Preconditioning rTMS of premotor cortex can reduce but not enhance short-term facilitation of primary motor cortex

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Running Head: Premotor rTMS and MEP Facilitation

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ABSTRACT

Introduction: Short trains of suprathreshold 5 Hz repetitive transcranial magnetic stimulation (rTMS) over primary motor cortex (M1) evoke motor potentials (MEPs) in hand muscles that progressively increase in amplitude via a mechanism that is thought to be similar to short term potentiation described in animal preparations. Long trains of subthreshold rTMS over dorsal premotor cortex (PMd) are known to affect the amplitude of single pulse MEPs evoked from M1. We tested whether PMd-rTMS affects short term facilitation in M1. We explored also the effect of PMd-rTMS on M1 responses evoked by single pulses TMS of different polarities.

Materials and Methods: We tested in 15 healthy subjects short term facilitation in left M1 (10 suprathreshold TMS pulses at 5 Hz) after applying rTMS to left PMd (1500 subthreshold pulses at 1 Hz and 5 Hz). In a sample of subjects we delivered single pulse TMS with different polarities, and paired-pulse TMS at short interval (SICI) after PMd-rTMS.

Results: Short term facilitation in M1 was reduced after applying 1 Hz to PMd, but was unaffected after 5 Hz PMd-rTMS. PMd 1Hz rTMS reduced the amplitude of MEPs evoked by monophasic PA or biphasic AP-PA but had little effect on MEPs by monophasic AP or biphasic PA-AP single pulse TMS. PMd-rTMS left SICI unchanged.

Discussion: 1 Hz PMd rTMS reduces short term facilitation in M1 induced by short 5 Hz trains. This effect is likely to be caused by reduced facilitation of I-wave inputs to corticospinal neurons.
INTRODUCTION

The dorsal premotor cortex (PMd) is thought to provide important information to primary motor cortex (M1) that enables the latter to select appropriate movements from a set of prepared possible responses (Cisek and Kalaska 2005). It has been suggested that input from the PMd cortex informs M1 which muscles to activate in a task. It must also suppress activity in other muscles to prevent inappropriate release of the other prepared responses.

Recent advances in TMS have allowed a number of investigators to develop ways of examining the connection between PMd and M1 in healthy subjects and to probe its activity during different types of movement. One approach has been with paired pulse designs in which a conditioning stimulus to PMd changes the amplitude of MEPs evoked from M1 (Civardi et al. 2001; Mochizuki et al. 2004, Baumer et al. 2006; Koch et al., 2007). Koch et al. (2006) recently used this method to show that in the reaction period of a 2-choice reaction time task, facilitation from PMd to M1 was enhanced onto corticospinal neurons involved in the forthcoming movement, whereas inhibitory connections were enhanced to corticospinal neurons involved in the non-selected movement.

A second approach has been to apply long trains of repetitive TMS (rTMS) over PMd in order to produce lasting changes in the influence of PMd on M1. Thus 1 Hz rTMS over PMd decreases the muscle twitches evoked by single pulse TMS to primary motor cortex (M1) for approximately 20 min, whereas they are increased after 5 Hz PMd rTMS (Gerschlager et al. 2001; Munchau et al. 2002; Baumer et al. 2003; Rizzo et al. 2004). PMd-rTMS is thought to transmit its effects through specific cortico-cortical connections acting on M1 (Gerschlager et al. 2001; Munchau et al. 2002; Baumer et al. 2003; Rizzo et al. 2004). Recent behavioural studies have also shown that rTMS over PMd may disrupt performance in complex motor tasks (Chouinard et al. 2005; Mochizuki et al. 2005; O’Shea et al. 2007).

