J Neurophysiol 123: 1969–1978, 2020.
First published April 15, 2020; doi:10.1152/jn.00607.2019.

RESEARCH ARTICLE | *Control of Movement*

High-intensity, low-frequency repetitive transcranial magnetic stimulation enhances excitability of the human corticospinal pathway

Jessica M. D'Amico,[1]* Siobhan C. Dongés,[1]* and Janet L. Taylor[1,2]
[1]*Neuroscience Research Australia, Sydney, Australia; and* [2]*University of New South Wales, Sydney, Australia*

Submitted 23 September 2019; accepted in final form 9 April 2020

D'Amico JM, Dongés SC, Taylor JL. High-intensity, low-frequency repetitive transcranial magnetic stimulation enhances excitability of the human corticospinal pathway. *J Neurophysiol* 123: 1969–1978, 2020. First published April 15, 2020; doi:10.1152/jn.00607.2019.—Paired corticospinal-motoneuronal stimulation (PCMS) is the repeated pairing of transcranial magnetic stimulation (TMS) with peripheral nerve stimulation to modify corticospinal synapses; however, it has yet to be determined whether PCMS modulates cortical excitability in a manner similar to paired-associative stimulation protocols. In this study, we first examined the effects of PCMS on adductor pollicis motor evoked potentials (MEPs). In *experiment 1*, on 2 separate days PCMS (repetitive, high-intensity TMS and ulnar nerve stimulation pairs; 1.5-ms interstimulus interval; 0.1 Hz) was compared with control conditioning of repetitive high-intensity TMS-only stimuli (0.1 Hz). Before and after conditioning, adductor pollicis MEPs were elicited using low-intensity TMS in three different coil orientations to preferentially activate corticospinal axons directly (thus bypassing cortical effects) or indirectly (cortical effects present). Unexpectedly, similar MEP increases were seen for all orientations on both PCMS (129 to 136% of baseline) and control days (108 to 129% of baseline). Given the common factor between conditioning protocols was repeated, high-intensity TMS, further experiments were performed to characterize this repetitive TMS (rTMS) protocol. In *experiment 2*, an intensity dependence of the rTMS protocol was revealed by a lack of change in MEPs elicited after repetitive low-intensity TMS (0.1 Hz; $P = 0.37$). In *experiment 3*, MEP recruitment curve and paired pulse analyses showed that the high-intensity rTMS protocol increased MEPs over a range of stimulus intensities but that effects were not accompanied by changes in intracortical inhibition or facilitation ($P > 0.12$). These experiments reveal a novel high-intensity, low-frequency rTMS protocol for enhancing corticospinal excitability.

NEW & NOTEWORTHY In this study, we present a novel, intensity-dependent repetitive transcranial magnetic stimulation (rTMS) protocol that induces lasting, plastic changes within the corticospinal tract. High-intensity rTMS at a frequency of 0.1 Hz induces facilitation of motor evoked potentials (MEPs) lasting at least 35 min. Additionally, these changes are not limited only to small MEPs but occur throughout the recruitment curve. Finally, facilitation of MEPs following high-intensity rTMS does not appear to be due to changes in intracortical inhibition or facilitation.

corticospinal; paired corticospinal-motoneuronal stimulation; plasticity; rTMS; transcranial magnetic stimulation

INTRODUCTION

Several techniques have been developed to induce plasticity in the human corticospinal pathway at either cortical or spinal levels. At the cortical level, repetitive transcranial magnetic stimulation (rTMS) over the motor cortex induces a frequency- and intensity-dependent change in motor cortical excitability (for review see Fitzgerald et al. 2006). Paired associative stimulation (PAS) paradigms repeatedly pair sensory afferent inputs elicited by peripheral nerve stimulation with transcranial magnetic stimulation (TMS) of the motor cortex (for review see Carson and Kennedy 2013), with facilitation or inhibition occurring when the afferent input arrives at the cortex just before or shortly after TMS, respectively (Classen et al. 2004; Stefan et al. 2000; Wolters et al. 2003). Similar paired protocols can be used to alter excitability at a spinal level (Cortes et al. 2011; Knikou 2017; Leukel et al. 2012; Shulga et al. 2016; Taylor and Martin 2009). One such technique, known as paired corticospinal-motoneuronal stimulation (PCMS), involves the delivery of repeated pairs of TMS and supramaximal peripheral nerve stimulation to induce plasticity at corticospinal-motoneuronal synapses. PCMS effectively modifies evoked muscle responses, enhances voluntary motor output during low-force and maximal voluntary contractions, and improves hand dexterity after spinal cord injury (Bunday and Perez 2012; D'Amico et al. 2018; Dongés et al. 2018; Fitzpatrick et al. 2016; Taylor and Martin 2009; Urbin et al. 2017).

While PCMS is designed to target the corticospinal-motoneuronal synapse through the convergence of descending corticospinal input and antidromic motoneuronal volleys, supramaximal stimulation of a mixed nerve also activates sensory afferent pathways. As occurs for cortically targeted PAS, it is possible that such afferent input, when combined with TMS, could alter cortical excitability. PCMS protocols designed to induce facilitation of corticospinal-motoneuronal synapses supplying hand muscles implement interstimulus intervals (ISIs) of ~5 ms (ulnar nerve stimulation at the wrist before TMS) (Bunday and Perez 2012; D'Amico et al. 2018). Although the ISI used for inhibitory PAS is 10 ms (median nerve stimulation at the wrist before TMS) (Wolters et al. 2003), both facilitatory PCMS and inhibitory PAS result in the arrival of sensory afferent inputs at the motor cortex after TMS (for somatosensory conduction times, see Macerollo et al. 2018). The window for inhibition after PAS is unlikely constrained to a single ISI of 10 ms, given that windows for facilitation and inhibition after paired stimulus protocols typically range from

* J. M. D'Amico and S. C. Dongés contributed equally to this manuscript. Correspondence: J. Taylor, (e-mail: janet.taylor@ecu.edu.au).

10 to ~100 ms (Feldman 2012). Indeed, in Wolters et al. the traces of a single individual, as well as group data (although not significant), suggest that inhibition also occurs for ISIs of 0 and 5 ms in at least some individuals (Wolters et al. 2003). Therefore, it is possible that PCMS designed to produce facilitation at a spinal level could have an inhibitory effect at the cortex. In support of this, a previous study reported an increase in responses evoked by cervicomedullary stimulation, but not MEPs when PCMS was compared with a TMS-only control protocol (Fitzpatrick et al. 2016). The lack of change in MEPs may reflect a combined effect of spinal cord facilitation and depression of the motor cortex.

TMS can elicit an early "D-wave" as well as later "I waves" through direct and indirect (transsynaptic) activation of corticospinal axons, respectively (Di Lazzaro and Ziemann 2013; Rusu et al. 2014; Triesch et al. 2015). Low-intensity TMS using a figure-eight coil preferentially elicits different D or I waves, depending on the coil's orientation and the resulting direction of induced current in the brain. In all coil orientations, as stimulus intensity is increased, other descending volleys begin to appear (Di Lazzaro et al. 2003; Di Lazzaro and Ziemann 2013). Inhibitory PAS has been shown to reduce the amplitude of later I-waves, while having no effect on the D or first I wave (I1) (Di Lazzaro et al. 2009). In *experiment 1*, we tested the idea that PCMS designed to induce spinal facilitation may result in cortical inhibition similar to that induced by inhibitory PAS. To do this we elicited MEPs using low-intensity TMS before and after PCMS and TMS-only conditioning. MEPs were obtained using three different coil orientations to induce current in a lateromedial (LM) direction, a 45° posterior-to-anterior (PA) direction (current approximately perpendicular to the central sulcus) (Janssen et al. 2015) and an anterior-to-posterior (AP) direction. Working under the assumption that PCMS has similar cortical effects to inhibitory PAS (i.e., later I-wave amplitude reduction and no effect on I1), and given that PCMS enhances corticospinal transmission at a spinal level, we hypothesized that PCMS would increase MEPs elicited in the LM (preferential for the D wave, thus bypassing cortical effects) and 45° PA (preferential for I1) orientations and would decrease (or increase by a smaller amount than the other orientations) MEPs elicited in the AP orientation (preferential for later I waves) (Di Lazzaro et al. 2003). Contrary to our hypothesis, *experiment 1* revealed similar increases in MEPs after PCMS regardless of the coil orientation used. Furthermore, the control protocol of repetitive high-intensity TMS-only stimuli resulted in MEP increases similar to those after PCMS. Given these unexpected findings, two additional experiments were performed to further explore the potential use of this repeated high-intensity TMS protocol as a novel rTMS protocol for enhancing excitability of the motor cortex. These experiments revealed that the rTMS protocol was intensity dependent and that its effects were not accompanied by alterations in intracortical inhibition or facilitation.

