Functional connectivity between secondary and primary motor areas
underlying hand-foot coordination

Winston D Byblow¹, James P Coxon¹, Cathy M Stinear¹, Melanie K Fleming¹, Garry Williams¹, J Florian M Müller² and Ulf Ziemann²

¹ Movement Neuroscience Laboratory, University of Auckland, New Zealand
² Department of Neurology, Johann Wolfgang Goethe-University Frankfurt, Germany

**Keywords**: primary motor cortex; dorsal premotor cortex; supplementary motor area; human; transcranial magnetic stimulation; interlimb coordination

**Abbreviations**: M1 primary motor cortex; SMA supplementary motor area; PMd dorsal premotor cortex; PMv ventral premotor cortex; TMS transcranial magnetic stimulation; MEP motor evoked potential; SICI short-latency intracortical inhibition; SEV stimulus evoked velocity

**Acknowledgements**: The authors thank Shane Warbrooke and Dr Richard Carson for technical assistance.

**Correspondence**:

Winston D. Byblow
Movement Neuroscience Laboratory
University of Auckland
Auckland, New Zealand
Fax: 64 9 373 7043
w.byblow@auckland.ac.nz
Abstract

Coincident hand and foot movements are more reliably performed in the same direction than in opposite directions. Using transcranial magnetic stimulation (TMS) to assess motor cortex function, we examined the physiological basis of these movements across three novel experiments. Experiment 1 demonstrated that upper limb corticomotor excitability changed in a way that facilitated isodirectional movements of the hand and foot, during phasic and isometric muscle activation conditions. Experiment 2 demonstrated that motor cortex inhibition was modified with active, but not passive, foot movement in a manner that facilitated hand movement in the direction of foot movement. Together, these findings demonstrate that the coupling between motor representations within motor cortex is activity-dependent. Since there are no known connections between hand and foot areas within primary motor cortex, Experiment 3 used a dual-coil paired-pulse TMS protocol to examine functional connectivity between secondary and primary motor areas during active ankle dorsiflexion and plantarflexion. PMd and SMA conditioning, but not PMv conditioning, produced distinct phases of task-dependent modulation of excitability of forearm representations within M1. Networks involving PMd-M1 facilitate isodirectional movements of hand and foot, whereas networks involving SMA-M1 facilitate corticomotor pathways non-specifically, which may help to stabilize posture during interlimb coordination. These results may have implications for targeted neurorehabilitation following stroke.
Introduction

Movements of the ipsilateral hand and foot are made more reliably when they are made in the same direction at the same time (Carson et al. 1995; Jeka et al. 1993; Jeka and Kelso 1995; Wenderoth et al. 2004). This can be experienced by flexing and extending the wrist and ankle together, and comparing the stability of movements made in the same direction (isodirectional) versus movements made in opposite directions (anisodirectional). The physiological origin of this preferred isodirectional mode of coordination is not well understood.

Within primary motor cortex (M1) there is no overlap or known anatomical connections linking arm and leg muscle representations (Brown et al. 1991; Huntley and Jones 1991). The synchrony of local field potential oscillations between M1 representations suggests common input arising from secondary motor areas rather than horizontal connectivity within M1 (Murthy and Fetz 1996). In secondary motor areas including dorsal and ventral premotor cortex (PMd, PMv), supplementary motor cortex (SMA) and cingulate, arm and leg areas overlap considerably (Fink et al. 1997). Given this overlap it is likely that coordination-related interactions between arm and leg originate upstream of M1.

The output of M1 contributes to the modulation of forearm H-reflexes to facilitate wrist movements that are isodirectional with respect to foot movement (Baldissera et al. 2002; Borroni et al. 2004; Cerri et al. 2003). However, the preference for isodirectional hand and foot movement reflects spatial rather than structural constraints. With the forearm pronated there is facilitation of wrist flexor pathways during plantarflexion, whereas with forearm resting supinated, wrist extensor pathways
are facilitated during plantarflexion, and wrist flexor pathways are facilitated during dorsiflexion of the foot (Borroni et al. 2004). This previously identified reciprocal pattern of corticomotor excitability of forearm pathways is used as a basis for the current study.

In the first experiment we explored whether lower limb muscle activation in the presence or absence of joint movement was sufficient to alter forearm motor cortical excitability. We hypothesized that the neurophysiological underpinning of isodirectional coupling is activity dependent. In experiment 2, we examined changes in excitability and intracortical inhibition of M1 arm regions during active versus passive foot movement. We hypothesized that intracortical inhibition of arm representations in M1 would be reduced by active foot movement in a direction-dependent manner, but not altered during passive movement. In experiment 3, we used a paired-pulse dual-coil technique to chart the modulation of functional connectivity between brain areas during motor performance. To date, very few studies have used this approach (Baumer et al. 2006; Civardi et al. 2001; Koch et al. 2006), and to our knowledge, none have done so during motor task performance. Given the strong functional connectivity between premotor areas and M1 (Civardi et al. 2001; Koch et al. 2006; Matsumura et al. 1992; Munchau et al. 2002), and the disruptive nature of repetitive TMS over SMA on the performance of difficult bimanual coordination patterns (Serrien et al. 2002; Steyvers et al. 2003), we hypothesized that PMd, PMv and SMA may have distinct functional roles during hand-foot coordination.

