCI. One possibility is to combine PCMS with a therapy which has its aftereffects mediated through similar neural mechanisms and would possibly boost PCMS-mediated spinal plasticity. PCMS pairs noninvasive stimulation of the primary motor cortex and a peripheral nerve, enhancing corticospinal transmission at the spinal cord level, likely by engaging spike-timing dependent plasticity (STDP) mechanisms that depend on N-methyl-D-aspartate (NMDA) receptor activation (Donges et al., 2017). PCMS predominantly affects low-threshold motoneurons (D'Amico et al., 2017), and its aftereffects in humans with SCI can be potentiated by decreasing the threshold for

control subjects (Note that data in aged-matched control obtained from Bunday and Perez, 2012; controls=0.5±0.3 mV, SCI=0.3±0.1 mV, p<0.001). Each subject underwent all testing sessions at the same time of the day and the majority of subjects were tested in the morning. All subjects (n=16) participated in the two main experimental sessions where we examined the effect of PCMS+AIH and PCMS+sham-AIH on the amplitude of MEPs elicited by TMS. Later, in a subgroup of subjects, we examined the effect of both interventions on the amplitude of MEPs elicited by electrical stimulation (n=9), and small levels of voluntary motor output and MVCs (n=10).

EMG and force recordings.

EMG was recorded from the first dorsal interosseous (FDI) through surface electrodes (Ag–AgCl, 10 mm diameter) in a muscle tendon montage with one electrode placed over the belly of the muscle. All signals were amplified, filtered (30–1000 Hz), and sampled at 2 kHz for off-line analysis (Cambridge Electronic Design 1401 with Signal software v4.1). At the start of the experiment, subjects were instructed to perform 3 brief MVCs for 3–5 s into index finger abduction, separated by 2 min of rest. Participants were verbally cued to commence the contraction by the experimenter who also provided strong verbal encouragement throughout the contraction. EMG data were processed offline calculating the mean rectified EMG over a 100 ms window centered over peak EMG. In brief, EMG digitized signals were rectified and smoothed using a 4th order Butterworth filter and low-passed at 5 Hz (Trumbower et al., 2012). Peak EMG was found through visual inspection and the mean value within a window ranging from 50 ms before to 50 ms after the peak was noted. Background EMG was subtracted from this value and the average of the 3 trials was noted. Three MVCs were also measured at baseline and 3 MVCs were collected 30 min after each intervention.

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 (Fig. 1A). Participants participated in two sessions in a randomized order separated by at least one week: PCMS combined with AIH (PCMS+AIH) and PCMS combined with sham AIH (PCMS+sham-AIH). During both sessions, participants remained at rest for all electrophysiological measurements taken before and after each protocol (Fig. 1B). PCMS was applied using a paired pulse paradigm previously described (Bunday and Perez, 2012; Taylor and Martin, 2009; Urbin et al., 2017).

PCMS.

A total of 180 pairs of stimuli were delivered every 10 s (~34 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 spikes evoked in motoneurons by supramaximal peripheral nerve stimulation (PNS) of the ulnar nerve. Magnetic stimuli were delivered with 100 % of the maximal stimulator output (MSO). PNS was set at an intensity of 150% of the intensity needed to evoke the maximal motor response (M-max) in the FDI muscle. Inter-stimulus intervals (ISIs) between TMS and PNS were tailored to individual subjects based on

central (CCT) and peripheral (PCT) conduction times calculated from latencies of MEPs, C-root, F-wave, and M-max (Fig. 2). In 6 out of 16 participants, the ISI was calculated based on F-waves instead of C-root stimulation. This was done upon participant's request based on preferred stimulation site and was kept consistent across all sessions. There was no significant difference in ISIs calculated based on F waves (6.1 ± 1.4) and C-root stimulation (5.1 ± 1.0 , $p=0.2$). At ISIs of ~ 5.5 the F-wave resulting from the antidromic activation of the motoneuron somas and potentials evoked from magnetic stimulation cannot be distinguished. The peak-to-peak amplitude of the combined response was analyzed and expressed in relation to M-max for each subject. For statistical comparisons the responses to the 180 paired pulses were pooled in blocks of 10 resulting in 18 data points for each participant (Fig. 3C; Bunday and Perez, 2012). MEP latencies were recorded during isometric 10% of MVC index finger abduction to determine the shortest and clearest response for our estimations. C-root potentials, F-waves and M-max latencies were investigated during rest. For MEPs, C-root potentials and M-max, 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, whereas the earliest onset latency of 40 F-waves was used. Cervical roots were stimulated using the same figure-of-eight coil with the outer edge of the coil placed between C7 and T1 spinous processes with the handle in a horizontal position pointing away from the cervical spine (Schmid et al., 1990; Inaba et al., 2002). Potentials evoked at the most proximal part of the C-roots were identified by increasing stimulation intensities in 5% increments until a consistent small response appeared. F-waves were measured using supramaximum stimulus intensity to the ulnar nerve at the wrist (200 μ s pulse duration, DS7A; Digitimer) with a monopolar bar electrode with the cathode positioned proximally. The stimuli were delivered at 1 Hz at an intensity of 150% of the M-max. The M-max was similar across sessions (PCMS+AIH=13.5 mV, PCMS+sham-AIH=12.9 mV, $p=0.4$). For each trial, we quantified each F-wave's latency. F-wave trials were filtered using a 2nd order Bessel high-pass filter (200 Hz) to 'flatten the tail of the M-wave' (Khan et al., 2012). M-max was acquired using our regular filter parameters (30–1000 Hz). Sixty F-waves were tested to determine PCMS ISIs. An F-wave was considered to be present if a response with a proper latency (minimum of 20 ms) had an amplitude >20 μ V above background. The PCT was calculated using one of the two following calculations: (C-root – M-max+0.5) or (F-wave– M-max) * 0.5 and the CCT was calculated as MEP – C-root +1.5 or MEP - (PCT + M-max). PCT and CCT and ISIs between TMS and PNS are summarized in Figure 2B and were not different between the two testing sessions (all $p>0.05$).

AIH.

AIH was delivered through a face-mask with 2 one-way valves restricting inspiration to the top valve and expiration to the bottom valve using an oxygen generator (EVEREST SUMMIT II model, Hypoxico Inc, New York, New York; Fig. 1A). The hypoxic ($F_iO_2 = 9 \pm 0.1$) and normoxic ($F_iO_2 = 20.9 \pm 3\%$) gas mixture was delivered through the top valve. The protocol consisted of 15 episodes of hypoxia/normoxia interspaced with 1 min inspiring ambient air (gas mixture supply unplugged). We monitored oxygen concentration before during, and after both sessions using a Handi⁺® oxygen monitor (maxtec, Salt Lake City, Utah, US). Participants breathed through the mask for 2 min before the first cycle

to familiarize them with procedures; heart rate and blood pressure were monitored for safety during the AIH protocol and values remained similar to baseline measurements at all times. Normal blood oxygen saturation levels (S_pO_2) and heart rate were assessed via pulse oximeter (Nonin 3150 WristOx2™, Nonin medical Inc, Plymouth, Minnesota, United States). SpO_2 nadir after each 60 s exposure was noted and is presented in Figure 3D. During sham AIH, subjects breathed throughout the same mask the protocol consisted of 15 episodes of normoxia/normoxia interspaced with 1 min inspiring ambient air.

TMS.