METHODS

Participants

All experiments were approved by the Human Research Ethics Committee at the University of New South Wales and conformed to the Declaration of Helsinki, except for registration in a database. All participants gave written, informed consent before participating in the study. Also before participation, a TMS safety screening questionnaire was carried out to ensure participants' safety and eligibility. Three sets of experiments were performed to determine changes in the corticospinal pathway following two different stimulation protocols in healthy individuals. Fourteen healthy participants were enrolled in *experiment 1* [3 women, 25 yr (SD 6)]. Another fourteen participants were enrolled in *experiment 2* [9 women, 22 yr (SD 3)]. Nine of the participants from *experiment 2* participated in *experiment 3*, along with an additional five participants [6 women, 23 yr (SD 2)].

Setup

In all experiments, participants were seated with their forearm resting on a table in a neutral position. The forearm and wrist were secured with straps to a padded L-shape support which was positioned along the dorsum of the forearm and hand. A padded plate was positioned over the palmar surface of the hand with the fingers fully extended. This plate clamped the hand and fingers to reduce activation of other hand muscles during recordings. The thumb was abducted and placed in a padded metal ring in a comfortable position.

Electromyogram

In all three experiments, surface EMG signals were obtained from adductor pollicis through adhesive surface EMG electrodes (10-mm diameter; Ambu WhiteSensor 7841P, Glen Burnie, MD) placed over the muscle belly and the first metacarpo-phalangeal joint. All EMG signals were amplified (×300) and bandpass filtered (16–1,000 Hz) using CED 1902 amplifiers (Cambridge Electronic Design, CED, Cambridge, UK) and sampled at 5 kHz using a 16-bit analog-to-digital converter (CED 1401) and Spike 2 (version 7, CED) or Signal (version 4, CED) software.

Experiment 1: Paired Corticospinal-Motoneuronal Stimulation and High-Intensity Transcranial Magnetic Stimulation Conditioning

Stimulation. TRANSCRANIAL MAGNETIC STIMULATION. A figure-eight coil (9.5-cm outside loop diameter; Magstim 200, Magstim, Whitland, UK) was used to elicit motor evoked potentials (MEPs) in adductor pollicis at rest. The coil was positioned in three different orientations throughout the experiment. These consisted of *1*) a 45° posterior-anterior (PA) orientation with the handle pointed backward and away from the midline, *2*) a lateromedial (LM) orientation with the handle pointed away from and perpendicular to the midline, and *3*) an anterior-posterior (AP) orientation with the handle pointed toward the front of the head parallel to the midline. The optimal scalp position to activate the right adductor pollicis muscle was identified in all three coil orientations during setup and was marked on a tight-fitting cap. The same coil holder was used for all experiments. Resting motor threshold (rMT; intensity required to elicit a MEP of ~50 μV in 50% of trials) was determined for each coil orientation using the TMS Motor Threshold Assessment Tool (MTAT 2.0) from www.clinical-researcher.org. An intensity of 1.1 × rMT [45° PA: 47% (SD 6) maximum stimulator output, MSO; LM: 52% (SD 8) MSO; AP: 60% (SD 9) MSO] was used for MEP testing in all three coil orientations before and after the conditioning protocols. 1.1 × rMT was chosen so that test MEPs could be reliably elicited while keeping intensity as low as possible to allow for preferential activation of D and I waves targeted by each respective coil orientation. Lastly, the intensity required to elicit a maximal MEP (MEPmax) was determined based on a rough MEP recruitment curve that was performed each day during setup in the 45° PA orientation only. Three stimuli were delivered at each intensity, and stimulus intensity was progressively increased until no further increase in MEP amplitude occurred (average of three responses). This high intensity [70 to 95% MSO; average

of 83% (SD 9) MSO on both days] and coil orientation were used during the conditioning protocols.

PERIPHERAL NERVE ELECTRICAL STIMULATION. Adductor pollicis maximal M waves were elicited through electrical stimulation of the ulnar nerve at the wrist (DS7AH stimulator, Digitimer, Welwyn Garden City, UK; pulse width 0.2 ms). Surface electrodes (10-mm diameter; Ambu WhiteSensor 7841P) were placed ~2 cm apart over the nerve with the cathode positioned distal to the anode, and ~2 cm proximal to the wrist. Stimulus intensity was increased until there was no further increase in the size of the adductor pollicis M wave, and 120% of this intensity was used [49 mA (SD 14)] to elicit maximal compound muscle action potentials (Mmax). This supramaximal ulnar nerve stimulation was used both for conditioning on one day (see *Protocol* for *experiment 1*) and to elicit Mmax before and after the conditioning protocols.

CERVICAL ROOT STIMULATION. Cervical root responses (Croot) were elicited with the same figure-eight coil used to elicit MEPs. The coil was held perpendicular to the spinal cord with the handle pointing left. The coil was centered over the spinous process of the first thoracic (T1) vertebra (stimulus intensity 50% MSO).

Protocol. Adductor pollicis MEPs were elicited using low-intensity TMS before and after two conditioning protocols: *1*) paired corticospinal-motoneuronal stimulation and *2*) repeated high-intensity TMS alone, designed as a control protocol and expected to have no effect.

Participants visited the laboratory on two different occasions, separated by at least 72 h. During one visit they received repeated, paired stimulation designed to facilitate corticospinal-motoneuronal synapses. This technique, known as paired corticospinal-motoneuronal stimulation (PCMS), consisted of 100 pairs (0.1 Hz) of high-intensity transcranial magnetic stimuli (45° PA orientation) and supramaximal ulnar nerve stimuli. Stimulus pairs were delivered at an interstimulus interval (ISI) that resulted in a 1.5-ms synaptic delay between the orthodromic corticospinal volley and the antidromic peripheral nerve volley at the corticospinal-motoneuronal synapse (corticospinal volley before peripheral nerve volley). This timing has been shown to facilitate the corticospinal pathway (Bunday and Perez 2012). The ISI [ulnar nerve stimulation 5.9 s (SD 1.2) before TMS] was calculated during setup on each day using latencies of the Mmax, MEPcontracting (MEP elicited during a weak adductor pollicis contraction), and Croot responses as follows:

$$Nerve\ to\ Synapse = Croot - Mmax + 0.5$$

$$Cortex\ to\ Synapse = MEPcontracting - \left(Croot + 1.5\right)$$

$$ISI = Nerve\ to\ Synapse - Cortex\ to\ Synapse - 1.5$$

During the other visit participants received a conditioning protocol of repeated high-intensity TMS alone, designed as a control protocol and expected to have no effect. This consisted of 100 high-intensity transcranial magnetic stimuli (45° PA orientation) at a rate of 0.1 Hz. The order of the visits was randomized.

On each day, three to four baseline measurement blocks were performed at 7-min intervals before conditioning. Immediately (~1 min) following each conditioning protocol, measurements resumed every 7 min for 35 min (Fig. 1, *top*). Each measurement block consisted of 10 MEPs in the 45° PA orientation, 10 MEPs in the LM orientation, and 10 MEPs in the AP orientation followed by 2 Mmax. Stimuli were delivered at 0.2 Hz.

Experiment 2: Low-Intensity TMS Conditioning

Experiment 2 tested whether MEPs elicited by low-intensity TMS were facilitated by conditioning with repeated low-intensity TMS.