**Method**

**Subjects**
Twenty-six subjects with no neurological impairments participated in three experiments. Informed consent was obtained prior to participation. The local Ethics Committees approved the procedures (University of Auckland, Experiment 1 and 2; JW Goethe University of Frankfurt, Experiment 3) in accordance with the Declaration of Helsinki.

**Experimental Procedure**

In all three experiments subjects were examined at rest, and in tasks requiring right ankle dorsiflexion (DF) and plantarflexion (PF). The left hemisphere was tested for all subjects. In all experiments, the forearm muscles remained quiescent with the hand and arm resting in a prone posture. Forearm muscle relaxation was monitored using custom software, by use of auditory feedback of the electromyogram (EMG) signal provided to subjects, and confirmed by quantitative offline analysis.

*Experiment 1.* Eight subjects (6 male; age 22 – 57 years; 7 right-handed) were tested. The aim was to investigate corticomotor excitability of *extensor carpi radialis* (ECR) and *flexor carpi radialis* (FCR) under two ipsilateral lower limb activation conditions: phasic DF and PF of the foot, and isometric activation of *tibialis anterior* (TA) and *soleus* (SOL) muscles. Subjects were seated with their right hand and foot in a custom built manipulandum (Figure 1A). The right knee and elbow were positioned at 135º and 90º flexion, respectively. The left foot was supported at the same height as the right foot and the left hand was at rest in the subject’s lap. The manipulandum consisted of hand and foot plates attached to horizontal spindles, aligned to the right wrist and ankle joint. Potentiometers were calibrated to record angular displacement of each joint. The spindle attached to the footplate was connected to a Brushless AC Servomotor.
(Baldor, Fort Smith, AR, USA) programmed to provide a resistive torque (Experiment 1) or to drive the foot passively through sinusoidal motion (Experiment 2). Foot motion was restricted to a 30° range of motion by the apparatus. The combined weight of the subject’s hand and the plate were opposed by an adjustable elastic support to set the wrist with equal and negligible resistance in flexion or extension (Figure 1A).

EMG was recorded from right ECR, FCR, TA and SOL via bipolar electrode configurations. EMG signals were amplified (Grass P511AC, Grass Instrument Division, West Warwick, RI, USA), bandpass filtered (30 – 1000 Hz), sampled at 2 kHz using a 16-bit A/D acquisition system (National Instruments, Austin, TX, USA), and displayed using custom LabVIEW software. Single-pulse TMS was applied using a figure-of-eight coil (90 mm wing diameter) connected to a Magstim 200 stimulator (Magstim, Whitland, UK). The coil was positioned to induce posterior-anterior current and optimally elicit motor evoked potentials (MEPs) in ECR/FCR (Kaneko et al. 1996). A test stimulus (TS) intensity of 150% resting motor threshold (RMT) (Rossini et al. 1994), was used throughout the experiment. RMT was defined as the minimum intensity that produced small MEPs (> 50 µV) in at least 5 of 10 consecutive trials. A trial consisted of 9 metronome beats (pitch 800 Hz, pulse duration 50 ms) at 1.5 Hz. Subjects were instructed to either stay completely relaxed, or to perform DF or PF movements in time with the metronome. In the Phasic condition subjects moved their foot rhythmically through 30° on each cycle, and synchronized with the metronome at peak DF or PF. The resistive torque was programmed for each subject so that they could obtain the full range of motion. In the Isometric conditions subjects generated enough force to overcome the same torque, but maintained an isometric contraction at peak DF or PF. In all trials the
TMS unit was triggered 150 ms prior to the 7th metronome beat (i.e., after some adaptation time for the subjects to synchronize with the metronome, and well before the end of the trial) such that TMS was delivered during SOL or TA activation in the Phasic conditions.

**Experiment 2.** Ten subjects participated in experiment 2 (6 male; age 21 – 39 years; 9 right-handed). Subject preparation and apparatus were as described in experiment 1. We used paired-pulse TMS to examine whether short-interval intracortical inhibition (SICI) of the ECR representation was modulated by foot movement during active and passive conditions. This allowed us to examine the possible contribution of afference elicited from foot movement (in the absence of any leg muscle activation) and to determine if the direction of foot movement under active conditions produces a differential effect on SICI that is not seen during passive conditions. A sub-threshold conditioning stimulus (CS) was delivered 3 ms prior to the test stimulus (TS) (Hanajima et al. 2003). The CS was set initially to 90% active motor threshold (AMT) defined as the minimum intensity required to produce a 100 µV MEP in 5 out of 10 consecutive trials (Rossini et al. 1994). This intensity was chosen to produce maximal inhibition (Orth et al. 2003; Ziemann et al. 1996b), and then decreased in 1% steps of stimulator output until the conditioned (C) MEP size was 50% of the non-conditioned (NC) MEP. This procedure ensured that both increases and decreases in SICI would be detectable (Stinear and Byblow 2003). TS was adjusted to produce ~1 mV MEP in ECR in each condition and then NC and C MEPs were recorded (Fisher et al. 2002). FCR MEPs were not investigated in this experiment due to the requirement of matching of NC ECR MEP amplitudes across conditions in order to assess changes in SICI. Trial duration and
stimulation delivery were identical to experiment 1. Active and Passive conditions were identical, except that the foot was moved by the servomotor in the Passive condition. Subjects were instructed to keep their leg muscles relaxed during the movement. Conditions were pseudo-randomized and counterbalanced across subjects. A behavioral measure of directional tuning within motor cortex was derived by examining the kinematics of TMS-evoked hand movements.