Transcranial magnetic stimuli were applied using a figure-of-eight coil (loop diameter 70 mm) through a Magstim 200² magnetic stimulator (Magstim, Whitland, Dyfed, UK) with a monophasic current waveform. Optimal position for eliciting a MEP in the FDI muscle (hot spot) was found by moving the coil with the handle pointing backward and 45° away from the midline in small steps across the hand representation of the contralateral primary motor cortex. With this coil position, the current flowed in a posterior-anterior direction and probably produced predominantly early I wave activation (Sakai et al., 1997). Coil position was monitored using a frameless stereotaxic neuro-navigation system (Brainsight 2, Rogue Research, Montreal, Canada). Two sets of 20 MEPs were collected at baseline and 30 min after each intervention.

MEPs elicited by TMS.

The resting motor threshold (RMT) was defined as the minimal stimulus intensity required to induce MEPs >50µV peak-to-peak amplitude above the background EMG in 5/10 consecutive trials in the relaxed muscle (Rothwell et al., 1999). MEPs were tested at rest at an intensity that evoked a MEP with amplitude of approximately 50% of MEP-max in both sessions (PCMS+AIH=75.5±11.7% of MSO, PCMS+sham-AIH=74.1±13.9% of MSO, p=0.8). 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 (PCMS+AIH=1.9±1.7 mV, PCMS+sham-AIH=2.5±3.8 mV, p=0.4). At baseline, TMS pulses were delivered at 4 s intervals in sets of 20 separated by resting periods as needed. MEPs were visually inspected and analyzed trial by trial. Two sets of 20 MEPs were collected at each time point.

MEPs elicited by electrical stimulation.

We measured corticospinal transmission and/or motoneuron excitability by stimulating the corticospinal tract using high voltage electrical current (200 µs duration, Digitimer DS7AH) passed between two small gold-cup electrodes (GRASS Technologies®, Astro Med®, Inc., Warwick, Rhode Island, USA) placed behind the mastoid process at the cervicomedullary level (n=3; Taylor and Gandevia, 2004a). We adjusted the stimulation intensity to produce a MEP of ~3% M-max. This response was not contaminated by activation of cervical spinal roots. This was verified by increasing intensity until a latency decrement of ~2 ms indicated that the ventral roots were stimulation directly and then turning it back down with an ensuing increment in latency (Taylor and Gandevia, 2004a). In 6 subjects the corticospinal tract was stimulated through the scalp with the cathode at the vertex and anode 7 cm lateral towards the external meatus acusticus (Day et al., 1989) using a stimulus intensity needed

to elicit MEPs of ~3% of M-max in the FDI muscle. The latency of MEPs elicited by cortical electrical stimulation (24.1 ± 2.2 ms) was shorter than the latency of MEPs evoked by TMS (26.0 ± 2.0 ms, $p=0.007$), suggesting that we stimulated corticospinal axons directly. Because MEPs elicited by cervico-medullary and cortical electrical stimulation showed the same changes across conditions we grouped all results together under MEPs elicited by electrical stimulation. Ten MEPs elicited by electrical stimulation were tested at baseline and 20 MEPs were collected 30 min after each protocol.

Small voluntary contractions.

Index finger abduction force and FDI EMG activity were measured during short, ballistic, isometric contractions into abduction using a custom LabView program ($n=10$). One cursor showed the target force (10% of MVC) and another cursor showed the force exerted by the subject. Subjects were instructed to perform index finger abduction to move the actual force to the target force as fast as possible without correcting the applied force production during the contraction based on the online visual feedback. After familiarization, four sets of 20 contractions were performed with partially simulated 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 $\pm 0.5\%$ 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). Forty contractions were performed before and 30 min after each intervention.

Statistics.

Sphericity was tested using Mauchly's test. The distribution of the dependent variables was examined using Shapiro Wilk's test and was natural log transformed (\ln) in cases where it was not normally distributed. Repeated-measures analysis of variance (ANOVA) was performed to examine the effect of INTERVENTION (PCMS+AIH and PCMS+sham-AIH) and TIME (baseline and 30 min after each intervention) on MEP size, small voluntary contractions, and MVC. Repeated-measures ANOVA was used to examine the effect of BLOCK (from 1 to 18) and INTERVENTION on the response size of measurements acquired during PCMS. Paired t-tests were used to compare the size of the baseline MEPs and MVCs as needed. Bonferroni's *post hoc* analysis was used to test for significant comparisons. Changes in SpO_2 from baseline over the course of 15 cycles were analyzed using two-way repeated measures ANOVA in a 2 by 16 design. Response amplitudes during the intervention were contrasted to the first block of 20 to reduce the risk of type 2 errors. This resulted in 17 within and 18 between group comparisons. \ln -transformed relative changes in electrically evoked potentials were compared between the two conditions using a paired t-test. Significance was set at $p < 0.05$. Group data are presented as the mean \pm SD in the text and figures.

Results

SpO₂ and evoked responses during PCMS

The PCMS protocol consisted of 180 pairs of stimuli delivered every 10 s (0.1Hz, 10 blocks; Fig. 3C). Two-way repeated measures ANOVA revealed no effect of BLOCK ($F_{3,6,50.8}=1.8$, $p=0.1$), INTERVENTION ($F_{1,14}=0.3$, $p=0.5$) nor their interaction ($F_{2,3,31.7}=1.3$, $p=0.2$) on response size, suggesting that the stimulus intensity was maintained constant across conditions. The AIH protocol consisted of 15 episodes of hypoxia/normoxia interspaced with 1 minute inspiring ambient air (Fig. 3D). Two-way repeated measures ANOVA revealed an effect of INTERVENTION ($F_{1,15}=553.3$, $p<0.001$), CYCLE ($F_{5,8,87.1}=45.3$, $p<0.001$) and their interaction ($F_{5,6,83.4}=46.8$, $p<0.001$) on SpO₂. *Post hoc* testing revealed between-group differences at all 15 cycles (all $p<0.001$) but not at baseline ($p=0.5$). SpO₂ was significantly lower during the PCMS+AIH versus PCMS+sham-AIH session in all cycles (all $p<0.001$) for PCMS+AIH, whereas no differences were observed during PCMS+sham-AIH (all $p=1.0$). The lowest average SpO₂ was observed after cycle 14 during the PCMS+AIH treatment (79.4 ± 0.9) and after the first cycle during PCMS+sham-AIH (96.0 ± 0.4). SpO₂ levels were different throughout both sessions (PCMS+AIH= 81.5 ± 3.2 , PCMS+sham-AIH= 96.4 ± 1.6 , $p<0.001$).

MEPs elicited by TMS

Figure 4A illustrates traces of MEPs elicited by TMS over the primary motor cortex in the FDI muscle from a representative SCI participant during both protocols. Here, the MEP amplitude increased largely after PCMS+AIH compared with PCMS+sham-AIH (Fig. 4B). Two-way repeated measures ANOVA revealed an effect of INTERVENTION ($F_{1,15}=26.3$, $p<0.001$), TIME ($F_{1,15}=8.8$, $p=0.01$), and in their interaction ($F_{1,15}=8.8$, $p=0.01$) on MEP amplitude. *Post Hoc* comparisons revealed increases in MEP amplitude at 30 min ($61.88\pm 50.97\%$; $p<0.001$) after PCMS+AIH. This facilitation was significantly higher than following PCMS+sham-AIH ($p=0.009$). Following PCMS+sham-AIH the MEP amplitude increased by $27.0\pm 30.0\%$ on average. MEPs were facilitated in most subjects after PCMS+AIH (14/16, range from 1.4% to 115.2%) comparing baseline to measurements at 30 min after PCMS+sham-AIH (Fig. 4C).

