Dorsal Premotor Cortex Exerts State-Dependent Causal Influences on Activity in Contralateral Primary Motor and Dorsal Premotor Cortex

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During voluntary action, dorsal premotor cortex (PMd) may exert influences on motor regions in both hemispheres, but such interregional interactions are not well understood. We used transcranial magnetic stimulation (TMS) concurrently with event-related functional magnetic resonance imaging to study such interactions directly. We tested whether causal influences from left PMd upon contralateral (right) motor areas depend on the current state of the motor system, involving regions engaged in a current task. We applied short bursts (360 ms) of high- or low-intensity TMS to left PMd during single isometric left-hand grips or during rest. TMS to left PMd affected activity in contralateral right PMd and primary motor cortex (M1) in a state-dependent manner. During active left-hand grip, high (vs. low)-intensity TMS led to activity increases in contralateral right PMd and M1, whereas activity decreases there due to TMS were observed during no-grip rest. Analyses of condition-dependent functional coupling confirmed topographically specific stronger coupling between left PMd and right PMd (and right M1), when high-intensity TMS was applied to left PMd during left-hand grip. We conclude that left PMd can exert state-dependent interhemispheric influences on contralateral cortical motor areas relevant for a current motor task.

Keywords: concurrent TMS-fMRI, effective connectivity, grip force, motor preparation, transcallosal inhibition

Introduction

The dorsal premotor cortex (PMd) is a key structure for selection and initiation of voluntary actions (Scott et al. 1997; Grafton et al. 1998; Rushworth et al. 2003; Hoshi and Tanji 2004; Cisek and Kalaska 2005; Churchland et al. 2006). It is reciprocally connected with ipsilateral (Lu et al. 1994; Wise et al. 1997; Dum and Strick 2005) and contralateral (Marconi et al. 2003) cortical motor areas, most notably primary motor cortex (M1), parietal cortex, and contralateral PMd. During simple unilateral hand movements, an increase in M1 activity is seen predominantly in the contralateral hemisphere (Evarts 1966), whereas the increase in PMd activity is typically bilateral in nonhuman primates (Tanji et al. 1988; Cisek et al. 2003) and humans (Ward and Frackowiak 2003). The potential importance of ipsilateral premotor cortex for voluntary action is further emphasized by findings that subcortical damage to corticospinal pathways can lead to a functionally relevant increase in PMd activity during movement of the affected ipsilateral hand (Johansen-Berg et al. 2002; Ward et al. 2006, 2007). To date however, it remains unclear by what routes and in what functional manner ipsilateral PMd may exert its influences over contralateral cortical motor structures.

Several lines of evidence suggest the particular importance of left PMd and its dominance for selection of both ipsilateral and contralateral actions (Schluter et al. 1998, 2001; Rushworth et al. 2003). Recent transcranial magnetic stimulation (TMS) experiments have used an interhemispheric conditioning-test paradigm, with 1 coil over premotor cortex and the other over M1 in opposite hemispheres, finding that left PMd may change excitability of contralateral M1, possibly directly via transcallosal projections (Mochizuki, Huang, et al. 2004a, 2004b; Koch et al. 2006, 2007). This method therefore assesses the causal impact of 1 brain region upon an interconnected area, at high temporal precision, noninvasively in healthy human subjects. However, conditioning-test TMS approaches for studying influences of PMd on other areas must typically rely on the induced motor response to an M1 pulse as their dependent measure and therefore must ultimately highlight influences converging on just M1 in particular. Moreover, they cannot disclose the spatial specificity across cortical regions of such influences. This is potentially important as left PMd also has anatomical connections with several cortical structures other than M1 in either hemisphere, notably with contralateral right PMd (Lu et al. 1994; Wise et al. 1997; Marconi et al. 2003).

A very different approach has been to combine TMS with physiological measures of induced changes in brain activity, such as via positron emission tomography (Paus et al. 1997, 1998; Paus 1999), electroencephalography (Paus et al. 2001; Massimini et al. 2005), or functional magnetic resonance imaging (fMRI) (Bohning et al. 1998, 1999, 2000; Baudewig et al. 2001; Kemna and Gembris 2003; Bestmann et al. 2004, 2005; Ruff and others 2006). Using such approaches, it has been shown that stimulating left PMd with TMS at rest may affect activity in putatively interconnected motor structures, including contralateral (right) PMd, bilateral SMA, plus frontal and parietal association cortices (Chouinard et al. 2003; Bestmann et al. 2005).

However, to date such studies have not addressed any dependence of remote causal influences from left PMd on the current state of putatively interconnected motor regions. These studies did not vary state due either to their use of prolonged TMS stimulation protocols prior to scanning (Chouinard et al. 2003) and/or the absence of any varied task requirements during scanning (Bestmann et al. 2005). Therefore, the nature and spatial distribution across brain regions of functional interplay between left PMd and interconnected cortical motor regions for the control of voluntary movement remain unclear.

Due to recent technical advances, such potentially state-dependent interplay between cortical regions can be studied

noninvasively by combining TMS on line with event-related fMRI (Bohning et al. 2000; Baudewig et al. 2001; Kemna and Gembris 2003; Bestmann et al. 2004; Sack et al. 2007). This approach applies short TMS events or stimulation trains that can have immediate effects on brain activity lasting only a few seconds (Romeo et al. 2000; Modugno et al. 2001) during scanning. By concurrently assessing evoked activity changes throughout the brain with fMRI, this approach can thus provide a means of assessing interregional influences directly by using the causal intervention or "perturbation" of TMS and then measuring its remote effects as well as any local influences (Paus 2005).