Experiment 3. Eight right-handed subjects participated in experiment 3 (3 male; age 24 - 42 years). EMG data were recorded from right ECR, FCR, TA and SOL using a belly-tendon montage for each muscle, and a common ground electrode. The EMG was amplified and filtered (10 Hz – 2 kHz, Counterpoint Mk2 Electromyograph, Dantec Electronics, Skovlunde, Denmark), passed through a CED Micro 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) digitized at 4 kHz and fed into a personal computer for online display and offline analysis, using customized data collection and conditional averaging software (Spike 2 for Windows, Cambridge Electronic Design, Cambridge, UK). Participants were seated comfortably, with their elbows flexed to approximately 90° and forearms resting pronated on the armrests of a chair. The wrist joints were supported in a neutral position, and the forearm muscles were maintained at rest throughout the experiment. The right foot was fully supported on a flat surface during the rest condition. During DF, the right heel remained on this surface while the ankle was fully dorsiflexed. During PF, the right heel was supported by the edge of the surface, allowing full plantarflexion without making contact with the floor. The left foot was supported on a flat surface at the same height as the right foot.
Pairs of magnetic stimuli were delivered with two small figure-of-eight coils (outer wing diameter 50 mm), each connected to a Magstim 200 stimulator. The test coil was oriented to induce a posterior to anterior current flow in the underlying cortex. The TS was delivered over the optimal location of the left M1 for evoking MEPs in right ECR. RMT and AMT were determined in ECR with leg muscles at rest. AMT was also evaluated during DF, but this did not differ from rest for any subject. Conditioning stimuli (CS) were delivered at 3 sites: dorsal (PMd) and ventral premotor (PMv) areas and supplementary motor area (SMA - encompassing pre-SMA and SMA proper) following procedures reported in previous studies (Civardi et al. 2001). For PMd stimulation, the scalp was marked 5 cm anterior to the ECR hotspot. To target SMA, the scalp was marked on the midline, 6 cm anterior to the ECR hotspot. For PMv the site was marked 5 cm anterior and 4 cm lateral of the ECR hotspot. The sites targeted are shown in Figure 1B using a 3D rendered and peeled anatomical MRI from a single participant examined in this experiment and specialised software and co-registration system (Brainsight™, Rogue Research, Montreal) to confirm placement of the stimulating coils with respect to each motor area. As in previous studies, the conditioning coil was oriented to induce an anterior to posterior current flow in the underlying cortex. The interstimulus interval (ISI) between CS and TS was 6 ms (Civardi et al. 2001).

The TS was set to produce a 1 mV MEP in ECR at rest in a block of 8 stimuli. The same TS were then delivered during DF and PF in separate blocks of 8 stimuli each, to confirm directional modulation of ECR and FCR MEPs. For DF and PF, participants were instructed to raise or lower their foot, in such a way that the stimuli occurred during isometric muscle activation at peak joint excursion. Participants were instructed to
maintain the contraction after each stimulus, before relaxing, returning to the starting position, and again contracting prior to the next stimulus. An experimenter monitored performance and was able to reject trials online. Subjects performed the task without difficulty. After these data were obtained, the three sites of CS (PMd, PMv, and SMA) were tested in a random order for each participant. The TS intensity was adjusted to produce 1 mV ECR MEPs in each Direction condition (Rest, DF, and PF). Within each block, CS was delivered at 8 different intensities from 70 – 140% AMT, in 10% increments. In each block 8 paired stimuli were delivered at each CS intensity, with a further 8 NC stimuli. This resulted in a total of 72 stimuli (64 C and 8 NC) per block delivered in a randomized order by custom software and a random inter-trial interval of 3.75 – 6.25 s. Stimulator intensity was automatically controlled by the PC through an 8-bit socket in the rear panel of the magnetic stimulator (resolution 1% of maximum stimulator output) through in-house customized software (Spike 2 for Windows, Cambridge Electronic Design, Cambridge, UK). A short break was taken at midpoint to allow for coil cooling, to confirm coil placement, and for subject rest. Altogether, 9 blocks (3 sites of CS x 3 Direction conditions) of 72 trials were undertaken with each subject.