In many of these experiments, the outcome effect on M1 has been measured in terms of the effect on the MEP in target muscles. However, given the possibility that PMd input
must prevent inappropriate release of unintended movement, it is also important to study possible effect on mechanisms of muscle recruitment in M1. One way of doing this is to use short trains of suprathreshold TMS pulses. When suprathreshold 5 Hz rTMS is delivered over M1 the MEP elicited by each single stimulus progressively increases in size during the train of stimulation (Pascual-Leone et al. 1994; Jennnum et al. 1995; Berardelli et al. 1998; Di Lazzaro et al. 2002). This excitatory phenomenon (MEP facilitation) resembles mechanisms of short-term synaptic facilitation mediated by NMDA receptors (Inghilleri et al. 2004, 2005; Gilio et al. 2007). In addition, as the train progresses, each TMS pulse gradually begins to recruit additional muscles into the response thereby “defocusing” the effect of stimulation (Lorenzano et al. 2002). Given the role of PMd-to-M1 input in “focusing” M1 output and the possible connection between MEP facilitation and “defocusing” of M1 output, we examined whether changes in PMd-to-M1 input might affect MEP facilitation in 5 Hz rTMS protocol.

Since short term facilitation was tested with biphasic 5 Hz rTMS whereas previous studies had examined M1 excitability with monophasic pulses (Gerschlager et al. 2001; Rizzo et al. 2004), we explored the effect of PMd-rTMS on responses evoked by single pulses TMS of different polarities.

Finally, we also examined short interval intracortical inhibition (SICI), a paired pulse paradigm of stimulation at short interstimulus interval (1-4 ms) (Kujirai et al. 1993) that is also thought to play a role in M1 motor output “focusing” (Ridding et al. 1995; Liepert et al. 1998; Stinear and Byblow 2003).

**METHODS**

**Subjects**
The study group comprised 18 right-handed normal subjects (eight male and ten female; mean age± SD: 27±3 years, range 24-35). None of the subjects were taking drugs acting on the central nervous system. All subjects gave their informed consent and the study was approved by the local ethical committee.
Stimulation techniques

In all experimental sessions a conditioning-test paradigm was used. The conditioning and test stimulation were both delivered over left hemisphere.

Test-rTMS

Test-rTMS was delivered over the left M1 through a high-frequency magnetic stimulator (Magstim Super Rapid –The Magstim Company Ltd, Whitland, South West Wales, UK) connected to a figure-of-eight coil with mean loop diameter of 9 cm. The magnetic stimulus had a biphasic waveform with a pulse width of ~ 300 µs. During the first phase of the stimulus, the current in the centre of the coil flowed toward the handle. The coil was held tangentially to the scalp with the handle pointing back and away from the midline at 45° inducing postero-anterior followed by antero-posterior (PA-AP) current in the brain. The coil was placed over the optimum scalp position (hot spot) to elicit motor responses (MEPs) in the contralateral first dorsal interosseus (FDI) muscle. Motor threshold was calculated at rest (RMT) and considered as the lowest intensity able to evoke a MEP of more than 50 µV in at least 5 out of 10 consecutive trials in the FDI muscle. Active motor threshold (AMT) was calculated as the lowest intensity able to evoke a MEP of 200 µV during slight contraction of FDI muscle. Test-rTMS consisted of a train of 10 stimuli at 5 Hz (2 s of stimulation). To avoid cumulative after-effects (Gilio et al. 2007) 15 trains were delivered in a time period of 30 min (intertrain interval of 1-2 min). The stimulation intensity was set at 120% RMT. 5 Hz-rTMS over M1 was delivered before and after conditioning-rTMS ended.

Conditioning-rTMS

Conditioning-rTMS was delivered over the left PMd through a high-frequency magnetic stimulator (Magstim Super Rapid –The Magstim Company Ltd, Whitland, South West Wales, UK) connected to a figure-of-eight coil with mean loop diameter of 9 cm. The left PMd was considered as being located at a site 2.5 cm anterior to the M1 hot spot (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). The coil was held tangentially to the scalp with the handle pointing antero-medially from the midline at 45° inducing antero-posterior followed by postero-anterior (AP-PA) current in the brain (Kammer et al. 2001; Gerschlager et al. 2001; Rizzo et al. 2004).
Conditioning PMd-rTMS was delivered in 15 subjects at 1 Hz and 5 Hz in two different sessions randomly assigned with an interval between each session of at least 5 days. 5 Hz PMd-rTMS consisted of a total of 1500 stimuli delivered in five blocks each of 300 pulses separated by intertrain intervals of 1 min (10 min in total) (Rizzo et al. 2004). 1 Hz PMd-rTMS consisted of 1500 stimuli delivered in two blocks each of 750 pulses separated by intertrain intervals of 1 min (25 min in total) (Rizzo et al. 2004).