Stimulation. TRANSCRANIAL MAGNETIC STIMULATION. A figure-eight coil (Magstim 200) in the 45° PA orientation only was used to

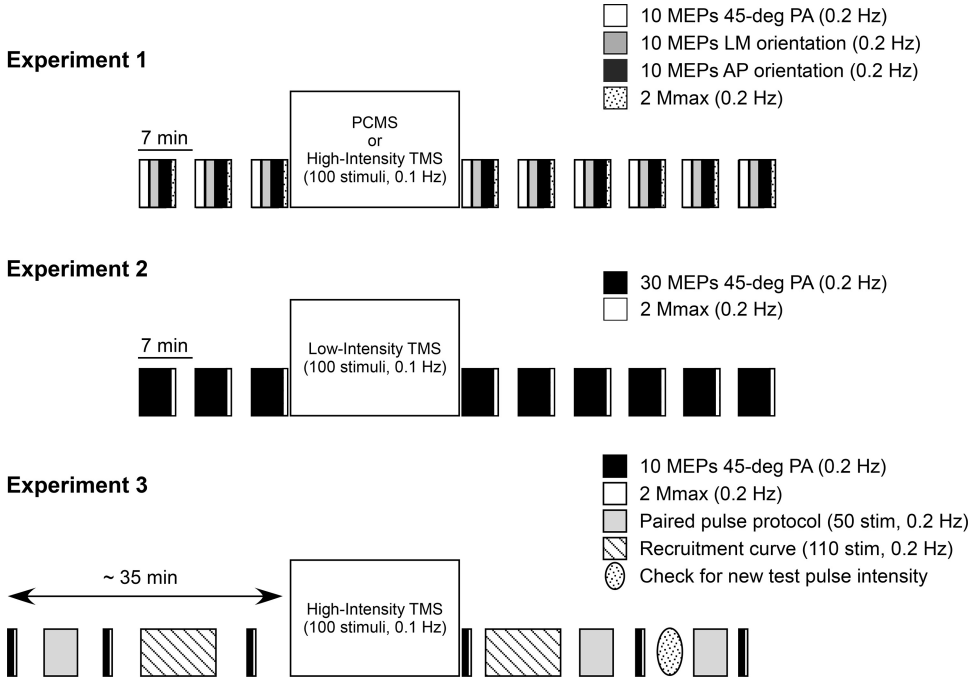

Fig. 1. Experimental design. *Top*: *experiment 1* examines the effects of PCMS or high-intensity TMS-only protocols on MEPs elicited with the coil in three different orientations: 45° PA, LM, and AP. At each time point, a set of 10 MEPs are elicited in each of the three coil orientations (1.1 × rMT), followed immediately by 2 Mmax measurements. Measurement blocks are repeated before and after the conditioning protocols. *Middle*: *experiment 2* examines the effects of low-intensity TMS-only conditioning on MEP areas. Each measurement block consisted of 30 MEPs (45° PA coil orientation; 1.1 × rMT) and 2 Mmax. Measurement blocks are repeated before and after the conditioning protocol. *Bottom*: *experiment 3* examines the effects of the high-intensity rTMS protocol on: MEPs elicited at 1.1 × rMT in the 45° PA coil orientation (black rectangles), TMS recruitment curves (white hatched boxes) and on intracortical inhibition and facilitation (gray rectangles). AP, anterior-to-posterior; LM, lateromedial; MEP, motor evoked potential; Mmax, maximal compound muscle action potentials; PA, posterior-to-anterior; PCMS, paired corticospinal-motoneuronal stimulation; rMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation; TMS, transcranial magnetic stimulation.

elicit MEPs in the right adductor pollicis muscle. The optimal scalp position as well as the rMT were determined as per *experiment 1*. An intensity of 1.1 × rMT was used both for testing and for the conditioning protocols [44% (SD 11) MSO].

PERIPHERAL NERVE ELECTRICAL STIMULATION. Adductor pollicis Mmax was elicited as per *experiment 1* using a supramaximal stimulus intensity of 40 mA (SD 16).

Protocol. Participants visited the laboratory only once and received a conditioning protocol which consisted of 100 low-intensity transcranial magnetic stimuli at 0.1 Hz. Three to four baseline measurement blocks were recorded 7 min apart. Measurement blocks resumed immediately (~1 min) after conditioning and every 7 min for 35 min (Fig. 1, *middle*). Each measurement block consisted of 30 MEPs followed by 2 Mmax. Stimuli were delivered at 0.2 Hz.

Experiment 3: High-Intensity TMS Conditioning Mechanisms

Experiment 3 tested whether conditioning with repeated high-intensity TMS facilitated MEPs regardless of their size and investigated the possibility of alterations in intracortical inhibition and facilitation.

Stimulation. TRANSCRANIAL MAGNETIC STIMULATION. A figure-eight coil (Magstim 200) in the 45° PA orientation only was used to elicit MEPs in the right adductor pollicis muscle at rest. The optimal coil position and rMT were determined as per *experiments 1* and *2*. In addition, rMT was also determined using a BiStim^2 (Magstim), which was used only for paired pulse testing. MEPmax was determined as per *experiment 1*.

Test responses included single MEPs, recruitment curves and paired pulse stimuli. Single MEP responses were evoked at 1.1 × rMT [44% (SD 8) MSO], similar to *experiments 1* and *2*. Recruitment curves were obtained using intensities which were set relative to rMT [39% (SD 8) MSO] and the intensity for MEPmax. These 11 intensities were 0.8, 0.9, 1.0, 1.1, and 1.2 × rMT, followed by 4 intensities equally distributed between 1.2 × rMT and MEPmax intensity, followed by MEPmax, and finally an intensity at the same increment above MEPmax [MEPmax intensity plus 0.2 × (MEPmax intensity − 1.2 × rMT)]. Distribution of intensities between each individual's MEPmax intensity and 1.2 × rMT was done to ensure that each individual received the same total number of stimuli during the recruitment curves. Stimuli were delivered every 5 s and at random intensities, with 10 stimuli at each intensity in the recruitment curve (total of 110 stimuli per recruitment curve).

For the paired pulse paradigms, the conditioning pulse intensity was set at 0.7 × rMT, and the test pulse was 1.2 × rMT [rMT = 44% (SD 9) MSO using Bistim^2]. Short-interval intracortical inhibition (SICI) was measured with both a 2- and 3-ms ISI. Intracortical facilitation (ICF) was measured with both a 10- and a 12-ms ISI. These ISIs were chosen based on previous work showing that a subthreshold conditioning stimulus can suppress or facilitate a MEP elicited by a suprathreshold stimulus delivered either ~1 to 4 ms (SICI) or ~8 to 15 ms (ICF) later (Kujirai et al. 1993; Ziemann et al. 1996). The paired pulse paradigm consisted of 10 pairs of stimuli at each ISI, and an additional 10 single test pulses delivered every 5 s, in a pseudo-random order (50 stimuli in total).

PERIPHERAL NERVE ELECTRICAL STIMULATION. Maximal M waves of adductor pollicis were elicited as per *experiments 1* and *2* using a supramaximal stimulus intensity of 47 mA (SD 19).

Protocol. Participants visited the laboratory on one day and received a conditioning protocol which consisted of 100 high-intensity transcranial magnetic stimuli at 0.1 Hz at the intensity required to elicit MEPmax during the setup [78% (SD 12) MSO]. Before and after conditioning, three sets of 10 single MEPs at 1.1 × rMT, followed by 2 Mmax, were delivered at 0.2 Hz. In addition, a single recruitment curve was carried out before and after the conditioning protocol. The paired pulse paradigm was performed once before conditioning and twice after conditioning. On the first occasion after conditioning, stimulus intensities were the same as before conditioning. If the averaged postconditioning test MEP differed by >50% from that measured before conditioning, test stimulus intensity was adjusted before the second set of paired pulse measurements. Test stimulus intensity was adjusted based on online measurement of individual MEP amplitudes to evoke a test MEP that was close to the test MEP amplitude before conditioning. The measurement blocks were performed as per Fig. 1, *bottom*. To ensure that all measurements were completed within a similar time frame to *experiments 1* and *2* and obtained during a time window where the facilitatory effects of the conditioning protocol had previously been demonstrated, the last outcome measure for *experiment 3* occurred at 35 min after conditioning.