Data Analysis

Due to the sometimes polyphasic nature of MEPs in forearm muscles (Stinear and Byblow 2004b), ECR and FCR MEP area was used as the primary dependent measure. MEP area was calculated over a 30 ms window from MEP onset determined for each individual. For each Direction, MEP area was normalized to rest and expressed as a
percentage. Trials with pre-trigger root mean squared EMG (rmsEMG) activity of either forearm muscle were discarded if greater than 10 µV for the 50 ms prior to TMS (experiment 3, 30 µV for the 90 ms prior to TMS). ECR and FCR pre-trigger rmsEMG were subject to the same repeated measures analysis of variance (RM ANOVA) as MEP data.

In experiment 1, RM ANOVA factors were Muscle (ECR, FCR), Direction (DF, PF) and Activation (Phasic, Isometric).

In experiment 2, SICI was expressed as a percentage using the formula, 
\[
\%\text{Inhibition} = 100 - (C/NC \times 100),
\]
where C and NC correspond to conditioned and non-conditioned ECR MEP area for each condition. RM ANOVA factors were Direction (DF, PF) and Condition (Passive, Active). The measure of \%\text{Inhibition} allows for increases or decreases in SICI relative to resting levels (approximately 50%) to be evaluated for each Direction and Condition. TMS-evoked hand movements were measured using displacement data obtained from the potentiometer signals, which were differentiated twice to derive wrist velocity and acceleration. The velocity at the time of the first acceleration peak following TMS, denoted as stimulus-evoked velocity (SEV), was used as an estimate of directional tuning. This event occurred within 100 ms of stimulation. SEVs during DF and PF were normalized to SEV at rest. SEVs were analyzed with factors Direction (DF, PF) and Condition (Active, Passive). Finally, rmsEMG of TA and SOL were monitored, and trials rejected online, to ensure complete relaxation of leg muscles (< 10 µV) during Passive conditions.
In experiment 3, MEP area was expressed as a percentage (C/NC) and analysed with a RM ANOVA. For C MEPs, factors were Direction (Rest, DF, PF) and CS intensity (70, 80, 90, 100, 110, 120, 130, 140% AMT) analyzed separately for each site of conditioning stimulation. For NC MEPs, factors were Muscle (ECR, FCR) and Direction (Rest, DF, and PF). The pre-trigger rmsEMG ANOVA design was 3 Direction (Rest, DF, PF) x 9 CS intensity (all CS Intensities and NC).

Planned contrasts were conducted using paired t-tests to compare DF and PF where there are interactions or trends between Direction and other factors. In order to prevent an escalation of the family-wise error rate, in experiment 3, paired t-tests were used to examine differences between rest, DF and PF at only selected low (80, 90% AMT) and high (120, 130% AMT) CS intensities. Statistical significance was set to $\alpha = 0.05$. Mean ± SD are reported in results.

**Results**

The procedures were well tolerated by all subjects.

*Experiment 1- Phasic Versus Isometric Muscle Activation*

Figure 2A illustrates ECR, FCR, TA, and SOL EMG and foot displacement for a single participant during phasic DF and PF trials.

For MEP area there was a main effect of Activation ($F_{1,7} = 7.1, p < 0.05$) with larger MEPs in phasic (1.67 ± 0.27 mV•s) versus isometric (1.3 ± 0.17 mV•s) conditions. There was an interaction between Muscle and Direction ($F_{1,7} = 10.8, p < 0.01$; see Figure 2B). ECR MEPs were larger during DF than PF, and FCR MEPs were larger during PF
than DF (both \( p < 0.01 \)). There were no other effects or interactions for MEP data (all \( p > 0.1 \)).

There were no effects or interactions for forearm pre-trigger rmsEMG (all \( p > 0.1 \)) and muscles of the forearm remained consistently at rest throughout testing.

**Experiment 2- SICI and TMS-evoked movements**

There was a main effect of Condition for the NC ECR MEPs (\( F_{1,9} = 54.5, p < 0.001 \); see Figure 3A) with larger MEPs obtained during active versus passive movement, and no other main effects or interactions. ECR MEPs were larger during active DF and PF than Rest. Conversely, MEPs were smaller during passive PF and DF than Rest (all \( p < 0.01 \)). Comparing Direction under active and passive conditions revealed no differences (all \( p > 0.05 \)) in ECR NC MEP area indicating that NC ECR MEP area was matched within activation conditions. For ECR SICI, %Inhibition at Rest was 42.8% (range 31-61%) as intended by the procedure. There was a Direction x Condition interaction (\( F_{1,9} = 5.1, p < 0.05 \); see Figure 3B) and no other effects (all \( p > 0.1 \)). ECR SICI was less during DF than PF for the active condition (\( p < 0.05 \)), but not the passive condition (\( p > 0.1 \)), and lower during active dorsiflexion than rest (\( p < 0.01 \)) but there was no difference with rest in any other condition (all \( p > 0.1 \)). ECR SICI expressed as %Inhibition was 40.8 ± 14.0% at rest, confirming sensitivity to factors that may increase or decrease SICI.