Conditioning PMd-rTMS with 1 Hz and 5 Hz were delivered in all sessions at 90% AMT (Rizzo et al. 2004).

The conditioning sham 1Hz rTMS stimulation was applied in 5 subjects over the left PMd with the coil held at 90° in an antero-medial position at 90% AMT.

To exclude spreading of non-synaptic current from PMd-to-M1 (Gerschlager et al. 2001; Munchau et al. 2002) we studied in 5 subjects the conditioning 1Hz M1\textsubscript{low intensity}-rTMS by positioning the coil over the left M1 inducing AP-PA current in the brain (Kammer et al. 2001; Gerschlager et al. 2001). Conditioning 1 Hz M1\textsubscript{low intensity}-rTMS was delivered at 60% AMT (Gerschlager et al. 2001; Munchau et al. 2002).

In order to evaluate the time course of the after effects of conditioning 1 Hz PMd-rTMS on the MEP facilitation we delivered in 5 subjects 15 trains of M1-rTMS before and after the end of conditioning 1 Hz PMd-rTMS at post 1 (0-30 minutes) and at post 2 (30-60 minutes after the end of conditioning PMd-rTMS).

Since M1 excitability was evaluated by biphasic pulses in this study, rather than the more usual monophasic pulses (Gerschlager et al. 2001; Rizzo et al. 2004), we evaluated in 5 subjects MEPs evoked by 25 single pulse TMS with interval between pulses of 4 sec. delivered over left M1 through two different TMS stimulators (monophasic and biphasic) and two different coil orientation inducing monophasic PA, monophasic AP, biphasic PA-AP and biphasic AP-PA current in the brain. Monophasic TMS was delivered through a high-power magnetic stimulator (Magstim 200–The Magstim Company Ltd, Whitland, South West Wales, UK) connected to a figure-of-eight coil with mean loop diameters of 9 cm. The magnetic stimulus delivered by Magstim 200 had a nearly monophasic pulse configuration with a rise time of ~ 100 µs, decaying back to zero over ~ 0.8 ms; the coil current during the rising phase of the
magnetic field flowed toward the handle. The intensity of single pulse TMS was set at the value able to evoke MEPs of 1mV amplitude before conditioning 1Hz PM-rTMS.

Finally, in 5 subjects we tested SICI (Kujirai et al. 1993) since it has been suggested that this circuit may be one physiological mechanism that contributes to the “focusing” of motor commands in M1 (Ridding et al. 1995; Liepert et al. 1998; Stinear and Byblow 2003). An interstimulus interval (ISI) of 3 ms was used between a conditioning and a test pulse delivered through two monophasic Magstim 200 stimulators connected by a Y-cable to a 9 cm diameter figure-of-eight coil. The coil was placed over the left M1 with the handle pointing back and away from the midline at 45° inducing monophasic PA current in the brain. The intensity of conditioning TMS pulse in SICI protocol was fixed to 80% of AMT as tested before conditioning-rTMS (Munchau et al. 2002; Rizzo et al. 2004). Forty-five pairs of pulses were applied. Before and after conditioning PM-rTMS the intensity of the test pulse was fixed to evoke MEPs of 1mV amplitude (Munchau et al. 2002; Rizzo et al. 2004).

**Recording Techniques and Measurements**
The electromyographic (EMG) activity was recorded through a pair of surface electrodes (Ag/AgCl) placed over the right FDI muscle, using a belly-tendon montage. EMG signals were recorded, amplified and filtered with a Digitimer D 360 (Digitimer Ltd, UK) (bandwidth 5 Hz-1 kHz), acquired at a sampling rate of 5 KHz through a 1401 plus AD laboratory interface (Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer for off-line analysis (Signal software; Cambridge Electronic Devices, Cambridge, UK). Experiments were performed with subjects fully relaxed and with their eyes opened. The level of baseline EMG activity before, during and after conditioning PMd-rTMS was controlled by visual-feedback through oscilloscope screen (Tektronic 5103N oscilloscope) and auditory-feedback through loudspeaker. Trials with background EMG activity were rejected. The amplitude of MEPs evoked by each of 10 stimuli of M1-rTMS were measured peak to peak (mV) and then averaged.
Statistical analysis