Data Analysis

All data analyses were performed offline using Signal 4 (Version 4, CED) and Excel software (Microsoft Corporation). The areas of the compound muscle action potentials (MEPs and Mmax) were calculated using built-in software functions. The MEP areas were then normalized to the average area of the corresponding Mmax either within the same stimulus set (for single MEPs), or in the stimulus set immediately before collection (for the recruitment curves and paired pulse paradigms). For the single pulse MEPs measured in all three experiments, a value was obtained for each time point by averaging either the 10 (*experiments 1* and *3*) or 30 (*experiment 2*) MEPs in each measurement block and coil orientation. The baseline measurements for each coil orientation were averaged together, and all measurement blocks were expressed as a percentage of this averaged baseline. For analysis of the recruitment curves, the 10 MEPs at each intensity were averaged. The difference curve (Post−Pre) was then calculated by subtracting the preconditioning averages (Pre) from the postconditioning averages (Post) at each intensity in the recruitment curves for each individual. For illustration, all intensities are expressed relative to rMT for each participant. For the paired pulse analysis, measurement windows were adjusted accordingly for each evoked response to encompass the potentials. The areas of the 10 MEPs in each condition (test, SICI2, SICI3, ICF10, and ICF12) were then averaged. SICI and ICF were then reported as a ratio of the conditioned MEP to the test MEP.

Statistics

Results are reported as means (SD) in the text and figures for averages at each time point. Differences between days and/or following the conditioning protocol are expressed as means [95% CI] in the text and figures. All statistical analyses were performed with SPSS Statistics 22 software. For each day of *experiment 1*, two-way repeated-measures (RM) ANOVAs were carried out to determine whether baseline MEP areas were well matched and whether there were differences in MEP latencies over time (3 time points) or between coil orientations (45° PA, LM, and AP). Two-way RM ANOVAs were carried out to determine the effect of conditioning protocol and time on MEPs elicited in each different coil orientation and Mmax in *experiment 1*. One-way RM ANOVAs were used to determine the effect of time (i.e., before and after conditioning) on normalized MEPs and Mmax in *experiments 2* and *3*. Where the assumption of normality was not met, nonparametric, one-way RM ANOVA on ranks (Friedman's test) was used. For the nine participants who completed both *experiments 2* and *3*, a two-way RM ANOVA was used to compare normalized MEPs after repetitive low- and high-intensity TMS at 1 and 35 min after conditioning. For analysis of the recruitment curves, a two-way RM ANOVA was carried out to determine the effect of time and stimulus intensity in *experiment 3*. Lastly, two-way RM ANOVAs were used to determine the effect of time and ISI on SICI and ICF in *experiment 3*. A one-way RM ANOVA was used

to determine whether the test MEP was significantly different following the conditioning protocol. Mauchly's test of sphericity was performed, and for any data which were not spherical, a Greenhouse–Geisser correction was used. For any significant interaction effects, post hoc Bonferroni-corrected *t* tests were used.

RESULTS

Experiment 1

Baseline MEP areas were well matched on both days within each coil orientation [45° PA TMS-only: 11.9% (SD 7.4); 45° PA PCMS: 8.5% (SD 5.2); LM TMS-only: 11.2% (SD 7.1); LM PCMS: 8.4% (SD 5.8); AP TMS-only: 8.2% (SD 4.2); AP PCMS: 7.2% (SD 5.6); all expressed as % Mmax; all $P > 0.14$]. Mmax values increased over time on both days [postconditioning difference TMS-only: 0.28 mV (SD 0.34); PCMS: 0.35 mV (SD 0.43); $F_{1.345,\ 16.141} = 17.946$, $P < 0.001$]; however, there was no day or day × time effect (all $P > 0.35$). Two-way RM ANOVAs showed no differences in baseline MEP areas on the TMS-only day (coil orientation effect: $F_{2,\ 26} = 3.225$, $P = 0.056$; time effect: $F_{2,\ 26} = 0.095$, $P = 0.91$, coil orientation × time effect: $F_{4,\ 52} = 0.342$, $P = 0.86$) or PCMS day (coil orientation effect: $F_{2,\ 26} = 0.626$, $P = 0.543$, time effect: $F_{2,\ 26} = 2.326$, $P = 0.12$, coil orientation × time effect: $F_{4,\ 52} = 1.380$, $P = 0.254$) but did show differences in baseline MEP onset latencies between coil orientations on both the TMS-only day (coil orientation effect: $F_{1.023,\ 13.304} = 13.581$, $P = 0.003$; time effect: $F_{2,\ 26} = 0.011$, $P = 0.989$, coil orientation × time effect: $F_{4,\ 52} = 0.282$, $P = 0.889$) and the PCMS day (coil orientation effect: $F_{1.031,\ 12.368} = 10.412$, $P = 0.007$; time effect: $F_{1.369,\ 16.422} = 4.146$, $P = 0.048$, coil orientation × time effect: $F_{4,\ 48} = 1.087$, $P = 0.374$). MEP latency was significantly longer in the AP orientation [TMS-only: 24.8 ms (SD 1.5); PCMS: 24.8 ms (SD 1.6)] than the 45° PA orientation [TMS-only: 23.0 ms (SD 1.5), $P = 0.007$; PCMS: 23.0 ms (SD 1.3), $P = 0.015$] or the LM orientation [TMS-only: 23.1 ms (SD 1.5), $P = 0.009$; PCMS: 23.2 ms (SD 1.3), $P = 0.031$] on both days.

MEPs elicited in the 45° PA coil orientation increased both on the TMS-only and PCMS day [TMS-only: 125% (SD 32); PCMS: 129% (SD 36) baseline]. A two-way RM ANOVA revealed a significant time effect ($F_{8,\ 104} = 3.75$, $P = 0.001$), but no significant treatment ($F_{1,\ 13} = 0.123$, $P = 0.73$) or treatment × time interaction effects ($F_{8,\ 104} = 0.41$, $P = 0.92$, Figs. 2 and 3*A*). When the difference between days was calculated for each individual and averaged for the group, the mean difference (PCMS − TMS-only) in MEPs was 4.4% [−20.1, 28.9] of baseline (Fig. 3*B*).

MEPs elicited in the LM coil orientation were increased following the TMS-only [108% (SD 39)] and the PCMS protocols (134% (SD 42) baseline]. Two-way RM ANOVA revealed a significant time effect ($F_{8,\ 104} = 2.38$, $P = 0.02$) but no significant treatment ($F_{1,\ 13} = 3.71$, $P = 0.08$), or treatment × time interaction effects ($F_{8,\ 104} = 1.78$, $P = 0.09$, Fig. 3*C*). The mean difference between days was 25.8% [−0.6, 52.2] of baseline (Fig. 3*D*).

MEPs elicited by the AP coil orientation displayed similar changes to those elicited with the 45° PA coil orientation. MEPs increased on both days [TMS-only: 129% (SD 51); PCMS: 136% (SD 55) baseline]. A two-way RM ANOVA revealed a significant time effect ($F_{2.982,\ 38.761} = 4.795$, $P = 0.006$, Greenhouse–Geisser corrected), with no treatment ($F_{1,\ 13} = 0.234$, $P = 0.64$) or treatment × time interaction effects ($F_{3.262,\ 42.407} = 0.351$, $P = 0.81$, Greenhouse–Geisser corrected, Fig. 3*E*). The mean difference between days was 7.8% [−24, 39.6] of baseline (Fig. 3*F*).

Experiment 2

Baseline MEPs were 5.3% (SD 3.4) Mmax in the 45° PA orientation and were stable before conditioning. Mmax values were 14.4 mV (SD 3.1) at baseline and did not change over time ($P = 0.20$). A one-way RM ANOVA on ranks revealed no change [χ^2 (6) = 6.490, $P = 0.37$] in MEPs following repetitive low-intensity TMS at 0.1 Hz [mean postconditioning MEPs were 106% (SD 25) of baseline] (Fig. 4*A*).

Experiment 3

Baseline MEPs were similar in size to those in *experiment 2* and were 4.7% (SD 3.1) Mmax. Similar to *experiment 1*, Mmax values were 12.8 mV (SD 2.2) at baseline and increased over time (average postconditioning: 13.2 mV (SD 2.5), $F_{1.394,\ 18.126} = 4.45$, $P = 0.04$). Following repetitive high-intensity TMS stimuli at 0.1 Hz, mean MEPs increased to 130% (SD 44) of baseline. A one-way RM ANOVA revealed a significant time effect ($F_{2.953,\ 38.394} = 3.42$, $P = 0.03$, Greenhouse–Geisser corrected, Fig. 4*B*). For the nine participants who completed both *experiments 2* and *3*, there was a significant difference in MEPs between repetitive low- and high-intensity TMS conditioning protocols ($F_{1,\ 8} = 14.96$, $P = 0.005$).