MEPs elicited by electrical stimulation

Figure 5A illustrates traces of MEPs elicited by electrical stimulation over the cervicomedullary junction in the FDI muscle from a representative SCI participant during both protocols. MEP amplitude increased more after PCMS+AIH versus PCMS+sham-AIH. Two-way repeated measures ANOVA revealed an effect of INTERVENTION ($F_{1,6}=6.9$, $p=0.03$), TIME ($F_{1,6}=14.7$, $p=0.009$), and in their interaction ($F_{1,7}=6.9$, $p=0.03$) on MEP amplitude. *Post hoc* comparisons revealed increases in MEP amplitude 30 min ($57.2\pm 28.2\%$) after PCMS+AIH. This facilitation was significantly higher than after PCMS+sham-AIH ($15.2\pm 36.5\%$, $p=0.03$). MEPs were facilitated in most subjects after PCMS+AIH (6/7, range from 5.05% to 156.9%) versus measurements taken after the PCMS+sham-AIH protocol (Fig. 5C).

Voluntary motor output

Figure 6 illustrates rectified raw EMG recordings from the FDI muscle from a representative SCI participant. Note that in this participant the magnitude of EMG increased to a similar extent after PCMS combined with AIH or sham-AIH. Repeated measures ANOVA revealed an effect of TIME ($F_{1,9}=9.1$, $p=0.001$), but not INTERVENTION ($F_{1,9}=0.2$, $p=0.6$) or their interaction ($F_{1,9}=1.4$, $p=0.2$), on mean EMG amplitude during small voluntary contractions. The mean EMG was facilitated in the subjects after PCMS+AIH (5/10, range from 1.5% to 32.3%) versus PCMS+sham-AIH. Similar results were found in peak EMG amplitude after both protocols [TIME ($F_{1,9}=8.7$, $p=0.01$, but not INTERVENTION ($F_{1,9}=0.2$, $p=0.6$) or their interaction ($F_{1,9}=1.5$, $p=0.2$)]. Peak EMG was facilitated after PCMS+AIH (5/10, range from 1.1% to 37.6%), versus PCMS+sham-AIH.

Figure 7 illustrates MVCs in the FDI muscle tested before and after PCMS+AIH and PCMS+sham-AIH in a representative SCI participant. Baseline MVC measurements were similar between the two protocols (PCMS+AIH= 0.3 ± 0.2 mV, PCMS+sham-AIH= 0.3 ± 0.1 mV, $p=0.1$). A two-way repeated measures ANOVA revealed no effect of INTERVENTION ($F_{1,9}=1.7$, $p=0.2$), TIME ($F_{1,9}=1.7$, $p=0.2$) or their interaction ($F_{1,9}=0.01$, $p=0.9$) on MVC.

Discussion

Our findings support the view that AIH is an effective strategy to enhance PCMS-induced spinal synaptic plasticity in humans with chronic incomplete SCI. The amplitude of MEPs evoked by magnetic and electrical stimulation were further increased when PCMS was applied in combination with AIH, suggesting a spinal origin for these effects. This is consistent with previous results showing that both protocols elicit spinal plasticity. Combined PCMS with AIH or sham-AIH increased EMG activity to a similar extent in a finger muscle during brief, ballistic voluntary finger contractions. In contrast, neither protocol resulted in MVC changes. We argue that the fact that combined PCMS plus AIH potentiated physiological outcomes, but not voluntary motor output suggests that physiological effects have a lower threshold compared with effects on voluntary motor output, which needs to be considered when using combinatorial treatment approaches in humans with SCI.

Physiological effects of PCMS and AIH

Our results agree with previous findings that PCMS increases corticospinal excitability in upper limb muscles in normal subjects (Christiansen et al., 2018; D'Amico et al., 2017; Donges et al., 2017; Fitzpatrick et al., 2016; Taylor and Martin, 2009) and in humans with chronic SCI (Bunday and Perez, 2012; Bunday et al., 2018; Jo and Perez, 2020). The magnitude of PCMS effects on MEP amplitude elicited by magnetic and electrical stimulation (~20% increase) in our SCI participants was similar our previous studies (Bunday and Perez, 2012; Bunday et al., 2018; Jo and Perez, 2020). Because MEPs elicited by anodal electrical stimulation of the primary motor cortex activates axons of pyramidal tract neurons at the axon initial segment (Day et al., 1989), and stimulation at the cervicomedullary junction activates corticospinal axons at the pyramidal decussation (Taylor and Gandevia, 2004), increases in MEP amplitude likely have a subcortical origin.

The synergistic effects of PCMS and AIH on MEP amplitude are consistent with previous results in control subjects showing that combined PCMS and AIH had additive facilitatory effects on corticospinal excitability (Christiansen et al., 2018). PCMS enhances corticospinal transmission at the spinal cord level, likely engaging STDP mechanisms that depend on NMDA receptor activation (Donges et al., 2017). NMDA receptors are often critical to generate persistent activity arising from neural associations (Welch et al., 2020). One possibility is that the synergistic effects of PCMS and AIH on spinal synaptic plasticity relate to new BDNF synthesis (Welch et al., 2020). AIH increases BDNF expression in somatic motoneurons (Lovett-Barr et al., 2012; Satriotomo et al., 2016) and BDNF is necessary for NMDA receptor-dependent synaptic plasticity (Crozier et al., 2008). Motoneuron TrkB protein expression is necessary for AIH-induced, BDNF-dependent long-term facilitation of motor neurons (Dale et al., 2017), and TrkB plays an important role in neuroplasticity following SCI (Garraway and Huie, 2016). BDNF effects on spinal synaptic transmission can be either presynaptic or postsynaptic at the same connection (Arvanian and Mendell, 2001). For example, BDNF facilitates monosynaptic excitatory postsynaptic potentials in neonatal rat motoneurons via postsynaptic NMDA receptors (Arvanian and Mendell, 2001). In fact, PCMS is contingent upon the timely somatic/dendritic invasion of the antidromic volley in the motoneurons causing a depolarization of the postsynaptic membrane 1–2 ms after presynaptic end-boutons are depolarized by descending volleys triggered by the magnetic stimulation. Thus, the postsynaptic terminal is a likely site for potentiating AIH/PCMS combinatorial plasticity.

It is also possible that it is the serotonin induced BDNF synthesis (Baker-Herman et al., 2004) that might have contributed to our findings. Since spinal serotonin release and postsynaptic receptor activation is necessary and sufficient for AIH-induced phrenic motor plasticity (Baker-Herman and Mitchell, 2002; MacFarlane and Mitchell, 2009), it may play a similar role in the facilitatory effect of AIH on STDP-like plasticity. Serotonin modulates the excitability of motoneurons through both presynaptic and postsynaptic actions (Rekling et al., 2000). AIH triggers phasic activity in serotonergic neurons from the raphe nuclei projecting to the cervical spinal cord (Hayashi et al., 1993; Morris et al., 2008) where various 5-HT receptor subtypes are expressed in the active zone of postsynaptic ventral horn neurons (Ridet et al., 1994). Specifically, AIH-induced long-term facilitation in cervical motoneurons is contingent on 5-HT₂ receptor activity (Tadjalli and Mitchell, 2019). Binding of 5-HT to both 5-HT₂ receptors triggers intracellular cascades that combine to elicit long-term facilitation (Baker-Herman and Mitchell, 2002; Devinney et al., 2015; Tadjalli and Mitchell, 2019). Serotonin binding to Gq-metabotropic receptors also inhibits outward potassium currents, thereby amplifying NMDA-receptor dependent calcium influx (Gu, 2002; Johnson and Heckman, 2014). Consistent with the idea that both serotonin and BDNF modulate STDP-like plasticity in humans (Batsikadze et al., 2013; Cheeran et al., 2008). It is important to consider that we used a fixed FiO₂ of 9% in all SCI participants; regardless of how responsive each individual was to hypoxia. Since individuals with SCI have low blood pressures (Krassioukov and Claydon, 2006), it is certainly possible that the same arterial oxygen saturation could lower spinal tissue PO₂ more than in control subjects and, thus, alter the expression of plasticity.

