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# More conditioning stimuli enhance synaptic plasticity in the human spinal cord

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HIGHLIGHTS

- Paired corticospinal–motoneuronal stimulation (PCMS) induces spinal plasticity.
- More PCMS produces longer lasting, more reliable facilitation.
- PCMS has potential to improve motor output in patients with insufficient descending drive.

ABSTRACT

*Objective:* To examine whether more paired corticospinal–motoneuronal stimulation (PCMS) is more effective at inducing spinal level plasticity.
*Methods:* To produce facilitation, corticospinal volleys evoked by motor cortical transcranial magnetic stimulation (TMS) were timed to arrive at corticospinal–motoneuronal synapses prior to antidromic potentials evoked in motoneurones by electrical brachial plexus stimulation. Paired stimuli were delivered repeatedly. 50-pair conditioning (50-PCMS) was compared to 100 pairs in single block (100-$PCMS_{single}$) and spaced (2 blocks of 50, 15-min break; 100-$PCMS_{spaced}$) patterns, and to 50 single, unpaired TMS (50-TMS). Biceps responses to cervicomedullary stimulation (cervicomedullary motor evoked potentials, CMEPs) and TMS (motor evoked potentials, MEPs) were measured before and for 1 h after conditioning (recorded each 5 min).
*Results:* After 100-PCMS, average CMEP areas were increased by 46 ± 55% (mean ± SD; $n = 10$; 100-$PCMS_{single}$) and 71 ± 99% (100-$PCMS_{spaced}$). 50-PCMS produced a non-significant 6 ± 40% increase. After 100-$PCMS_{single}$ and 100-$PCMS_{spaced}$, CMEPs were larger than those after 50-TMS from 0 to 60 min ($p < 0.05$). 100-$PCMS_{single}$ and 100-$PCMS_{spaced}$ produced more reliable changes than 50-PCMS. Overall, MEPs were larger at 35-60 min; however there were no differences between conditioning protocols.
*Conclusions:* More PCMS produces more reliable enhancement of corticospinal transmission.
*Significance:* This technique has therapeutic potential to improve muscle control in patients with reduced descending drive.


## 1. Introduction

The nervous system has an extraordinary capacity to change the structure and organisation of its neural networks, leading to subsequent changes in function. One mechanism of change involves modifications to the strength of neuronal connections through synaptic plasticity. Experimentally, synaptic changes can be induced by delivering repeated, paired pre- and postsynaptic stimuli at specific timing intervals. Such changes are known as spike-timing-dependent plasticity (STDP). Repetitive stimulus pairs timed such that presynaptic volleys arrive at a synapse just prior to antidromically induced postsynaptic potentials typically result in long term potentiation (LTP), whereas the reverse order of stimuli typically causes long term depression (LTD) (for reviews see: Caporale and Dan, 2008; Dan and Poo, 2006; Feldman, 2012).

*Abbreviations:* CMEP, cervicomedullary motor evoked potential; PCMS, paired corticospinal–motoneuronal stimulation; STDP, spike-timing-dependent plasticity.

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The corticospinal tract conveys the main component of control over voluntary muscle contraction in humans. This pathway has a monosynaptic component for many muscles, with presynaptic corticospinal axons synapsing directly onto spinal motoneurones (de Noordhout et al., 1999; Palmer and Ashby, 1992). If the pathway is interrupted through spinal cord injury or stroke, motor function can be severely impaired. In people with incomplete injury, strengthening of remaining corticospinal–motoneuronal synapses may provide a useful therapeutic target to enhance descending drive and improve muscle strength.

The use of paired stimulus protocols to induce STDP-like effects in the human brain has been well defined, particularly at the motor cortex (Carson and Kennedy, 2013). However few studies have used paired stimulus protocols to induce plasticity at a spinal level in humans. Repeated pairing of TMS to the primary motor cortex with low-intensity peripheral nerve stimulation to activate Ia afferents can induce spinal cord plasticity. An increase in 'conditioned H-reflex' size (H-reflex paired with cortical or cervicomedullary stimulation), but not unconditioned H-reflexes suggests changes in the corticospinal pathway in the spinal cord and not in the reflex circuit itself (Cortes et al., 2011; Leukel et al., 2012). Another way to induce STDP-like effects in the human spinal cord is to pair descending volleys in corticospinal neurones with antidromic volleys in motoneurones in a technique referred to here as paired corticospinal–motoneuronal stimulation (PCMS). This technique can modify motor responses to direct corticospinal stimulation in able-bodied (Taylor and Martin, 2009) and spinal cord injured individuals (Bunday and Perez, 2012). These changes may reflect STDP-like mechanisms at corticospinal–motoneuronal synapses. Importantly, voluntary motor output is also modified by PCMS, and spinal cord injured participants improved on a test of manual dexterity. (Bunday and Perez, 2012; Taylor and Martin, 2009). These studies highlight the technique's potential for strengthening the corticospinal pathway and enhancing descending drive to muscles, which could lead to functionally relevant improvements. However only these two studies have demonstrated PCMS and further optimisation of methodology and characterisation of effects are required.