MEP recruitment curves were obtained before and after conditioning using a range of intensities from 0.8 to 2.2 (SD 0.4) × rMT depending on the spread of each individual's curve. Following conditioning, the averaged group curve displayed a slight increase in MEPs at all intensities above threshold, including MEPmax (Fig. 5*A*). Two-way RM ANOVA revealed significant time ($F_{1,\ 13} = 5.594$, $P = 0.034$) and stimulation intensity effects ($F_{1.161,\ 15.095} = 33.07$, $P < 0.001$, Greenhouse–Geisser corrected), with no significant stimulation intensity × time inter-

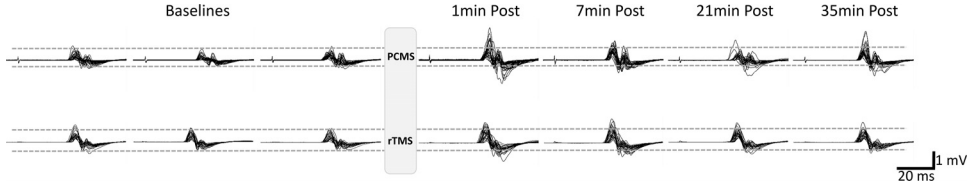

Fig. 2. Raw EMG traces following PCMS and rTMS protocol in a single individual. Figure shows 30 overlaid MEP traces elicited with the coil in the 45° PA, LM, and AP orientations (10 MEPs each) before (Baselines) and 1, 7, 21, and 35 min after (Post) the PCMS (top traces) and rTMS (bottom traces) protocols in the same individual. AP, anterior-to-posterior; LM, lateromedial; MEP, motor evoked potential; Mmax, maximal compound muscle action potentials; PA, posterior-to-anterior; PCMS, paired corticospinal-motoneuronal stimulation; rTMS, repetitive transcranial magnetic stimulation.

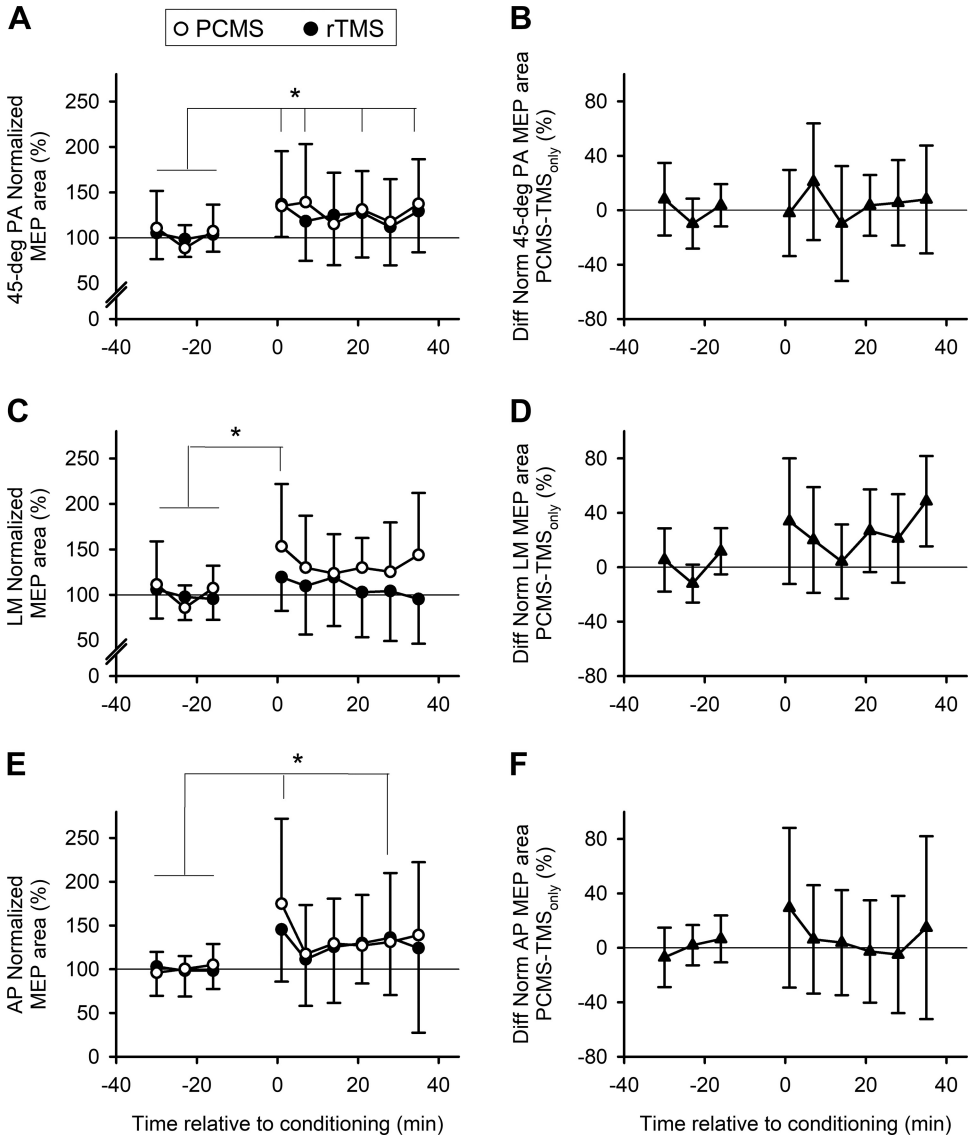

Fig. 3. Effect of PCMS and high-intensity TMS-only (TMS_{only}; rTMS) protocols on MEPs evoked in the 45° PA, LM, and AP orientations. *A*, *C*, *E*: group average ($n = 14$) of MEP areas normalized to the average of the three baselines before and after high-intensity rTMS (black circles) and PCMS (white circles) protocols. *B*, *D*, *F*: difference (PCMS – rTMS) in averaged MEP areas across time. *$P < 0.05$ post hoc comparisons for time effect when compared with the average of the three baselines. AP, anterior-to-posterior; Diff, difference; LM, lateromedial; MEP, motor evoked potential; Norm, normalized; PA, posterior-to-anterior; PCMS, paired corticospinal-motoneuronal stimulation; rTMS, repetitive TMS; TMS, transcranial magnetic stimulation.

action effects ($F_{2.714,\ 35.280} = 1.986$, $P = 0.139$, Greenhouse–Geisser corrected). The differences in MEP area following conditioning at each intensity averaged across individuals appeared to be greatest at the 1.2 to 1.35 × rMT intensities (Fig. 5*B*), with the greatest difference of 6.5% Mmax [3.3, 9.7] occurring at ~1.35 × rMT. The overall average difference postconditioning was 2.8% Mmax [0.5, 5.2].