Effect of Conditioning PMd-rTMS
The effect of conditioning PMd-rTMS (1 Hz / 5 Hz) on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS was analyzed with a three-way repeated-measures ANOVA with “Frequency” (1 Hz / 5 Hz conditioning PMd-rTMS), “Time” (before / after conditioning PMd-rTMS) and “Number of Stimuli” (1 / 2, 3, 4, 5, 6, 7, 8, 9 and 10) as main factors. The RMT values and the amplitude of the first MEP evoked by M1-rTMS were both tested with a two-way ANOVA with “Frequency” (1 Hz / 5 Hz) and “Time” (before / after) as main factors.

Effect of Conditioning 1 Hz sham PMd-rTMS
The effect of conditioning 1 Hz sham PMd-rTMS on the amplitude of MEPs evoked by each stimulus of the M1-rTMS was tested with a three-way ANOVA with “Conditioning” (1 Hz PMd / 1 Hz sham PMd-rTMS), “Time” (before / after conditioning PMd-rTMS) and “Number of Stimuli” (1 / 2, 3, 4, 5, 6, 7, 8, 9 and 10) as main factors. The RMT values and the amplitude of the first MEP evoked by M1-rTMS were both tested with a two-way ANOVA with “Conditioning” (1 Hz PMd / 1 Hz sham PMd-rTMS) and “Time” (before / after conditioning PMd-rTMS) as main factors.

Effect of Conditioning 1 Hz M1\textsuperscript{low intensity-rTMS}
The effect of conditioning 1 Hz M1\textsuperscript{low intensity-rTMS} on the amplitude of MEPs evoked by each stimulus of the M1-rTMS was tested with a three-way ANOVA with “Conditioning” (1 Hz PMd / 1 Hz M1\textsubscript{low intensity-rTMS}), “Time” (before / after conditioning rTMS) and “Number of Stimuli” (1 / 2, 3, 4, 5, 6, 7, 8, 9 and 10) as main factors. The RMT values and the amplitude of the first MEP evoked by M1-rTMS were both tested with a two-way ANOVA with “Conditioning” (1 Hz PMd / 1 Hz M1\textsubscript{low intensity-rTMS}) and “Time” (before / after conditioning rTMS) as main factors.

Time course of the after-effects of conditioning 1 Hz PMd-rTMS
The time course of the after-effects of conditioning 1 Hz PMd-rTMS was analysed
testing the amplitude of MEPs evoked by each stimulus of the M1-rTMS at different time after conditioning stimulation ended by using a two-way ANOVA with “Time” (before / after conditioning 1 Hz PMd-rTMS at post1 and post2) and “Number of Stimuli” (1 / 2, 3, 4, 5, 6, 7, 8, 9 and 10) as main factors. The RMT values and the amplitude of the first MEP evoked by M1-rTMS trains were both tested with one-way ANOVA with “Time” (before / after conditioning 1 Hz PMd-rTMS at post1 and post2) as main factor.

Effect of Conditioning 1 Hz PMd-rTMS on MEPs amplitude evoked by single pulse TMS
The effect of conditioning 1 Hz PMd-rTMS on the amplitude of MEPs evoked by single pulse TMS delivered by monophasic and biphasic magnetic stimulator with two different coil orientations, was tested with a three-way ANOVA with “Stimulator” (monophasic / biphasic), “Coil orientation” (postero-lateral / antero-medial) and “Time” (before / after conditioning 1Hz PMd-rTMS) as main factors of analysis.

Effect of Conditioning 1 Hz PMd-rTMS on MEPs amplitude evoked by paired pulse TMS delivered at 3 ms ISI (SICI)
The effect of conditioning 1 Hz PMd-rTMS on the amplitude of MEPs evoked by paired pulse TMS delivered at 3ms ISI (SICI) was tested with a two-way ANOVA with “Time” (before / after conditioning 1 Hz PMd-rTMS) and “ISI” (Test / SICI) as main factors of analysis.