Representative traces depicting TMS-evoked movements of the hand for each Direction are shown in Figure 4A. For SEV there was a main effect of Direction (\( F_{1,9} = 10.0, p < 0.01 \); see Figure 4B) but no effect of Condition (\( p > 0.1 \)). The Direction x Condition interaction did not reach conventional levels of significance (\( F_{1,9} = 4.2, p = 0.07 \)). To explore the effect of Direction in the presence of this Direct x Condition trend,
we examined the effect of Direction for Active versus Passive movements separately. During active DF, TMS evoked wrist movements toward extension whereas during active PF, TMS evoked wrist movements toward flexion \( (p < 0.005) \). There was no effect of Direction on SEVs during passive foot movement \( (p = 0.5) \). Across all subjects, muscles and conditions ECR pre-trigger EMG ranged between 5 and 8 µV confirming relaxation throughout the experiment.

**Experiment 3- Conditioning of ipsilateral PMd, PMv and SMA**

The data from one participant were excluded, as this person was unable to maintain forearm muscle relaxation.

With conditioning stimulation applied over PMd there was a main effect of CS Intensity \( (F_{7,42} = 2.85, p < 0.02) \) and a Direction x CS Intensity interaction \( (F_{14,84} = 2.13, p < 0.02; \) see Figure 5, PMd). The source of this interaction was investigated using post-hoc comparisons. It was found that ECR MEP area was larger during DF than Rest with conditioning at 90% AMT \( (p < 0.05) \) and 130% AMT \( (p < 0.05) \) but not at 80% or 120% AMT (both \( p > 0.1 \)). MEPs were larger during DF than PF with conditioning at 130% AMT only \( (p < 0.05, \) other \( p > 0.1 \)). MEP areas did not differ between PF and Rest with either CS intensity (all \( p > 0.09 \)).

With conditioning stimulation applied over SMA there was a Direction x CS Intensity interaction \( (F_{14,84} = 1.91, p < 0.05; \) see Figure 5, SMA). From post-hoc comparisons, ECR MEPs were larger during both DF and PF than Rest with conditioning at 90% AMT (both \( p < 0.05 \)) but did not differ from rest at other CS intensities (all \( p > 0.1 \)). ECR MEPs were larger during PF than DF with conditioning at 130% AMT only \( (p < 0.01) \).
There were no significant effects or interactions of ECR MEPs with conditioning applied over PMv (all $p > 0.1$). The analysis of pre-trigger rmsEMG of ECR confirmed there were no effects of CS Intensity, Direction, or any interactions (all $p > 0.1$) during stimulation of any site, confirming that subjects maintained ECR and FCR relaxation during all testing.

NC ECR MEPs were deemed to be successfully matched in the paired-pulse sessions as confirmed by the Conditioning Site x Direction RM ANOVA, which revealed no effects or interactions (all $p > 0.1$; see Figure 6A). For single-pulse trials, the ANOVA of unmatched NC ECR and FCR MEPs revealed the expected Muscle x Direction interaction ($F_{1,6} = 3.3$, $p < 0.01$; see Figure 6B). ECR MEPs were larger during DF than PF, and FCR MEPs were larger during PF than DF (both $p < 0.05$) confirming the result of experiment 1 (Figure 2B). The analysis of pre-trigger rmsEMG of leg muscle activation revealed an effect of Muscle, Direction, and Muscle x Direction interaction ($F_{2,12} = 14.4$, $p < 0.001$). As expected, activation of TA was greater during DF than PF ($p < 0.01$), and activation of SOL was greater during PF than DF ($p < 0.05$), indicating successful task performance by the group during the experiment.

Discussion

Our results extend previous findings (Cerri et al. 2003) by demonstrating that upper limb corticomotor excitability and SICI were altered by movement conditions involving leg muscle activation, but not during passive leg movement. These neurophysiological changes occurred in parallel with TMS-evoked hand movements which revealed an isodirectional coupling between hand and foot. As there are no known anatomical connections between arm and leg regions within M1 (Huntley and Jones
1991), we used a novel dual-coil paired-pulse TMS protocol to examine functional connectivity between secondary and primary motor areas during active ankle dorsiflexion and plantarflexion. Our results demonstrate that networks involving PMd-M1 facilitate the production of isodirectional movements between the hand and foot. Conversely, networks involving SMA-M1 facilitate forearm corticospinal excitability in a non-specific manner, perhaps as a mechanism underlying postural stability. Each of these findings and their implications are discussed in turn.

**SICI and Activity-dependent coupling**

ECR SICI was significantly reduced during active dorsiflexion compared to plantarflexion, thereby facilitating isodirectional movement patterns between hand and foot. This supports earlier findings that foot movement produces changes in excitability within upper limb regions of M1 (Baldissera et al. 2002; Borroni et al. 2004) and extends these findings by demonstrating modulation of GABA-mediated inhibitory networks (Ilic et al. 2002; Ziemann et al. 1995; Ziemann et al. 1996a; Ziemann et al. 1998) during these movements. In M1, the inhibitory networks responsible for SICI play a crucial role in spatial and temporal modulation of corticospinal output prior to (Reynolds and Ashby, 1999), during (Stinear and Byblow 2004a; 2003) and at the termination of movement (Buccolieri et al. 2004). Interestingly, SICI of hand muscle representations increases during the production of more difficult coordination patterns compared to easy coordination patterns (Byblow and Stinear 2006; Stinear and Byblow 2002). We propose that PMd networks may interact with these inhibitory networks within M1 (Munchau et al. 2002) to facilitate corticospinal excitability in support of isodirectional movements.
**Functional connectivity between PMd and M1**