For the paired pulse protocol, baseline test MEPs were 11.8% (SD 9.9) Mmax. For SICI, baseline conditioned MEPs were 4.9% (SD 4.5) and 6.4% (SD 7.3) Mmax for the 2 and 3 ms ISIs, respectively. From this, the SICI ratios were 0.45 (SD 0.24) and 0.48 (SD 0.24), respectively. For ICF, baseline conditioned MEPs were 16.0% (SD 12.4) and 15.8% (SD 10.9) Mmax for the 10- and 12-ms ISIs, respectively. The ICF ratios were 1.44 (SD 0.53)

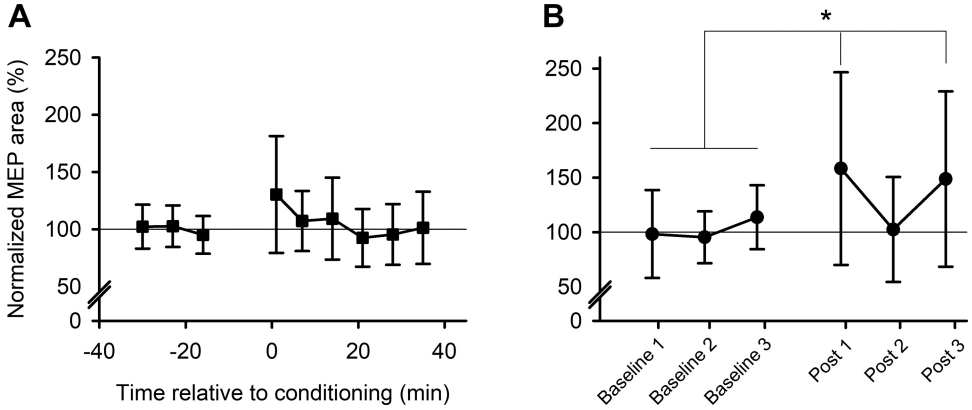

Fig. 4. Effect of low- and high-intensity rTMS protocols on MEP areas. *A*: group ($n = 14$) averaged MEP areas, normalized to the average of the three baselines, following low-intensity rTMS protocol (*experiment 2*). *B*: group ($n = 14$) averaged MEP areas, normalized to the average of the three baselines at ~1, 22, and 35 min (Post 1, Post 2, Post 3), following high-intensity rTMS protocol (*experiment 3*). *$P < 0.05$ post hoc comparisons for time effect when compared with the average of the three baselines. MEP, motor evoked potential; rTMS, repetitive transcranial magnetic stimulation.

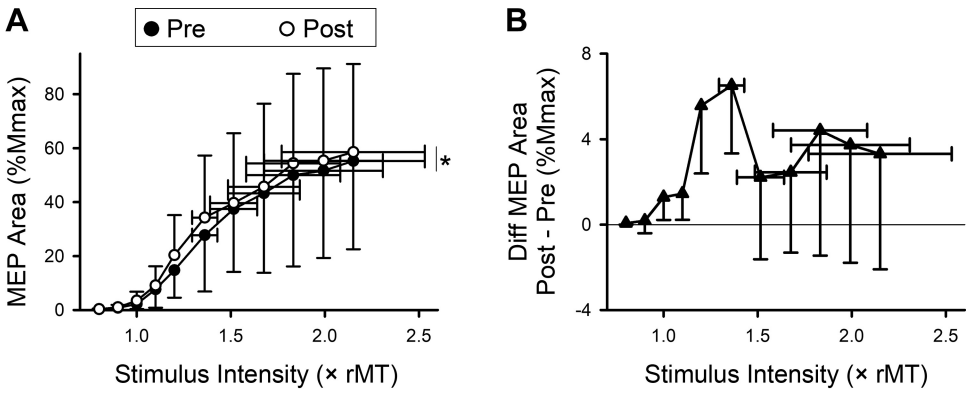

Fig. 5. Effect of high-intensity rTMS protocol on MEP recruitment curve. *A*: group ($n = 14$) averaged MEP recruitment curves before (Pre; black circles) and after (Post, white circles) high-intensity rTMS protocol. MEP areas are expressed as a percentage of Mmax and plotted versus stimulation intensity (× rMT). *$P < 0.05$ post hoc comparisons for time effect. Significant post hoc comparisons for stimulation intensity are not shown. *B*: difference (Post−Pre) in group-averaged MEP areas across stimulation intensities (× rMT). Diff, difference; MEP, motor evoked potential; Mmax, maximal compound muscle action potentials; rMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation.

and 1.48 (SD 0.53), respectively. The above baseline measurements indicate that the intensities used for the conditioning and test pulse, as well as the ISIs chosen, were appropriate to measure SICI and ICF in our participants. Following the conditioning protocol, the first block of paired-pulse stimuli (Post 1) revealed that 5 of the 14 participants required an adjusted test pulse because the test MEP was too large (>50% increase). A second block of paired-pulse stimuli (Post 2) was performed in all participants, and in those in whom the test MEP was too large the stimulus intensity was adjusted for the second postconditioning set. One-way RM ANOVA revealed no significant differences in the test MEP with time ($F_{2, 26} = 2.153$, $P = 0.14$). Separate two-way RM ANOVAs for SICI and ICF (ISI × time) initially revealed significant time effects for SICI only, with greater SICI at Post 1 (unadjusted) than before conditioning (time: $F_{2, 26} = 3.40$, $P = 0.049$, Fig. 6*B*). There were no time effects for ICF and no ISI or ISI × time interaction effects for either SICI or ICF (all $P > 0.12$). Once unadjusted test pulses that were too large were excluded from the statistical analysis across all three timepoints (only 9 participants included) the significant time effect for SICI was no longer significant (all $P > 0.53$), thus the difference was most likely due to a change in the size of the test response at the Post 1 timepoint. Therefore, high-intensity TMS conditioning does not appear to alter cortical SICI and ICF.

DISCUSSION

In the present study, MEPs elicited using three different coil orientations were similarly increased following paired corticospinal-motoneuronal stimulation (PCMS). Unexpectedly, similar increases in MEPs after conditioning with repetitive high-intensity TMS-only stimuli were also observed, revealing a novel rTMS protocol for enhancing corticospinal excitability. Further exploration of this rTMS protocol revealed that it was intensity dependent, as evidenced by a lack of effect following a conditioning protocol of repetitive low-intensity TMS-only stimuli. Additionally, MEP recruitment curve analysis demonstrated that MEP facilitation occurred at all intensities above threshold (including MEPmax) after the high-intensity rTMS protocol; however, these changes were not accompanied by alterations in intracortical inhibition or facilitation.

PCMS facilitates corticospinal transmission to the motoneurons, most likely through strengthening of the corticomotoneuronal synapse, as demonstrated by increases in cervicomedullary motor evoked potentials (Dongés et al. 2018; Fitzpatrick et al. 2016; Taylor and Martin 2009). However, a previous study reports that despite facilitation of subcortically evoked responses, there were no differences in MEPs when PCMS was compared with a TMS-only control protocol (Fitzpatrick et al. 2016). This variable effect may be explained by inhibition of the motor cortex similar to that which occurs following inhibitory PAS protocols (Di Lazzaro et al. 2009). In an attempt to discriminate cortical and spinal changes following PCMS targeted for adductor pollicis, we elicited MEPs using three different coil orientations. The LM coil orientation preferentially elicits D waves (Di Lazzaro et al. 2003; Di Lazzaro and Ziemann 2013) which are thought to have limited sensitivity to

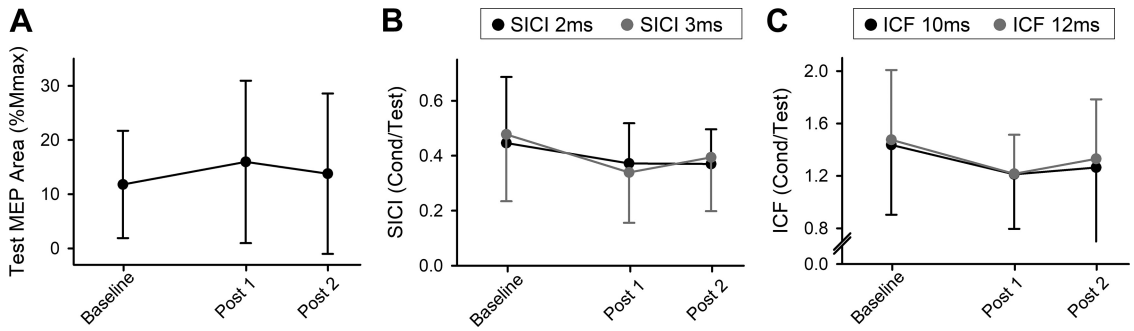

Fig. 6. Effect of high-intensity rTMS protocol on intracortical inhibition and facilitation. *A*: group averaged ($n = 14$) test MEP areas (as a percentage of Mmax) before (Baseline) and ~17 and 30 min after (Post 1, Post 2) rTMS protocol. *B*: effect of high-intensity rTMS protocol on short interval intracortical inhibition (SICI). SICI is measured as the group averages of the ratios of the conditioned MEP area/test MEP area before and after the rTMS protocol and was measured using both a 2-ms (black circles) and 3-ms ISI (gray circles). *C*: effect of high-intensity rTMS protocol on intracortical facilitation (ICF). ICF is measured as the group averages of the ratios of the conditioned MEP area/test MEP area before and after the rTMS protocol and was measured using both a 10-ms (black circles) and 12-ms ISI (gray circles). Cond, conditioned; ISI, interstimulus interval; MEP, motor evoked potential; Mmax, maximal compound muscle action potentials; rTMS, repetitive transcranial magnetic stimulation.