Post hoc analysis was tested with the Tukey Honest Significant Difference test. The Greenhouse-Geisser correction was used when necessary to correct for non-sphericity. P value < 0.05 was considered significant for all statistical analysis.

RESULTS

None of the subjects experienced any adverse effects.
The detailed statistical results are provided below; however, the data can be summarized by saying that long trains of 1 Hz rTMS over PMd reduced the MEP facilitation evoked
by short trains of 5 Hz-rTMS over primary motor cortex (M1) for up to 30 minutes after conditioning (post 1 v. post 2). In contrast, long trains of 5 Hz rTMS over PMd had no effect on MEP facilitation. A puzzling result was that 1 Hz PMd-rTMS had no effect on the amplitude of the first MEP evoked by M1-rTMS, even though this had been expected from previous work (Gerschlager et al. 2001). Control experiments showed that the difference was due to the fact that the present study used a biphasic TMS pulse to test M1 excitability whereas Gerschlager et al. (2001) had used a monophasic pulse.

Effect of conditioning PMd-rTMS

Three-way ANOVA showed a significant effect of factors “Time” ($F_{(1.14)}=15.85; P < 0.01$) and “Number of Stimuli” ($F_{(9.126)}=6.03; P < 0.01$), with a significant two way interaction of “Time” by “Number of Stimuli” ($F_{(9.126)}=4.82; P < 0.01$), and a significant three way interaction of “Time”, “Number of Stimuli” and “Frequency” ($F_{(9.126)}=2.43; P = 0.01$). Post hoc analysis showed that conditioning 5 Hz PMd-rTMS left the MEP facilitation evoked by M1-rTMS unchanged (FIG. 1). On the other hand conditioning 1 Hz PMd-rTMS significantly reduced the degree of MEP facilitation as shown by significant two way interaction of “Time” by “Number of Stimuli” ($F_{(9.126)}=6.47; P < 0.01$) (FIG. 2).

Two-way ANOVA showed that both 1 Hz and 5 Hz PMd-rTMS left RMT and the amplitude of the first MEP evoked by M1-rTMS unchanged (FIG. 3-4).

Effect of conditioning 1 Hz sham PMd-rTMS

Three-way ANOVA showed a significant effect of factors “Time” ($F_{(1.4)}=8.19; P = 0.046$), and “Number of Stimuli” ($F_{(9.36)}=2.59; P = 0.02$), with a significant two way interaction of “Time” by “Number of Stimuli” ($F_{(9.36)}=2.19; P = 0.046$), and a significant three way interaction of “Conditioning”, “Time” and “Number of Stimuli” ($F_{(9.36)}=2.16; P = 0.049$). Post hoc analysis showed that 1 Hz sham PMd-rTMS left the MEP facilitation evoked by M1-rTMS unchanged. Two-way ANOVA showed that RMT and the amplitude of the first MEP evoked by M1-rTMS were both similar before and after 1 Hz sham PMd-rTMS (FIG. 3-4).
**Effect of conditioning 1 Hz M1 low intensity-rTMS**

Three-way ANOVA showed a significant effect of factors “Conditioning” ($F_{(1.4)}=10.83; P < 0.05$), “Time” ($F_{(1.4)}=13.94; P = 0.02$), and “Number of Stimuli” ($F_{(9.36)}=10.19; P < 0.01$) and a significant two way interaction of “Conditioning” by “Number of Stimuli” ($F_{(9.36)}=4.21; P < 0.01$). Post hoc analysis showed that 1 Hz M1$_{low intensity}$-rTMS left the MEP facilitation evoked by M1-rTMS unchanged. Two-way ANOVA showed that RMT and the amplitude of the first MEP evoked by M1-rTMS were both similar before and after 1 Hz M1$_{low intensity}$-rTMS (FIG. 3-4).

**Time course of the after-effects of conditioning 1 Hz PMd-rTMS**

Two-way ANOVA showed a significant effect of factors “Time” ($F_{(2.8)}=9.77; P < 0.01$) and “Number of Stimuli” ($F_{(9.36)}=3.44; P < 0.01$), with a significant two way interaction of “Time” by “Number of Stimuli” ($F_{(18.72)}=2.08; P = 0.015$). Post hoc analysis showed that 1 Hz PMd-rTMS reduced the degree of MEP facilitation evoked by M1-rTMS in post 1 as shown by a significant two way interaction of “Time” by “Number of Stimuli” ($F_{(9.135)}=5.52; P < 0.01$). Conversely 1 Hz PMd-rTMS did not reduce the degree of MEP facilitation evoked by M1-rTMS in post 2 (FIG. 5).