At rest, ECR MEPs tended to be inhibited by conditioning applied over PMd at 90% AMT, in agreement with previous studies (Civardi et al. 2001). However, during ankle dorsiflexion, PMd conditioning *facilitated* ECR MEPs, whereas there was no evidence that ECR excitability was suppressed during plantarflexion. ECR SICI was modulated by movement direction in active conditions only, with a significant decrease in SICI during dorsiflexion compared to plantarflexion (experiment 2). Although there was no evidence for suppressive influences on ECR M1 excitability to countermand anisodirectional movement patterns, this is not surprising given that the intention of the participants was to move only the foot (while the forearm remained stationary and at rest). Facilitation along the PMd - M1 pathway specifies the *preferred* coordination mode, but form the current study, it cannot be determined if this is the sole pathway through which one mode or the other is *selected* during interlimb coordination.

The PMd-M1 pathway facilitates the production of isodirectional patterns via a direct facilitatory pathway between PMd and M1, the modulation of inhibitory projections between PMd and M1 circuits mediating SICI, or both. ECR MEP facilitation occurred across two widely separated CS intensity ranges. Separate PMd neuronal populations may have given rise to the two distinct peaks of facilitation observed in Figure 5-PMd. This result is consistent with reports of independent PMd-M1 networks (Baumer et al. 2006; Baumer et al. 2003; Civardi et al. 2001; Koch et al. 2006). Subthreshold conditioning of ipsilateral PMd produces inhibition of M1 at rest (Civardi et al. 2001). The first phase of facilitation observed with PMd conditioning during dorsiflexion may reflect a dis-facilitation of inhibitory networks within M1. At rest,
higher PMd conditioning intensities did not produce facilitation of ECR MEPs in contrast to (Civardi et al. 2001). Since these authors investigated an intrinsic hand muscle, this may reflect differences between PMd-M1 networks that operate on hand versus forearm muscle representations. However, higher PMd conditioning intensities facilitated ECR MEPs during dorsiflexion, again suggesting that PMd-M1 cortico-cortical connections are modulated in a manner that facilitates isodirectional movement. At this time, it is not known whether low versus high conditioning stimulation operates through separate intracortical inhibitory versus facilitatory mechanisms within M1. To ascertain the interaction between these putative independent networks between PMd and M1, further investigation utilizing triple-pulse paradigms (Koch et al. 2007) in the context of interlimb coordination is warranted. The idea that ECR MEP facilitation during dorsiflexion is mediated through PMd activation is also consistent with FMRI studies of hand and foot coordination (Heuninckx et al. 2005). PMd contains neural representations of relative position codes (Pesaran et al. 2006) and thus, may provide a general solution to the spatial problem of coordinating different body parts.

**Functional connectivity between SMA and M1**

At rest, ECR MEPs were inhibited by conditioning SMA at 90% AMT in agreement with previous studies (Civardi et al. 2001). However, ECR MEPs were facilitated by SMA conditioning during dorsiflexion and plantarflexion relative to rest (90% AMT). This non-specific facilitation of ECR during both movement directions may reflect a cortico-cortical mechanism which subserves postural stability. SMA is known to be involved in the maintenance of anticipatory postural adjustments (Aruin and Latash
1995; Aruin et al. 1996; Aruin et al. 2001) and may also stabilize posture during coincident hand and foot movements (Cerri et al. 2003).

The SMA is involved in the stabilization of difficult coordination patterns as demonstrated by the disruption of difficult, but not easy, bimanual coordination patterns following repetitive TMS over SMA (Serrien et al., 2002; Steyvers et al., 2003), as well as maximum intensity single-pulse TMS (Meyer-Lindenberg et al. 2002; Serrien et al. 2002; Steyvers et al. 2003). When SMA was conditioned at 130% AMT, ECR MEPs were significantly larger during plantarflexion than dorsiflexion or rest, which did not differ from each other. Interpreting this result is difficult. Although SMA-M1 networks may subserve the production of difficult coordination patterns, we do not favor this interpretation on the basis of the present findings, since the observed difference in MEPs between conditions resulted from suppression during rest and dorsiflexion, rather than ECR MEP facilitation during plantarflexion (compare Fig 5-SMA with Fig 5-PMd). A more detailed examination of SMA-M1 functional connectivity during the production of difficult coordination patterns does seem warranted.

*Functional connectivity between PMv and M1*

PMv conditioning had no effect on ECR MEPs at rest, nor was it modulated by foot direction. It is known from animal studies that PMv is involved in transformations from extrinsic to intrinsic coordinates which are required in the context of certain goal-directed movements of the upper limb (Kakei et al. 2001; Kakei et al. 2003). PMv neurons with terminations in M1 may convey information linking representations within the upper limb, for example linking digits with hand and arm regions (Dancause et al. 2001; Dancause et al. 2002).
However, our results lend no support for PMv involvement in linking representations of ipsilateral hand and foot.