changes in cortical excitability, while the AP coil orientation preferentially elicits later I waves that may be more sensitive to modulation by inhibitory PAS (Di Lazzaro et al. 2009). However, when these coil orientations were tested in the present study, MEPs elicited in the LM and AP orientations were similarly increased following PCMS. Using low-intensity TMS, and based on previous work (Di Lazzaro et al. 2003), we assumed that the three different coil orientations preferentially elicited certain D and I waves. This was further supported by MEP onset latency analysis, which showed longer latencies for the AP orientation than the LM orientation, suggesting that different neural elements were likely activated. However, without independently observing D and I waves using epidural recordings or single motor unit studies, we cannot be certain of the precise waves elicited. Indeed, MEPs elicited by any coil orientation result from the summation of multiple descending volleys and are likely to have contributions from both direct and indirect activation of corticospinal axons. MEP size can also be influenced by changes in corticospinal transmission to motoneurons at a spinal level, and by changes in motoneuronal excitability. Furthermore, although coil position was marked on a tight-fitting cap and the same coil holder was used between experiments, we cannot rule out a possible contribution of small, unintentional changes in coil position to MEP variability. Another possible reason we did not see the expected differences between MEPs elicited in the LM and AP orientations is that any inhibitory effect of PCMS at the cortex may have been overshadowed by an apparent facilitatory effect of the repetitive high-intensity TMS that formed part of the PCMS protocol, an effect that was also seen when high-intensity TMS was used on its own as a control protocol. While surprising, these results reveal a potential novel rTMS protocol for enhancing corticospinal excitability.

rTMS protocols vary greatly in the number of stimuli delivered and the frequency and intensity of stimulation. Overall, the literature points toward a facilitatory effect of high-frequency stimulation (2–20 Hz) and an inhibitory effect of low-frequency stimulation ($\leq$1 Hz) (Fitzgerald et al. 2006). Relevant to the current findings, previous studies investigating the effects of 0.1 Hz motor cortical rTMS revealed no change in cortical excitability (Chen et al. 1997; Ziemann et al. 1998). In contrast, we observed an increase in MEP size after 0.1 Hz stimulation. However, the intensities used in the current study were much higher than those used previously, and studies of various plasticity-inducing protocols show that long-term depression can switch to long-term potentiation or vice versa when the amplitude of the inputs and resulting calcium influx to the postsynaptic cell are increased or decreased, respectively (Cash et al. 2017a; Cummings et al. 1996; Doeltgen and Ridding 2011; Li et al. 2012). The intensity dependence of this rTMS protocol is further supported by the results of *experiment 2*, which showed no change in MEP size when 0.1 Hz stimulation was delivered at submaximal intensities (1.1 $\times$ rMT).

Although the effect of high-intensity rTMS was initially observed for test MEPs elicited by low-intensity TMS (1.1 $\times$ rMT), recruitment curve analyses revealed that the effect was not confined to small MEPs and low intensities. Indeed, there were similar increases in MEP size over a wide range of intensities, with the greatest absolute increase at 1.35 $\times$ rMT. *Experiment 3* also showed that the high-intensity rTMS protocol had no effect on ICF, and, while there was a small increase in the amount of SICI at Post 1 after rTMS, this change was not seen when participants with test stimuli that were too large were excluded, or when the test stimulus was adjusted to keep the test MEP amplitude similar to that before rTMS. Thus, the rTMS protocol is unlikely to exert its facilitatory effect at the same populations of neurons that have been suggested to play a role in SICI and ICF. This rules out the involvement of cortico-cortical connections responsible for later I-waves, as these are reduced by SICI (Di Lazzaro et al. 1998). Therefore, the facilitatory rTMS protocol reported here probably acts via different mechanisms to those of higher-frequency, lower-intensity facilitatory rTMS protocols such as 5 Hz rTMS, which is shown to reduce SICI and increase later I waves (Di Lazzaro et al. 2002a, 2002b). In contrast, ICF has no effect on the number and amplitude of I waves (Di Lazzaro et al. 2006); however, there are probably both cortical (Cash et al. 2017b) and spinal (Wiegel et al. 2018) contributions to ICF, involving circuits that do not contribute to I-wave generation. Neither SICI nor ICF affect I1 (Di Lazzaro et al. 1998, 2006), and thus the excitatory monosynaptic connections to corticospinal neurons thought to be responsible for I1 are a potential location for the rTMS-induced facilitation seen here. These may be connections between superficial, layer II and III pyramidal neurons and large, layer V pyramidal tract neurons (Di Lazzaro and Ziemann 2013). It is also possible that the rTMS-induced facilitation could result from modifications at other cortical and subcortical locations that do not contribute to later I-waves and are not involved in ICF.

The results of this study should not be taken as evidence against the efficacy of PCMS. Despite the complex effects of PCMS at the motor cortex, with potential inhibition caused by the arrival of afferent inputs after TMS and the facilitatory effects of high-intensity rTMS reported here, PCMS has repeatedly been shown to enhance corticospinal transmission at a spinal level (Dongés et al. 2018; Fitzpatrick et al. 2016; Taylor and Martin 2009) and to produce functionally relevant improvements in voluntary muscle activity and manual dexterity (Bunday and Perez 2012; D'Amico et al. 2018; Taylor and Martin 2009; Urbin et al. 2017). There are reasons to suggest that these improvements are not solely caused by the facilitatory effects of high-intensity rTMS. First, the rTMS protocol reported here is dependent on the use of high-intensity stimulation; however, several studies have shown PCMS-induced facilitation using lower, submaximal TMS intensities (Dongés et al. 2018; Fitzpatrick et al. 2016; Taylor and Martin 2009). Second, studies using high-intensity TMS for PCMS protocols show an increase in evoked potentials after facilitatory protocols and not protocols with inhibitory or control interstimulus timings (Bunday and Perez 2012; Urbin et al. 2017). If the high-intensity TMS component of PCMS was solely responsible for the facilitation, an increase in evoked potentials would be expected to occur similarly for facilitatory, inhibitory, and control protocols.

In conclusion, the similar increases in MEPs elicited in three different coil orientations following PCMS do not support the original hypothesis that PCMS would increase MEPs elicited in the LM and 45° PA orientations and would decrease (or increase by a smaller amount than other orientations) MEPs elicited in the AP orientation. However, the study did reveal a novel rTMS protocol for inducing corticospinal facilitation, using higher intensities but lower frequencies than previously

reported facilitatory rTMS protocols. This technique may have therapeutic potential for enhancing motor control after stroke and other motor impairments.

ACKNOWLEDGMENTS

J.M.D. has a current affiliation with the Kentucky Spinal Cord Injury Research Centre, Department of Neurological Surgery, University of Louisville, Louisville, KY and J.L.T. has a current affiliation with Edith Cowan University, Perth, Australia.

GRANTS

This research was supported by a National Health and Medical Research Council of Australia (NHMRC) Grant 1055084, "Motor Impairment."

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.M.D. and J.L.T. conceived and designed research; J.M.D. and S.C.D. performed experiments; J.M.D. and S.C.D. analyzed data; J.M.D., S.C.D., and J.L.T. interpreted results of experiments; J.M.D. and S.C.D. prepared figures; J.M.D., S.C.D., and J.L.T. drafted manuscript; J.M.D., S.C.D., and J.L.T. edited and revised manuscript; J.M.D., S.C.D., and J.L.T. approved final version of manuscript.

REFERENCES

Bunday KL, Perez MA. Motor recovery after spinal cord injury enhanced by strengthening corticospinal synaptic transmission. *Curr Biol* 22: 2355–2361, 2012. doi:10.1016/j.cub.2012.10.046.

Carson RG, Kennedy NC. Modulation of human corticospinal excitability by paired associative stimulation. *Front Hum Neurosci* 7: 823, 2013. doi:10.3389/fnhum.2013.00823.