Lower tissue PO₂ is expected to shift the balance between serotonin-dependent versus adenosinergic mechanisms in motor circuitry, undermining, cancelling or even replacing serotonin-dependent effects (depending on the tissue PO₂; Perim and Mitchell, 2020). Thus, variations in the degree of facilitation in response to PCMS+AIH could be, at least in part, related to different degrees of between-subject tissue hypoxia in response to the same AIH protocol. It is less likely that these variations were related to the degree of spared corticospinal tract fibers since similar effects were found in participants with larger and smaller MEP amplitudes and/or more or less impaired AIS levels.

Effects of PCMS and AIH on Voluntary Motor Output

Consistent with previous findings, PCMS combined with sham-AIH increased small levels of voluntary motor output in individuals with SCI (Bunday and Perez, 2012; Urbin et al., 2017). One critical question is why combined PCMS and AIH did not further increase MVCs and/or small level voluntary output. Using a similar AIH protocol, Trumbower and collaborators (2012) showed increased voluntary ankle strength in a similar group of SCI participants. It is possible that AIH exerts differential actions on upper vs lower limb muscles. Hand muscles, as tested in our study, are under a strong cortical control, and even a single AIH session induces changes at subcortical (not cortical) levels (Christiansen et al., 2018). However, AIH also improves hand function in people with SCI (Trumbower et al., 2017). It is also possible that PCMS prevented AIH-induced increases in maximal voluntary output and/or that the lack of sufficient synergy to change voluntary motor output may reflect the fact that AIH and PAS involve similar pathways. This possibility is unlikely since AIH potentiated PCMS effects on corticospinal excitability in this and an earlier publication from our group (Christiansen et al., 2018), suggesting additive effects between these plasticity protocols.

A parsimonious explanation is that PCMS and AIH target different sizes of motoneurons, consistent with evidence that motoneurons of different size have different sensitivities to different plasticity mechanisms (Krishnan, 1983). This concept is supported by findings that a single PCMS session increases the magnitude of small levels of voluntary motor output (likely mediated by small motor units), but not MVCs (likely mediated by small and large motor units) in humans with and without SCI (Taylor and Martin, 2009; Bunday and Perez, 2012; D'Amico et al., 2018; Donges et al., 2019; Jo and Perez, 2020). Further support for this idea is provided by our observation that larger MEPs showed greater increases in amplitude versus smaller MEPs with combined PCMS and AIH. Since TMS recruits contralateral motor units in sequence from small to large, small and large motor units likely contribute to larger MEPs (Bawa and Lemon, 1993; Gandevia and Rothwell, 1987; Mills, 1991). Thus, if PCMS favors low threshold motoneurons whereas AIH favors high threshold motoneurons, we might expect that their combination increases motor output during small and large contractions as with physiological outcomes. These differential actions might be explained by threshold differences in physiological versus motor output gains. The responsiveness of motoneurons right before (Federico and Perez, 2017) and during (Vastano and Perez, 2020) voluntary activity decreases in humans with SCI versus control participants, which could contribute to the different thresholds between physiological vs motor output outcomes.

The lack of increases in voluntary motor output may suggest that changes in serotonin release and/or BDNF levels, which are needed to promote synaptic plasticity (Cotman et al., 2007; Baker-Herman et al., 2004), were not adequate. This idea is supported by observations that chronic protocols (1 week) of more intense intermittent hypoxia elicit greater and/or more enduring BDNF-related plasticity (Fuller et al., 2003; Navarrete-Opazo et al., 2017) and that AIH can result in aberrant plasticity under some conditions (Warren et al., 2018). Multiple PCMS sessions are also needed to increase MVCs in humans with SCI (Jo and Perez, 2020). In the present study, we only measured EMG activity during maximal voluntary contraction. Previous studies demonstrated parallel changes in torque and EMG activity following PCMS and AIH (Bunday and Perez, 2012; Trumbower et al., 2012; Sandhu et al., 2019), but, considering the complex relationship between EMG-force output (Paquin and Power, 2018), future studies will benefit from including both outcomes. It is less likely that other factors such as the time of measurements contributed to our findings. In the study by Trumbower and collaborators (2012), MVC in plantarflexor muscles was increased 30 and 60 minutes after AIH; our measurements were completed 30 min after AIH. Similarly, PCMS effects on small levels of voluntary contraction in upper and lower limb muscles were present 30 min post-stimulation, and returned to baseline ~60–80 min after the end of the stimulation (Bunday and Perez, 2012; Bunday et al., 2018; Urbin et al., 2017). We also highlight that either PCMS or AIH alone increases corticospinal excitability and voluntary motor output in people with SCI. Although we did not test the effect of AIH alone on corticospinal excitability in the present study, unpublished data from our laboratory indicates that the amplitude of corticospinal responses elicited by TMS increases after a single PCMS and AIH session in people with SCI. A direct comparison of the physiological and motor output magnitudes between protocols is difficult since the nature of protocols differs. Our results indicate that the beneficial effects of these protocols are complementary on corticospinal excitability, but this does not extrapolate to voluntary motor outcomes, suggesting it is difficult to determine which protocol might represent the best therapy.

Functional considerations

We combined PCMS with AIH to engage and facilitate residual corticospinal-motoneuronal connections in humans with SCI. This approach is supported by a number of studies showing that AIH combined with activity-dependent plasticity protocols triggers recovery of motor function in humans with incomplete SCI (Lynch et al., 2016; Navarrete-Opazo et al., 2016; Navarrete-Opazo et al., 2017; Trumbower et al., 2012; Hayes et al., 2014; Sandhu et al., 2019). This idea agrees with multiple combinatorial, non-invasive neurostimulation strategies that successfully potentiated outcomes in humans with SCI (Angeli et al., 2014; Belci et al., 2004; Gad et al., 2018; Gerasimenko et al., 2015; Gomes-Osman and Field-Fote, 2015; Harkema et al., 2011). An important observation is that, whereas combined AIH and PCMS potentiated physiological outcomes, they failed to impact volitional motor behaviors. Similarly, after STDP-like plasticity protocols, physiological outcomes that rely on excitability changes are modulated without changes in voluntary motor output (Elahi et al., 2014; Hussain et al., 2016). Additional studies demonstrate disparities between physiological and behavioral changes after STDP-like plasticity (Rajji et al., 2011; Stefan et al., 2006). Future studies of plasticity-inducing protocols that prime activity-dependent plasticity in controls (Hurley and Machado, 2017) and participants with SCI (Jo and Perez,

2020; Potter-Baker et al., 2018) should incorporate outcomes that reflect both physiological and voluntary motor output improvements.