**Cortico-cortical connectivity between PMd, SMA and M1**

The present study is the first to show that networks involving PMd-M1 facilitate isodirectional tendencies between the hand and foot. There is evidence for left PMd involvement in action selection involving limbs on either side of the body (Schluter et al. 2001), whereas a preferential role of right PMd has been implicated in the maintenance of bimanual coordination (Meyer-Lindenberg et al. 2002; Sadato et al. 1997). Although the extent of lateralisation of PMd-M1 networks for movements made on either side of the body, or across the midline, is not yet known, the modulation of cortico-cortical connectivity between PMd and M1 provides directionally specific facilitation to support isodirectional movements of hand and foot. It is conceivable that leg motor practice may induce lasting effects on upper limb M1 excitability. This may be of potential value in the context of motor rehabilitation given the importance of PMd during recovery of motor function following stroke (Frost et al. 2003; Johansen-Berg et al. 2002a; Johansen-Berg et al. 2002b; Seitz et al. 1998; Ward et al. 2003).

**Limitations and Significance of the present findings**

One limitation of the paradigm employed in the current studies is that the direction-dependent modulation of ECR/FCR MEPs during foot muscle activation occurs in the absence of forearm muscle activation. Therefore, the importance of modulation of excitability of these pathways in the production of hand-foot coordination is assumed. We infer that increases in M1 excitability and decreases in SICI reflect changes that may facilitate voluntary movement of the upper limb (Reynolds and Ashby 1999). The fact
that TMS-evoked hand movements were also direction-dependent lends support to this assumption.

In the present studies, the excitability of motor pathways was not assessed by applying stimulation below the level of the cortex. Although it is possible that segmental or propriospinal networks may have caused alterations in motor neuron excitability in these experiments, this is unlikely for several reasons. First, sub-threshold conditioning applied in the evaluation of SICI, and with two-coil paired-pulse TMS (up to 90% AMT) are unlikely to reflect, or induce, effects at or below the corticospinal elements within M1 (Di Lazzaro et al. 1999). Therefore changes in excitability occurring below the motor cortex cannot explain the differential modulation of conditioned MEPs across foot movement conditions. Second, it has been demonstrated that there are no changes to H-reflexes recorded in intrinsic hand muscles in response to conditioning over PMd or SMA up to 120% AMT (Civardi et al. 2001). The extent to which stimulation intensities above 120% AMT applied over secondary areas directly influence spinal motor neuron excitability cannot be known from the present study. However, the finding of differential modulation with foot movement tasks, and no facilitation at rest support the interpretation that the effects from this stimulation protocol reflect cortico-cortical interactions.

It is possible that the conditioning stimulation in experiment 3 may have spread to adjacent cortical areas, but this seems unlikely given the low CS intensities used in this study. Although the effects of stimulation spread cannot be discounted entirely, direct connections from prefrontal or inferior frontal areas to M1 are known to be sparse. The time course of effects given our chosen ISI suggest direct cortico-cortical connections
between the targeted premotor /SMA areas and M1, where anatomically it is known there are direct connections between these areas (Godschalk et al. 1995).

Another point of difference with the study of Civardi and colleagues is the muscle examined, which was FDI in their study and ECR in ours. There may be important differences between the cortico-cortical connections for these muscle representations that might explain slight differences between studies in conditioning of PMd or SMA at rest.

In conclusion, the present experiments identify a context of limb movement which modulates the functional connectivity between PMd, SMA and M1. Modulation of M1 excitability by PMd may subserve the preferred isodirectional pattern of hand-foot coordination. This may be of particular relevance in motor rehabilitation following stroke, considering the involvement of PMd during motor tasks in patients who exhibit good recovery (Frost et al. 2003; Johansen-Berg et al. 2002a; Johansen-Berg et al. 2002b; Seitz et al. 1998; Ward et al. 2003).
References


Figure Captions

Figure 1  **A.** Experimental paradigm of experiments 1 and 2.  **B.** Three conditioning stimulation sites (PMd blue; PMv red; SMA yellow) and the arm representation of M1 (green) for eliciting responses in ECR from a single participant examined in experiment 3. The locations are shown as they would appear at the scalp surface but projected above a 3D rendered brain peeled to a depth of 9 mm from the cortical surface. Because of distortions of perspective, the locations appear as approximations only. Actual distances were measured at the scalp surface between each site and the hotspot as reported in the text.

Figure 2  **A.** ECR and FCR EMG illustrating MEPs, SOL and TA EMG and ankle displacement averaged over 8 trials, for a single participant during the phasic movement tasks of Experiment 1. The gray horizontal lines indicate the amplitude of the MEP obtained in ECR and FCR at rest.  **B.** There was a significant Muscle x Direction interaction for NC ECR and FCR MEP area in both phasic (left) and isometric (right) activation conditions (*p* < 0.01). Bars represent the mean from eight subjects. Error bars indicate SEM.