One-way ANOVA showed that 1 Hz PMd-rTMS left RMT and the first MEP amplitude evoked by M1-rTMS both unchanged in post 1 and post 2 (FIG. 3-4).

**Effect of Conditioning 1 Hz PMd-rTMS on MEPs amplitude evoked by single pulse TMS**

Three-way ANOVA showed a significant effect of factor “Time” ($F_{(1.4)}=19.51; P = 0.012$). Post hoc analysis showed that 1 Hz PMd-rTMS reduced MEPs evoked by monophasic PA ($P < 0.01$) and biphasic AP-PA ($P = 0.018$) but not by monophasic AP and biphasic PA-AP TMS (FIG. 6).

**Effect of Conditioning 1 Hz PMd-rTMS on MEPs amplitude evoked by paired pulse TMS delivered at 3 ms ISI (SICI)**

Two-way ANOVA showed significant effect of factor “ISI” ($F_{(1.4)}=26.29; P = 0.007$) confirming that paired pulse TMS at 3 ms ISI reduced the amplitude of test MEPs (SICI). There was no other main effect or interaction term suggesting that 1 Hz PMd-rTMS has no effect on the amount of SICI (FIG. 7).
DISCUSSION

The present study in normal subjects shows that long trains of 1 Hz rTMS over dorsal premotor cortex (PMd) reduce the MEP facilitation evoked by short trains of 5 Hz-rTMS over primary motor cortex (M1). This inhibitory change was seen at up to 30 minutes after the end of 1 Hz PMd-rTMS conditioning but not later. Conversely 5 Hz, 1 Hz sham PMd-rTMS and 1 Hz M1_{low intensity}-rTMS left short term facilitation in M1 unchanged. In apparent contrast to previous reports, 1 Hz PMd rTMS had no effect on the amplitude of the first MEP of the short term train. The second set of experiments showed that this was due to the fact that although 1 Hz PMd-rTMS reduces the amplitude of MEPs evoked by monophasic PA and biphasic AP-PA single pulse TMS over M1 it has no effect on MEPs evoked by monophasic AP and biphasic PA-AP TMS. The latter was used in the first set of experiments to examine short term facilitation in M1. Finally, 1 Hz PMd-rTMS left SICI unchanged.

Several mechanisms might explain why 1 Hz PMd-rTMS reduces the MEP facilitation from M1. Possible factors include differences in the background EMG activity because subjects are not at complete rest (Berardelli et al. 1999), sleep induction (Bertini et al. 2004; De Gennaro et al. 2004; Salih et al. 2005), attentional level (Rossini et al. 1999; Stefan et al. 2004), and volitional inhibition (Sohn et al. 2002). However, we think none of these played an important role in the present experiments. Thus, all subjects were asked to sit comfortably and to relax with the eyes opened. No background EMG activity during the experimental session was noted. In addition, M1 cortical excitability tested with RMT and the first MEP evoked by M1-rTMS did not change after conditioning-rTMS; moreover 1 Hz sham PMd-rTMS had no after effect on MEP facilitation. Spreading of non-synaptic induced current from PMd-to-M1 (Rizzo et al. 2004) also cannot explain our results since 1 Hz M1_{low intensity}-rTMS (Gerschlager et al. 2001; Munchau et al. 2002) had no after effect on the MEP facilitation. Other factors such as activation of premotor cortico-spinal direct projections (Dum and Strick 2002; Chouinard and Paus 2006) are unlikely to contribute to the after-effects of PMd-rTMS as discussed previously by Gerschlager et al. (2001), Munchau et al. (2002) and Rizzo et al. (2004).
Primate experiments have demonstrated the existence of strong facilitatory and inhibitory connections between PMd and M1 (Tokuno and Nambu 2000; Picard and Strick 2001; Dum and Strick 2002, 2005), and previous authors (Gerschlager et al. 2001; Munchau et al. 2002; Baumer et al. 2003; Rizzo et al. 2004) have therefore suggested that direct PMd-to-M1 cortico-cortical connections in humans are involved in the after-effects of PMd-rTMS on M1 excitability as tested with single pulse TMS. We think that similar mechanisms may be involved in the present results. That is, PMd-rTMS may activate PMd-to-M1 output directly and produce after effects on specific M1 neural circuits involved in short term facilitation of M1 MEPs to 5 Hz rTMS (Munchau et al. 2002; Rizzo et al. 2004). Alternatively, even if PMd-rTMS does not activate the projections directly it could nevertheless change tonic levels of PMd-to-M1 output to the same circuits and have similar effects (Bestmann et al. 2005).