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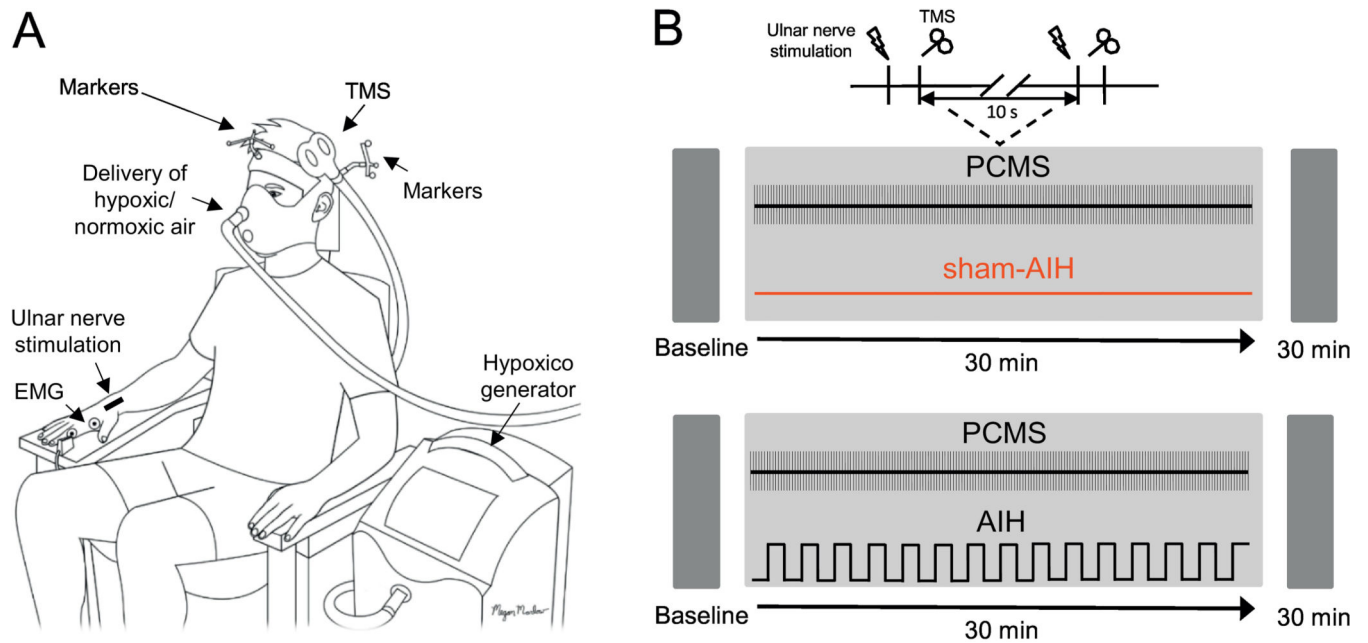
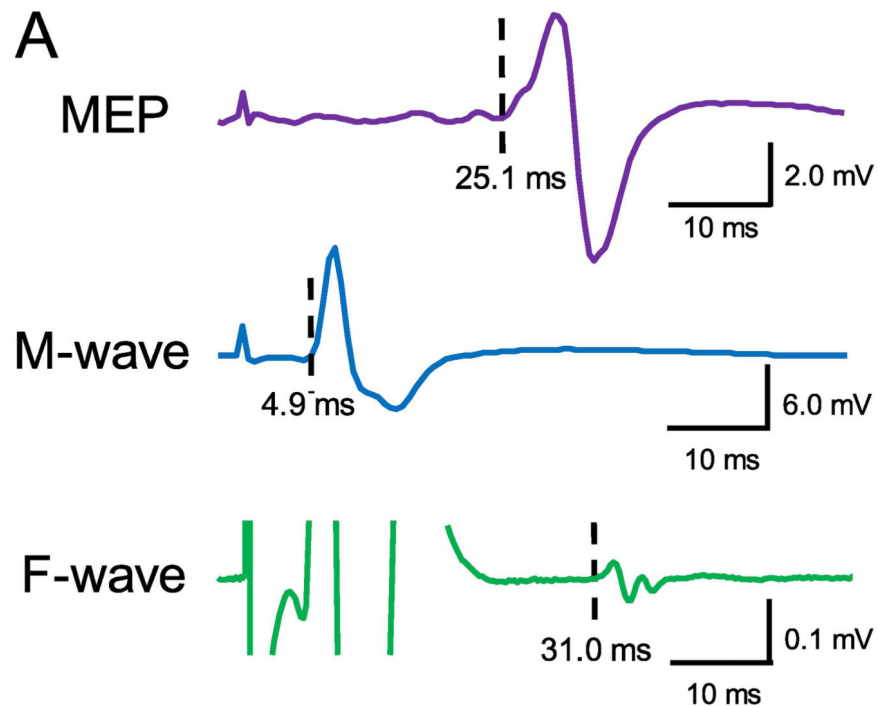


Figure 1. Experimental set-up.

(A) Participants were seated in a customized chair during paired corticospinal-motoneuronal stimulation (PCMS) combined with acute intermittent hypoxia (PCMS+AIH) and sham AIH (PCMS+sham-AIH). PCMS was delivered using transcranial magnetic stimulation (TMS) over the hand representation of the primary motor cortex and supramaximal peripheral nerve stimulation (PNS) over the ulnar nerve with 180 pairs of stimuli delivered every 10 s (0.1 Hz). The position of the TMS coil was monitored using a frameless stereotaxic system and electromyographic (EMG) activity was recorded from the first dorsal interosseous (FDI) muscle to record electrophysiological outcomes. AIH was administered by using the Hypoxico Inc. (EVEREST SUMMIT II, New York) and the protocol consisted of 15 cycles of 1 min of inspiring ambient air with 1 min of hypoxic air. (B) Timeline of the experimental procedures. Electrophysiological outcomes were measured before (Baseline) and 30 min after each protocol.



B

Time (ms)	Spinal Cord Injury
Response Latencies	
M-wave (ms)	4.9±0.5
F-wave (ms)	32.9±5.1
MEP (ms)	25.0±4.0
Conduction Times	
CCT (ms)	5.9±1.3
PCT (ms)	12.9±2.0

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 subject from the FDI muscle. (B) MEP, F-wave, and M-max latencies were used to calculate central (CCT) and peripheral conduction time (PCT) used to estimate time the arrival of pre- and postsynaptic volleys at the cortico-motoneuronal synapses for PCMS in SCI subjects.