Previous studies have highlighted methods to enhance and prolong plastic changes in neural activity. In humans, paired associative stimulation (PAS), a protocol targeting the motor cortex, can produce larger EMG responses to motor cortical stimulation when more stimulus pairs are delivered (Elahi et al., 2013). Similarly, doubling the duration of transcranial direct current stimulation (tDCS), which also induces plasticity, produces longer lasting changes in motor responses (Monte-Silva et al., 2010). Another method to prolong the duration of after-effects is to deliver conditioning stimuli in a spaced pattern of multiple blocks separated by optimal windows of time rather than as a single block. This can be seen in rat hippocampal slices (Huang and Kandel, 1994), the xenopus visual system (Zhou et al., 2003) and in human visual and motor cortical studies (Goldsworthy et al., 2011; Monte-Silva et al., 2010; Nyffeler et al., 2006).

Here we determined whether the delivery of more PCMS or the delivery of a spaced pattern of PCMS could lead to more reliable, longer lasting induction of plasticity at corticospinal–motoneuronal synapses within the human spinal cord.

## 2. Materials and methods

### 2.1. Participants

Participants were accepted into the study if they displayed sufficient biceps brachii responses to motor cortical stimulation (>0.5 mV; ~5% of $M_{max}$, described below) and tolerated cervicomedullary stimulation. Fourteen healthy volunteers (4 F) aged 26 ± 9 years (mean ± SD) participated in the study. All participants gave informed written consent and procedures were approved by the Human Research Ethics Committee of the University of New South Wales. The study was conducted according to the Declaration of Helsinki.

### 2.2. Experimental setup

Participants sat with their right arm secured in a sling so that the elbow was at a 90° angle, with the arm and shoulder relaxed. Electromyograms (EMG) were recorded from the right biceps brachii through Ag–AgCl surface electrodes (20 mm diameter, Conmed, NY, USA) placed over the muscle in a belly-tendon configuration. EMG signals were amplified and filtered at 16–1000 Hz (CED 1902 amplifier; Cambridge Electronic Design, Cambridge, UK). Data were sampled at 2 kHz, and recorded on computer for analysis (CED 1401 with Signal software; Cambridge Electronic Design).

### 2.3. Peripheral nerve stimulation (PNS)

A constant current stimulator (Model DS7AH, Digitimer, Welwyn Garden City, UK) delivered single stimuli (200 μs pulse width) to peripheral nerves supplying right elbow flexors. The cathode was placed in the supraclavicular fossa over the brachial plexus and the anode over the acromion. On each day, stimulus intensity was increased until no further increase was seen in the compound muscle action potential recorded from biceps, and 120% of this intensity was used (58 ± 25 mA; mean ± SD) to elicit maximal compound muscle action potentials ($M_{max}$). The $M_{max}$ (Fig. 1A) indicates maximal orthodromic activation of motor axons and implies antidromic activation of all motoneurones.

### 2.4. Transcranial magnetic stimulation (TMS)

TMS was used to stimulate the corticospinal tract at the level of the primary motor cortex. A circular coil (13.5 cm outside diameter, Magstim 200, Magstim, Whitland, UK) was positioned over the vertex and oriented to preferentially activate the left motor cortex. Motor evoked potentials (MEPs) were recorded from right biceps (Fig. 1A). TMS intensity (74 ± 13% of maximum stimulator output; mean ± SD) was set to elicit MEPs of 0.5–1 mV (~5% of $M_{max}$), and remained constant for each subject throughout the study.

EMG responses to cervical nerve root ($C_{root}$) stimulation (Fig. 1A) were elicited with the same circular coil centered over the spinous process of the 7th cervical vertebra (stimulus intensity 50% maximum stimulator output).

### 2.5. Cervicomedullary stimulation

To stimulate corticospinal axons at the level of the cervicomedullary junction, single electrical pulses (200 μs duration; Digitimer DS7AH) were delivered through surface electrodes fixed 1-2 cm posterosuperior to the tips of the mastoid processes (Taylor et al., 2002; Ugawa et al., 1991). Cervicomedullary motor evoked potentials (CMEPs) were recorded from the right biceps muscle (Fig. 1A). Changes in the size of the CMEP indicate changes occurring in the corticospinal pathway at a subcortical level. CMEP onset latency was monitored throughout the study to ensure that responses were a result of corticospinal axon stimulation, as a latency ~2 ms earlier would represent activation of motoneurones at cervical roots (Taylor and Gandevia, 2004). Stimulus intensity (165 ± 33 mA; mean ± SD) was set on each day to elicit CMEPs of ~10% $M_{max}$.