Figure 3.  **A.** Non-conditioned ECR MEP matched for each Direction for active and passive conditions.  **B.** There was a significant Direction x Condition interaction obtained for ECR SICI expressed as %Inhibition (normalized to SICI values obtained at rest). Larger values are indicative of greater inhibition relative to rest. The asterisk indicates
that DF and PF differed from each other in active but not passive conditions ($p < 0.01$).

Bars represent the means of ten subjects. Error Bars indicate SEM.

Figure 4. **A.** Representative foot and hand displacement, and hand velocity and acceleration profiles from one subject in experiment 2 demonstrating TMS induced movements of the wrist during active foot movement and the method for determination of SEV (vertical dashed line). All traces begin 100 ms prior to stimulation. **B.** Group results (N=10) showing SEV of TMS-evoked movements about the wrist showing a significant difference between DF and PF for active but not passive movements. Positive values indicate wrist extension, and negative values indicate wrist flexion. Error bars indicate SEM.

Figure 5. Group data (N=7) of ECR MEP area for Dorsiflexion (DF) and Plantarflexion (PF) conditions plotted against CS Intensity for each conditioned target- PMd, SMA, PMv. The area between the two smoothed lines reflects ±1 SEM of conditioned responses at Rest. The dashed horizontal line represents the size of NC ECR MEPs. There was a significant CS Intensity x Direction interaction for PMd and SMA. The single asterisk indicates significant difference between DF and Rest with conditioning at 90% AMT (PMd, SMA). The double asterisk indicates a significant difference between PF and rest at 90% AMT (SMA). The triple asterisk indicates a significant difference between DF and PF with conditioning at 130% AMT (PMd, SMA). Notice that with PMd conditioning, MEPs are facilitated during DF. All $p < 0.05$. Error bars indicate SEM.
Figure 6 **A.** Stimulator intensity was adjusted to match NC ECR MEP area between conditions in the dual-coil paired-pulse experiment. **B.** With constant stimulator intensity across conditions the Muscle x Direction interaction was significant for NC MEP area (*p* < 0.01), confirming the direction-dependent changes observed in experiment 1 (Figure 2B). Bars represent group averages (N=7). Error bars indicate SEM.
A. Experimental paradigm of experiments 1 and 2. B. Three conditioning stimulation sites (PMd blue; PMv red; SMA yellow) and the arm representation of M1 (green) for eliciting responses in ECR from a single participant examined in experiment 3. The locations are shown as they would appear at the scalp surface but projected above a 3D rendered brain peeled to a depth of 9 mm from the cortical surface. Because of distortions of perspective, the locations appear as approximations only. Actual distances were measured at the scalp surface between each site and the hotspot as reported in the text.
A. ECR and FCR EMG illustrating MEPs, SOL and TA EMG and ankle displacement averaged over 8 trials, for a single participant during the phasic movement tasks of Experiment 1. The gray horizontal lines indicate the amplitude of the MEP obtained in ECR and FCR at rest. B. There was a significant Muscle x Direction interaction for NC ECR and FCR MEP area in both phasic (left) and isometric (right) activation conditions (* p < 0.01). Bars represent the mean from eight subjects. Error bars indicate SEM.
A. Non-conditioned ECR MEP matched for each Direction for active and passive conditions.

B. There was a significant Direction x Condition interaction obtained for ECR SICI expressed as %Inhibition (normalized to SICI values obtained at rest). Larger values are indicative of greater inhibition relative to rest. The asterisk indicates that DF and PF differed from each other in active but not passive conditions (p < 0.01). Bars represent the means of ten subjects. Error Bars indicate SEM.
A. Representative foot and hand displacement, and hand velocity and acceleration profiles from one subject in experiment 2 demonstrating TMS induced movements of the wrist during active foot movement and the method for determination of SEV (vertical dashed line). All traces begin 100 ms prior to stimulation. B. Group results (N=10) showing SEV of TMS-evoked movements about the wrist showing a significant difference between DF and PF for active but not passive movements. Positive values indicate wrist extension, and negative values indicate wrist flexion. Error bars indicate SEM.
Group data (N=7) of ECR MEP area for Dorsiflexion (DF) and Plantarflexion (PF) conditions plotted against CS Intensity for each conditioned target- PMd, SMA, PMv. The area between the two smoothed lines reflects ±1 SEM of conditioned responses at Rest. The dashed horizontal line represents the size of NC ECR MEPs. There was a significant CS Intensity x Direction interaction for PMd and SMA. The single asterisk indicates significant difference between DF and Rest with conditioning at 90% AMT (PMd, SMA). The double asterisk indicates a significant difference between PF and rest at 90% AMT (SMA). The triple asterisk indicates a significant difference between DF and PF with conditioning at 130% AMT (PMd, SMA). Notice that with PMd conditioning, MEPs are facilitated during DF. All p < 0.05. Error bars indicate SEM.

88x127mm (600 x 600 DPI)
A. Stimulator intensity was adjusted to match NC ECR MEP area between conditions in the dual-coil paired-pulse experiment. B. With constant stimulator intensity across conditions the Muscle x Direction interaction was significant for NC MEP area (* p < 0.01), confirming the direction-dependent changes observed in experiment 1 (Figure 2B). Bars represent group averages (N=7). Error bars indicate SEM.