As noted in the Introduction, the gradual increase in MEP amplitude during 5 Hz rTMS may involve mechanisms analogous to NMDA dependent short term facilitation of synaptic connections described in animal experiments (Inghilleri et al. 2004, 2005; Cooke and Bliss 2006; Gilio et al. 2007). 5 Hz-rTMS over M1 is also accompanied by a spread of excitation that gradually recruits MEPs in non target muscles (Pascual-Leone et al. 1994; Lorenzano et al. 2002). Both of these features, MEP facilitation and spread of excitation, are due to recruitment through cortico-cortical connections of higher threshold cortical columns in the target and non target muscle (Lorenzano et al. 2002) probably via lateral spread of excitation through reciprocal connections of layer II/III pyramidal neurons (Feldmeyer et al. 2002, 2006; Brecht et al. 2003; Wirth et al. 2004). Experimental studies in animals show that excitatory cortico-cortical connections from PMd project predominantly to layer II/III inhibitory interneurons of M1 (Ghosh and Porter 1988; Tokuno and Nambu 2000). Effectively, PMd-to-M1 output might “sculpt” the response of M1 to other inputs that affect the way M1 cortical columns modulate ongoing motor processing (Chouinard and Paus 2006). The effect we observed in the present experiments after 1 Hz PMd-rTMS may then be caused by modulation of activity in this circuitry.
We found that 1 Hz PMd-rTMS reduced the amplitude of MEPs evoked by monophasic PA and biphasic AP-PA but had no effect on MEPs evoked by monophasic AP and biphasic PA-AP single pulse TMS. Biphasic TMS pulses activate the brain preferentially on the reversing phase of current (Maccabee et al. 1998; Kammer et al. 2001; Di Lazzaro et al. 2001, 2004); this explains why monophasic PA stimulation responds in the same way as biphasic AP-PA, and why monophasic AP is the same as biphasic PA-AP. Direct recordings from the cervical spinal epidural space of descending cortico-spinal activity evoked by TMS have shown that monophasic PA and biphasic PA-AP both recruit preferentially I₁-waves; on the other hand monophasic AP and biphasic PA-AP both recruit preferentially I₃-waves together with a “proximal D-wave” (Di Lazzaro et al. 2001). Thus we suggest that 1 Hz PMd-rTMS might reduce M₁ cortical excitability by modulating predominantly the I-wave volleys, which could produce a larger decrease in MEPs evoked by monophasic PA and biphasic AP-PA in comparison to the activation produced by monophasic AP and biphasic PA-AP stimuli. This would be because monophasic AP and biphasic PA-AP contain excitatory input from “proximal D-wave” which would be uninfluenced by changes in I-wave recruitment.

We found unchanged SICI after 1 Hz PMd-rTMS as previous reports (Munchau et al. 2002; Rizzo et al. 2004). Direct epidural recordings of descending cortico-spinal activity have shown that SICI reduces MEP amplitude mainly by acting on I₃-wave inputs (Di Lazzaro et al. 1998). Although SICI play a role in “focusing” M₁ motor output (Ridding et al. 1995; Liepert et al. 1998; Stinear and Byblow 2003) our results suggest that it unlikely to be involved in PMd-to-M₁ after-effects (Munchau et al. 2002; Rizzo et al. 2004). Given that 1Hz PM-rTMS at a specific intensity of 80% AMT leads to facilitation of test MEPs at interstimulus intervals of 6 and 7ms (ICF) and at higher intensities decreases cortical silent period (CSP) duration without affecting SICI, we conclude that specific target circuits in M₁ can be modulated by PM-rTMS (Munchau et al. 2002; Rizzo et al. 2004).