Cash RFH, Jegatheeswaran G, Ni Z, Chen R. Modulation of the direction and amplitude of Hebbian plasticity in human motor cortex by stimulus intensity and concurrent inhibition. *Brain Stimul* 10: 83–90, 2017a. doi:10.1016/j.brs.2016.08.007.

Cash RFH, Noda Y, Zomorrodi R, Radhu N, Farzan F, Rajji TK, Fitzgerald PB, Chen R, Daskalakis ZJ, Blumberger DM. Characterization of glutamatergic and $GABA_A$-mediated neurotransmission in motor and dorsolateral prefrontal cortex using paired-pulse TMS–EEG. *Neuropsychopharmacology* 42: 502–511, 2017b. doi:10.1038/npp.2016.133.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology* 48: 1398–1403, 1997. doi:10.1212/WNL.48.5.1398.

Classen J, Wolters A, Stefan K, Wycislo M, Sandbrink F, Schmidt A, Kunesch E. Paired associative stimulation. *Suppl Clin Neurophysiol* 57: 563–569, 2004. doi:10.1016/S1567-424X(09)70395-2.

Cortes M, Thickbroom GW, Valls-Sole J, Pascual-Leone A, Edwards DJ. Spinal associative stimulation: a non-invasive stimulation paradigm to modulate spinal excitability. *Clin Neurophysiol* 122: 2254–2259, 2011. doi:10.1016/j.clinph.2011.02.038.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC. Ca^{2+} signaling requirements for long-term depression in the hippocampus. *Neuron* 16: 825–833, 1996. doi:10.1016/S0896-6273(00)80102-6.

D'Amico JM, Dongés SC, Taylor JL. Paired corticospinal-motoneuronal stimulation increases maximal voluntary activation of human adductor pollicis. *J Neurophysiol* 119: 369–376, 2018. doi:10.1152/jn.00919.2016.

Di Lazzaro V, Dileone M, Profice P, Pilato F, Oliviero A, Mazzone P, Di Iorio R, Capone F, Ranieri F, Florio L, Tonali PA. LTD-like plasticity induced by paired associative stimulation: direct evidence in humans. *Exp Brain Res* 194: 661–664, 2009. doi:10.1007/s00221-009-1774-9.

Di Lazzaro V, Oliviero A, Berardelli A, Mazzone P, Insola A, Pilato F, Saturno E, Dileone M, Tonali PA, Rothwell JC. Direct demonstration of the effects of repetitive transcranial magnetic stimulation on the excitability of the human motor cortex. *Exp Brain Res* 144: 549–553, 2002a. doi:10.1007/s00221-002-1106-9.

Di Lazzaro V, Oliviero A, Mazzone P, Pilato F, Saturno E, Dileone M, Insola A, Tonali PA, Rothwell JC. Short-term reduction of intracortical inhibition in the human motor cortex induced by repetitive transcranial magnetic stimulation. *Exp Brain Res* 147: 108–113, 2002b. doi:10.1007/s00221-002-1223-5.

Di Lazzaro V, Oliviero A, Pilato F, Mazzone P, Insola A, Ranieri F, Tonali PA. Corticospinal volleys evoked by transcranial stimulation of the brain in conscious humans. *Neurol Res* 25: 143–150, 2003. doi:10.1179/016164103101201292.

Di Lazzaro V, Pilato F, Oliviero A, Dileone M, Saturno E, Mazzone P, Insola A, Profice P, Ranieri F, Capone F, Tonali PA, Rothwell JC. Origin of facilitation of motor-evoked potentials after paired magnetic stimulation: direct recording of epidural activity in conscious humans. *J Neurophysiol* 96: 1765–1771, 2006. doi:10.1152/jn.00360.2006.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Exp Brain Res* 119: 265–268, 1998. doi:10.1007/s002210050341.

Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. *Front Neural Circuits* 7: 18, 2013. doi:10.3389/fncir.2013.00018.

Doeltgen SH, Ridding MC. Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. *Clin Neurophysiol* 122: 1411–1416, 2011. doi:10.1016/j.clinph.2010.12.034.

Dongés SC, D'Amico JM, Butler JE, Taylor JL. Involvement of N-methyl-d-aspartate receptors in plasticity induced by paired corticospinal-motoneuronal stimulation in humans. *J Neurophysiol* 119: 652–661, 2018. doi:10.1152/jn.00457.2017.

Feldman DE. The spike-timing dependence of plasticity. *Neuron* 75: 556–571, 2012. doi:10.1016/j.neuron.2012.08.001.

Fitzgerald PB, Fountain S, Daskalakis ZJ. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin Neurophysiol* 117: 2584–2596, 2006. doi:10.1016/j.clinph.2006.06.712.

Fitzpatrick SC, Luu BL, Butler JE, Taylor JL. More conditioning stimuli enhance synaptic plasticity in the human spinal cord. *Clin Neurophysiol* 127: 724–731, 2016. doi:10.1016/j.clinph.2015.03.013.

Janssen AM, Oostendorp TF, Stegeman DF. The coil orientation dependency of the electric field induced by TMS for M1 and other brain areas. *J Neuroeng Rehabil* 12: 47, 2015. doi:10.1186/s12984-015-0036-2.

Knikou M. Spinal excitability changes after transspinal and transcortical paired associative stimulation in humans. *Neural Plast* 2017: 6751810, 2017. doi:10.1155/2017/6751810.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD. Corticocortical inhibition in human motor cortex. *J Physiol* 471: 501–519, 1993. doi:10.1113/jphysiol.1993.sp019912.

Leukel C, Taube W, Beck S, Schubert M. Pathway-specific plasticity in the human spinal cord. *Eur J Neurosci* 35: 1622–1629, 2012. doi:10.1111/j.1460-9568.2012.08067.x.

Li L, Stefan MI, Le Novère N. Calcium input frequency, duration and amplitude differentially modulate the relative activation of calcineurin and CaMKII. *PLoS One* 7: e43810, 2012. doi:10.1371/journal.pone.0043810.

Macerollo A, Brown MJN, Kilner JM, Chen R. Neurophysiological changes measured using somatosensory evoked potentials. *Trends Neurosci* 41: 294–310, 2018. doi:10.1016/j.tins.2018.02.007.

Rusu CV, Murakami M, Ziemann U, Triesch J. A model of TMS-induced I-waves in motor cortex. *Brain Stimul* 7: 401–414, 2014. doi:10.1016/j.brs.2014.02.009.

Shulga A, Zubareva A, Lioumis P, Mäkelä JP. Paired associative stimulation with high-frequency peripheral component leads to enhancement of corticospinal transmission at wide range of interstimulus intervals. *Front Hum Neurosci* 10: 470, 2016. doi:10.3389/fnhum.2016.00470.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 123: 572–584, 2000. doi:10.1093/brain/123.3.572.

Taylor JL, Martin PG. Voluntary motor output is altered by spike-timing-dependent changes in the human corticospinal pathway. *J Neurosci* 29: 11708–11716, 2009. doi:10.1523/JNEUROSCI.2217-09.2009.

Triesch J, Zrenner C, Ziemann U. Modeling TMS-induced I-waves in human motor cortex. *Prog Brain Res* 222: 105–124, 2015. doi:10.1016/bs.pbr.2015.07.001.

Urbin MA, Ozdemir RA, Tazoe T, Perez MA. Spike-timing-dependent plasticity in lower-limb motoneurons after human spinal cord injury. *J Neurophysiol* 118: 2171–2180, 2017. doi:10.1152/jn.00111.2017.

Wiegel P, Niemann N, Rothwell JC, Leukel C. Evidence for a subcortical contribution to intracortical facilitation. *Eur J Neurosci* 47: 1311–1319, 2018. doi:10.1111/ejn.13934.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R, Classen J. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol* 89: 2339–2345, 2003. doi:10.1152/jn.00900.2002.

Ziemann U, Corwell B, Cohen LG. Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *J Neurosci* 18: 1115–1123, 1998. doi:10.1523/JNEUROSCI.18-03-01115.1998.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol* 496: 873–881, 1996. doi:10.1113/jphysiol.1996.sp021734.