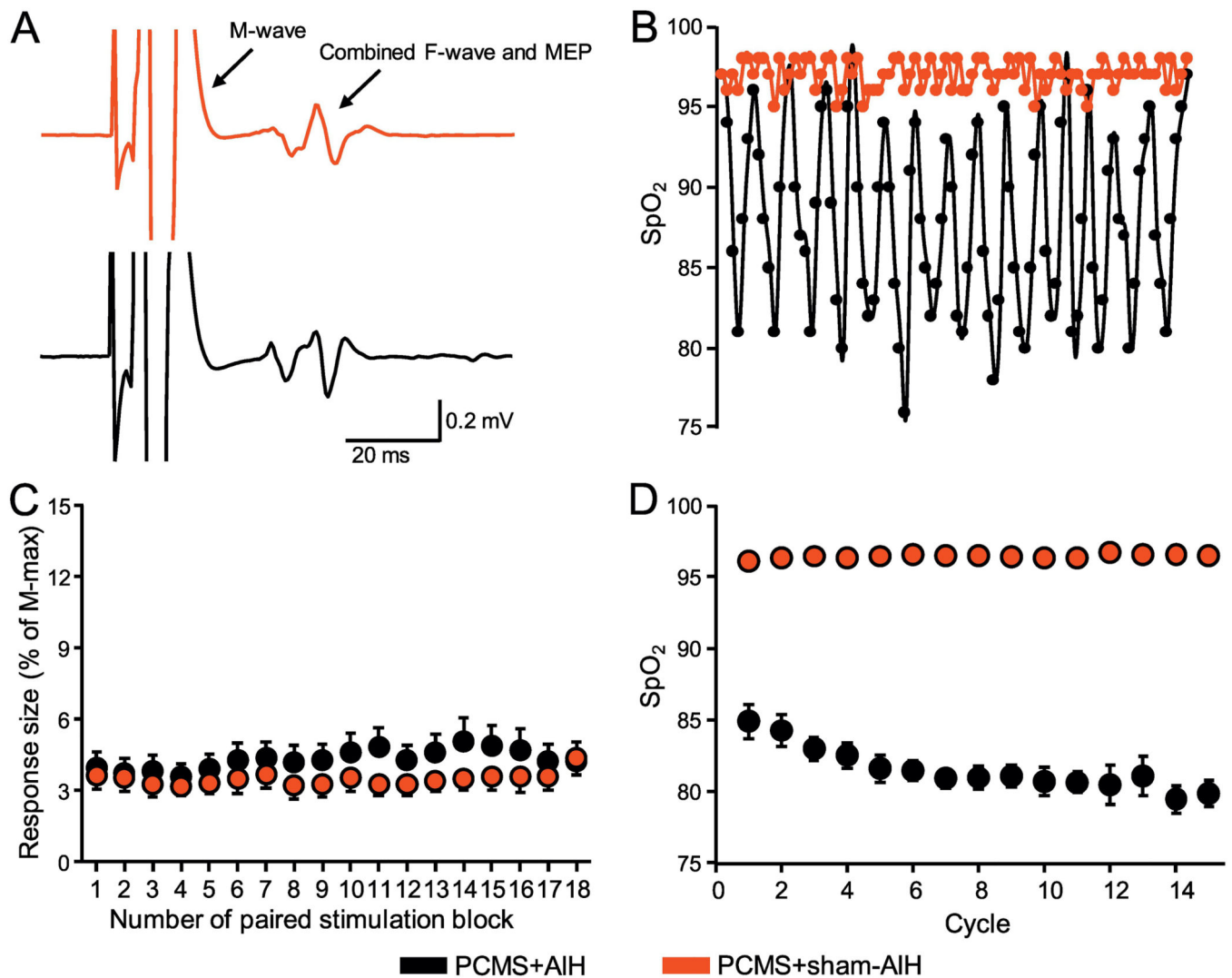


Figure 3. Monitoring during protocols.

(A) Raw traces from the FDI muscle during PCMS+sham-AIH and PCMS+AIH. Note that the M-wave is followed by an F-wave that is likely to be combined with a MEP elicited by TMS. (B) SpO₂ from a representative participant during the PCMS+sham-AIH (red) and PCMS+AIH (black) protocols. The AIH protocol consisted of 15 cycles of 1 min of inspiring ambient air (20.9% O₂) with 1 min of hypoxic air (9.4% O₂). (C) The abscissa shows the number of pairs of stimuli during each protocol (a total of 180 pairs of stimuli). At each point, the average of 10 responses is shown. The ordinate shows the size of the conditioned response expressed as % of the M-max in SCI participants during PCMS+sham-AIH and PCMS+AIH. (D) Group data showing the SpO₂ observed during the 15 cycles in the PCMS+sham-AIH (red) and PCMS+AIH (black) protocol.

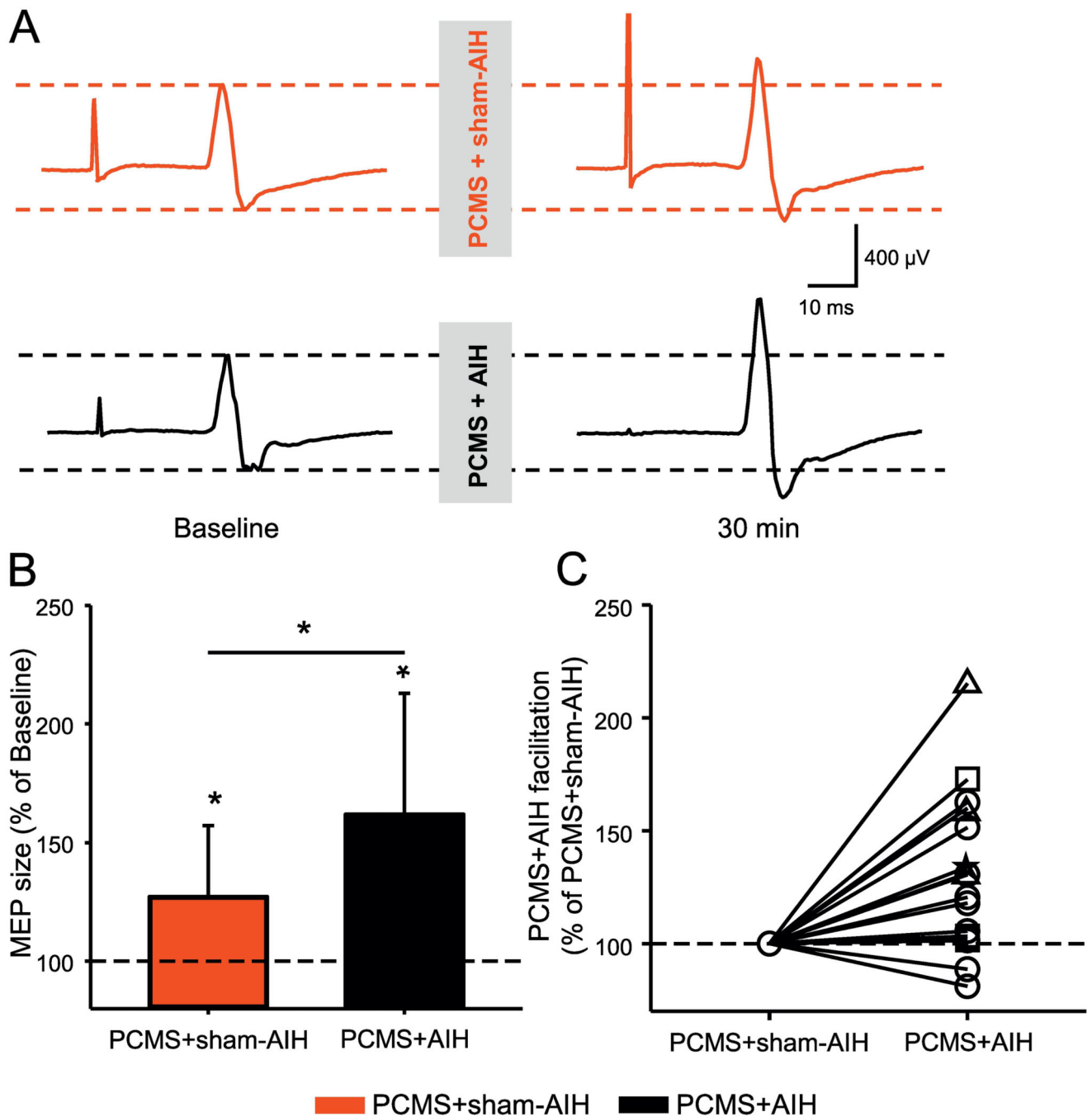


Figure 4. MEPs elicited by TMS.