In our study 5 Hz PMd-rTMS failed to modify the degree of MEP facilitation evoked by 5 Hz rTMS over M₁. One possibility is a “ceiling effect” that is the M₁ intracortical
interneurons driving the MEP facilitation could not be further activated by this specific PMd-rTMS. However, as noted below, if the role of PMd input is to select appropriate combinations of muscle activity in certain tasks, then it may well be that PMd input can only reduce short term facilitation and not enhance it.

Given the role for PMd-to-M1 pathway in generation and selection of movements (Tokuno and Nambu 2000; Dum and Strick 2002, 2005; Chouinard and Paus 2006) and the observation that PMd rTMS may interfere with both selection of intended or suppression of unintended movement (Dum and Strick 2002, 2005; Chouinard et al. 2005; Mochizuki et al. 2005; Chouinard and Paus 2006; Koch et al. 2007; O’Shea et al. 2007) we suggest as behavioural counterpart for our findings that reducing short term facilitation and spread of activation between muscle groups may be one mechanism by which M1 activity is “focused” appropriately for a selected task.

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FIGURES AND LEGEND

**Fig 1.** MEP Facilitation evoked by 5Hz M1-rTMS before and after conditioning 5Hz PMd-rTMS. Each point corresponds to the mean amplitude of MEPs. Vertical bars denote SE.

**Fig 2.** MEP Facilitation evoked by 5Hz M1-rTMS before and after conditioning 1Hz PMd-rTMS. Each point corresponds to the mean amplitude of MEPs. Vertical bars denote SE. Significant reduction of MEPs is marked by an asterisk.

**Fig 3.** RMT before and after conditioning PMd-rTMS. Histograms are mean values and error bars denote SD.

**Fig 4.** First MEP evoked by 5 Hz M1-rTMS before and after conditioning PMd-rTMS. Histograms are mean values and error bars denote SD.

**Fig 5.** MEP Facilitation evoked by 5Hz M1-rTMS before and after conditioning 1Hz PMd-rTMS at Post 1 and Post 2 (time course). Each point corresponds to the mean amplitude of MEPs. Vertical bars denote SE.

**Fig 6.** MEPs evoked by single pulse TMS before and after conditioning 1Hz PMd-rTMS. PA = monophasic postero-anterior; AP = monophasic antero-posterior; PA-AP = biphasic postero-anterior and antero-posterior; AP-PA = biphasic antero-posterior and postero-anterior. Histograms are mean values and error bars denote SD. Significant reduction of MEPs is marked by an asterisk.

**Fig 7.** MEPs evoked by single pulse (Test) and paired pulse TMS at 3 ms ISI (SICI) before and after conditioning 1Hz PMd-rTMS. Histograms are mean values and error bars denote SD. Significant reduction of MEPs is marked by an asterisk.
MEP Facilitation before and after 5Hz PMd-rTMS

- **Before**
- **After**

Number of Stimuli

MEP Amplitude (mV)
MEP Facilitation before and after 1Hz PMd-rTMS

Before

After
First MEP evoked by 5Hz-rTMS over M1

**Condition**
- Before 5 Hz PMd cond.
- After 5 Hz PMd cond.
- Before 1 Hz PMd cond.
- After 1 Hz PMd cond.
- Before 1 Hz Sham cond.
- After 1 Hz Sham cond.
- Before 1 Hz M1 Low int.
- After 1 Hz M1 Low int.

**MEP Amplitude (mV)**
- 0.0
- 0.2
- 0.4
- 0.6
- 0.8
MEP Facilitation-Time Course

Number of Stimuli: 1 to 10

- Before
- Post 1
- Post 2

MEP Amplitude (mV)

0.0 0.2 0.4 0.6 0.8
MEP amplitude before and after 1 Hz PMd-rTMS

Before

After

Condition

PA
AP
PA-AP
AP-PA

MEP amplitude (mV)

0
0,5
1
1,5
2