(A) Representative MEP traces recorded from the FDI muscle in a SCI participant before and 30 min after PCMS+sham-AIH (red traces) and PCMS+AIH (black traces). Waveforms represent the average of 20 MEPs. (B) Graph shows group data (n=16). The abscissa shows the protocols tested (PCMS+sham-AIH=red bar and PCMS+AIH=black bar) and the ordinate shows the size of MEPs expressed as a % of MEPs at baseline. (C) Graph shows individual data (AIS A=triangle, AIS B=star, AIS C=square, and AIS D=circle). The ordinate shows the % of changes in MEP amplitude after PCMS+AIH expressed as % of the

changes after PCMS+sham-AIH. The dotted line indicates the baseline. Error bars indicate SDs. * $p < 0.05$, comparison with baseline; † $p < 0.05$, comparison between PCMS+sham-AIH and PCMS+AIH protocols.

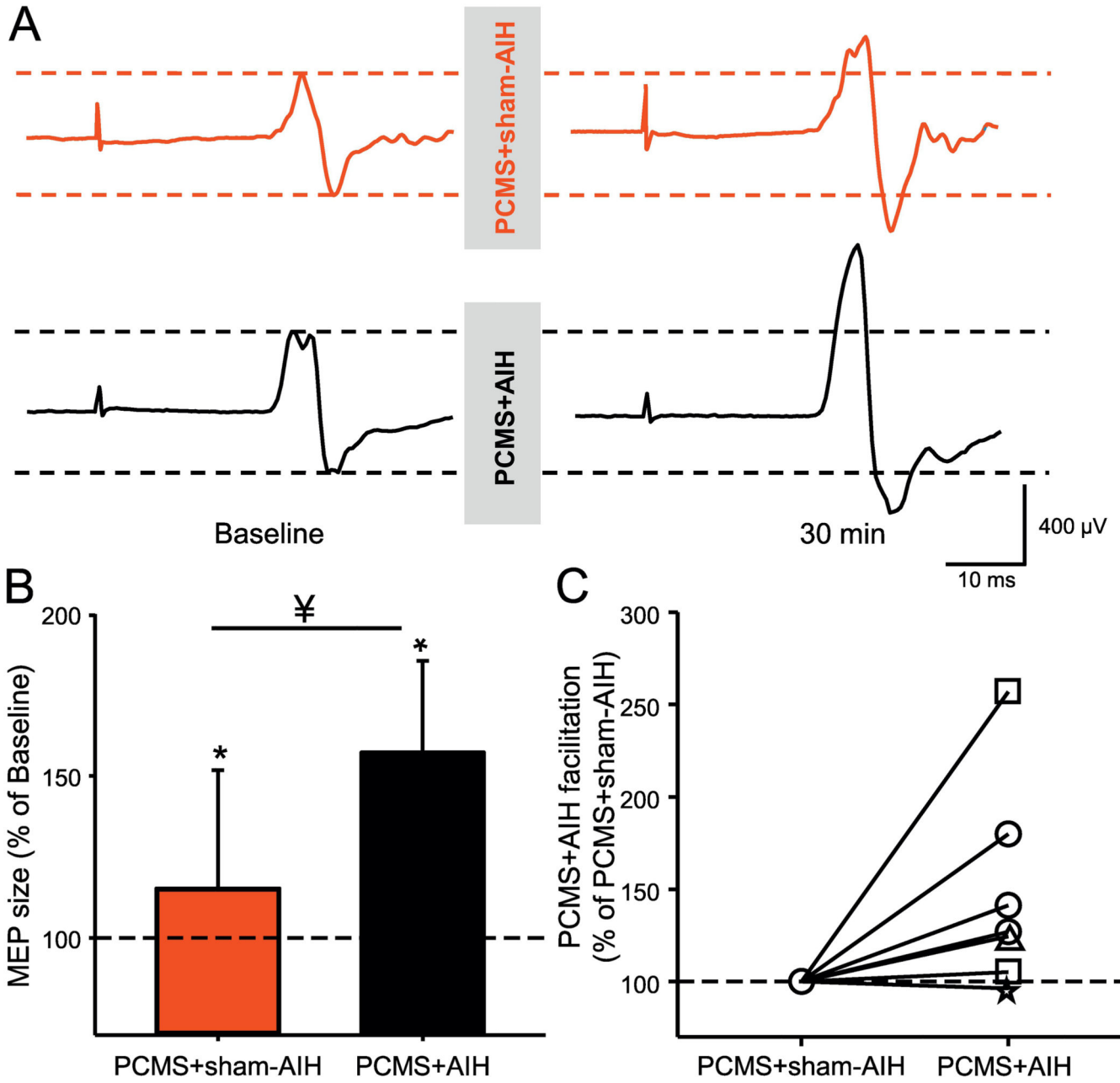


Figure 5. MEPs elicited by electrical stimulation at the cervicomedullary level.
 (A) Raw MEPs traces recorded from the FDI muscle of a representative SCI participant before and 30 min after PCMS+sham-AIH (red traces) and PCMS+AIH (black traces). Waveforms represent the average of 20 MEPs. (B) The graph shows group data (n=9) with the abscissa showing the protocols tested (PCMS+sham-AIH=red bar and PCMS+AIH=black bar) and the ordinate showing the size of MEPs expressed as % of MEPs at baseline. (C) Graph shows individual data (AIS A=triangle, AIS B=star, AIS C=square, and AIS D=circle). The ordinate shows the % of changes in MEP amplitude after PCMS+AIH expressed as a % of the facilitation after PCMS+sham-AIH. The dotted line

indicates the baseline. Error bars indicate SDs. * $p < 0.05$, comparison with baseline; † $p < 0.05$, comparison between PCMS+sham-AIH and PCMS+AIH protocols.

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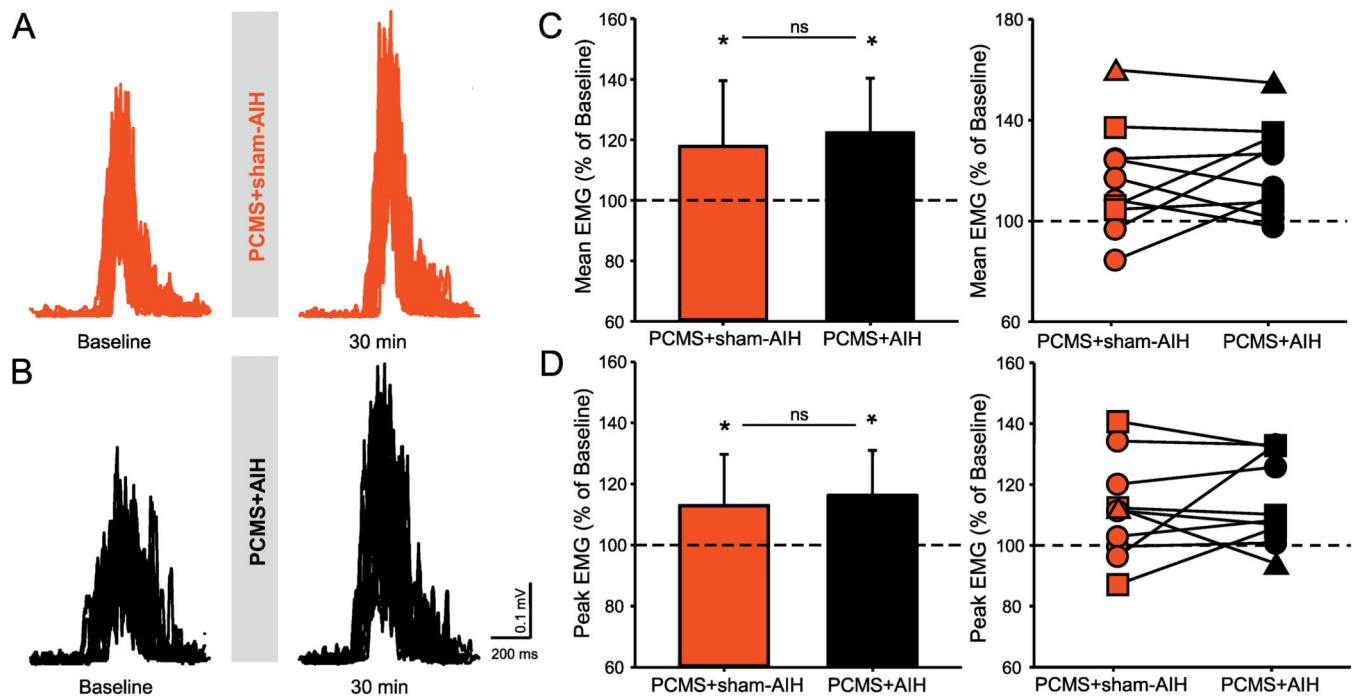


Figure 6. Small level of voluntary contraction.

Representative EMG traces recorded during small level of voluntary contraction in a participant with SCI before and after **(A)** PCMS+sham-AIH (red traces) and **(B)** PCMS+AIH (black traces). Graphs show the group (n=10) and individual (AIS A=triangle, AIS C=square, and AIS D=circle) data. The abscissa shows the protocols tested (PCMS+sham-AIH=red bars and PCMS+AIH=black bars) and the ordinate shows the mean **(C)** and peak **(D)** EMG activity expressed as % of baseline EMG in the FDI muscle during small level of voluntary contractions (10% MVC) tested. The dotted line indicates the baseline. Error bars indicate SDs. *p<0.05, comparison with baseline.

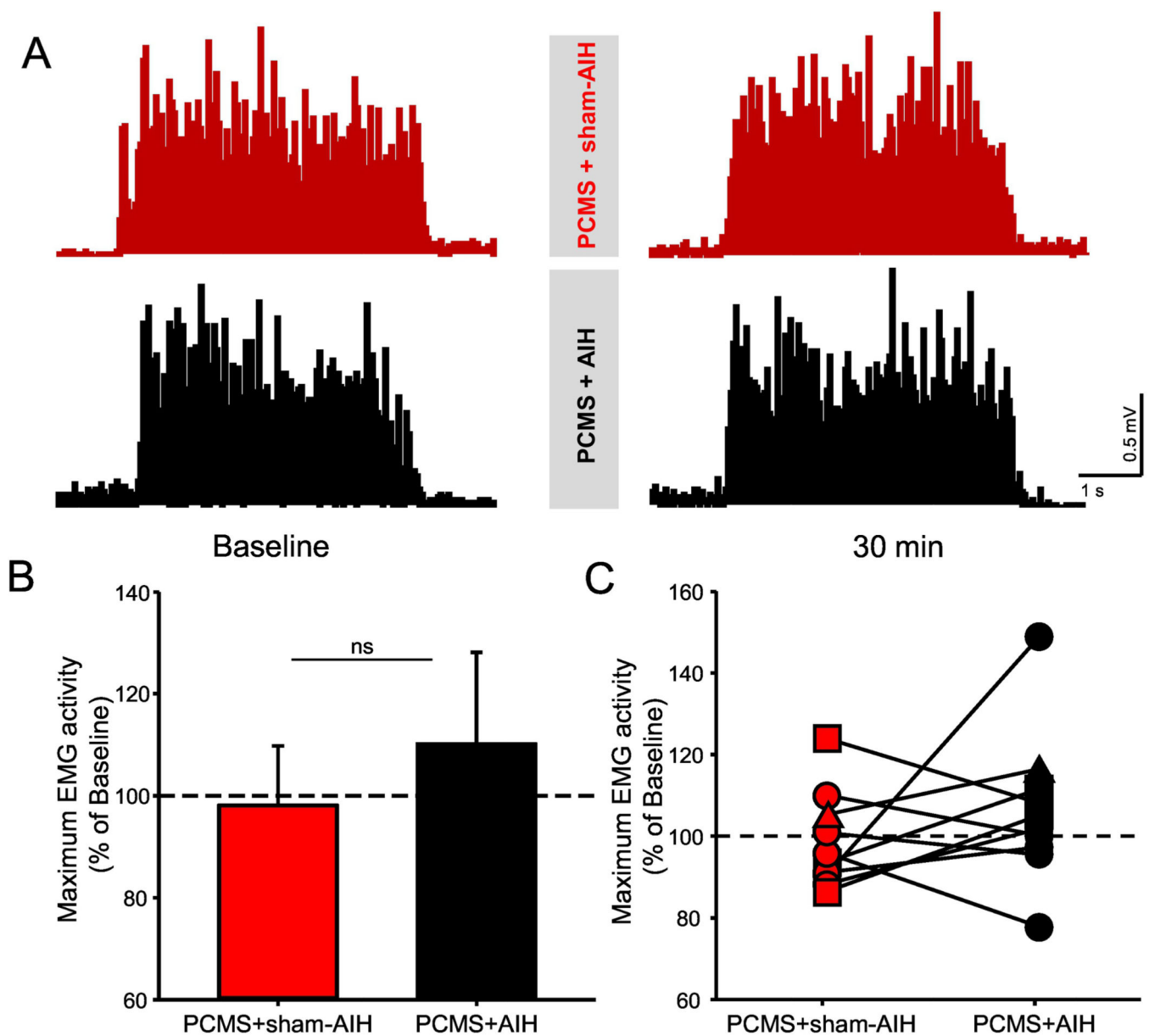


Figure 7. Maximum voluntary contraction (MVC).

(A) Representative EMG traces recorded during MVC (measured as the highest mean rectified EMG activity found in 1 s during the MVC burst) in a participant with SCI before and after PCMS+sham-AIH (red traces) and PCMS+AIH (black traces). (B) Graph shows the group data (n=10). The abscissa shows the protocols tested (PCMS+sham-AIH=red bars and PCMS+AIH=black bars) and the ordinate shows the mean EMG activity expressed as % of baseline during MVC. (C) Graph shows individual data (AIS A=triangle, AIS C=square, and AIS D=circle). The ordinate shows the magnitude change in MVC after 30 min of PCMS+sham-AIH (red circles) and PCMS+AIH (black circles). The dotted line indicates the baseline. Error bars indicate SDs. * $p < 0.05$, comparison with baseline.

Table 1:

Spinal cord injury participants

Participants	Age	Sex	Etiology	Level of lesion	AIS	Years since injury
1	67	M	T	C2	C	11
2	50	M	T	C5	D	4
3	53	F	T	C5	D	14
4	39	F	NT	C1	D	25
5	34	M	T	C7	C	2
6	38	M	T	C7	C	3
7	38	M	T	C5	C	11
8	55	M	T	C7	A	18
9	51	F	T	C2-6	A	10
10	33	M	T	C2	C	9
11	66	M	T	C4	D	6
12	69	M	T	C2	C	5
13	48	F	T	C6	C	7
14	60	M	NT	C1	D	3
15	36	M	T	C5	C	2
16	66	M	NT	C5-7	A	6

AIS=American Spinal Injury Association Impairment Scale, F=Female, M=Male, T=Traumatic, NT=Non-traumatic

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