Accordingly, we examined the causal impact of left PMd stimulation upon activity throughout the brain during 2 different contexts (ipsilateral grip task or no-grip rest). We assessed any state-dependent remote effects of left PMd TMS, and whether they might be distributed across all putatively interconnected areas (potentially including, e.g., ipsilateral left M1) or instead might be topographically restricted to task-relevant brain regions during the left-hand movement (including contralateral right M1 and right PMd). Such an outcome would indicate some state specificity of causal influences from left PMd during control of voluntary ipsilateral movement. We specifically chose stimulation of left PMd because of its suggested dominance for the selection and preparation of hand movements (Schluter et al. 2001; Astafiev et al. 2003; Rushworth et al. 2003; Davare et al. 2006).

We applied short bursts of left PMd stimulation either during an active force generation grip task with the ipsilateral left-hand or during no-grip rest. We chose the grip task as its neural substrates (in the absence of TMS) are already well characterized (e.g., Ehrsson et al. 2000, 2001; Ward and Frackowiak 2003). Moreover, it allowed TMS application to overlap temporally with the active grip (see below). Our data reveal that causal interactions of left PMd TMS on activity in interconnected regions (including contralateral right PMd and right M1) vary strongly with the current motor state in a manner that is specific to relevant task-related motor cortical areas contralateral to the gripping hand and thus contralateral to the applied left PMd TMS.

Materials and Methods

Participants

Twelve right-handed healthy volunteers participated (3 females, mean age 45.33 years, range 28–73 years) with neither history of neurological or psychiatric illness nor regular medication. Participants were naive to the purpose of the experiment. Full written consent was obtained from all participants, in accord with local ethics approval. The wide age range of participants (chosen here with a view for future possible clinical studies of patients) was taken into account in analysis of the results, which considered age as 1 possible factor.

Experimental Paradigm

A continuous scanning session (25 min 44 s) was conducted to measure TMS influences in a 2 × 2 factorial event-related design with motor state (*grip, no grip*) and TMS input to left PMd (TMS_{high}, TMS_{low}) as experimental factors. In addition to 20 experimental trials per condition, we also recorded 20 null events. These comprise trials without experimental treatment and thus provide a baseline measure of activity. All trials were pseudorandomized so that each trial type occurred twice within 8 consecutive trials, with an average intertrial interval of 16.11 s to minimize any carryover effects of TMS bursts between successive trials (Modugno et al. 2001).

We varied the context in which TMS was applied using a simple left-hand dynamic isometric grip task (Ward and Frackowiak 2003; Ward et al. 2007). The grip manipulandum consisted of 2 force transducers (Honeywell FSG15N1A; Honeywell, Harristown, NJ) moulded between 2 plastic bars (width 6 cm). Compression of the 2 bars during isometric handgrip generated a differential voltage linearly proportional to the exerted force. The signal was fed into an amplifier (CED 1902), digitized (CED 1401; both Cambridge Electronic Design, Cambridge, UK), and stored on a personal computer. A video projector at the bore of the magnet projected visual stimuli onto a frosted screen, viewed via a mirror system mounted atop the MR head coil. A centrally presented visual columnar display gave visual feedback about any dynamic changes in recorded voltage (and hence grip force) vertically. During scanning, a visual cue indicated that participants should either perform a single isometric left-hand grip or maintain rest. Target force levels for all grip trials were set to 20% of individual maximum voluntary contraction (MVC) and displayed as a horizontal yellow bar on the red feedback column (Fig. 1A). This grip task was chosen based on pilot work showing that participants could perform the task well even in the presence of TMS (thus avoiding contamination of any neural effects of TMS by overt behavioral changes, see Discussion). Furthermore, the extended duration of each handgrip in the present task allowed TMS to overlap with the active motor state, as confirmed below. Finally, the neural substrates of this particular grip task have been well characterized by prior fMRI studies (Ward and Frackowiak 2003).

On an unpredictable 50% of trials (grip condition), a left-hand arrow as well as the grip-force target level were presented at trial onset (see Fig. 1A). This cue started the trial, indicating that participants should perform a brief single left-hand grip, until the column representing force reached the horizontal target bar. The cue and target level were displayed for 4 s, superimposed on the force column that was present throughout the experiment. Participants were explicitly instructed that speed was not critical and that they should generate a nonballistic handgrip to approximately match the displayed target bar using a gentle pace without major corrective movements.

On the other unpredictable 50% of trials (no-grip condition), a cross ("×" character) was shown instead of an arrow at trial onset (Fig. 1A). This indicated that participants should withhold from making a left-hand grip and instead keep the hands relaxed (no-grip rest condition). This forewarning for a no-grip trial also served to prevent unintentional grip force being generated when TMS pulses occurred. Only left-hand grips were performed. However, participants held 1 manipulandum in the right-hand to allow the assessment of any unintended mirror movements or of undesired twitches during TMS. Neither was observed here.

Two TMS intensities were applied over left PMd: for the TMS_{high} condition, TMS was applied at 110% of individual resting motor (M1) threshold (RMT; mean intensity [± SD] of 74% [±7%] of maximal stimulator output, MSO), which is known to be effective in stimulating PMd (Mochizuki, Huang, Rothwell 2004; Bestmann et al. 2005; Praeg et al. 2005; Baumer et al. 2006; Koch et al. 2006; O'Shea et al., 2007). RMT was determined for the right-hand when stimulating over left M1. An unpredictable 50% of all trials during the fMRI experiment were in this TMS_{high} condition (orthogonally to the grip/no-grip manipulation). The other unpredictable half of trials during scanning provided the TMS_{low} condition, which had the TMS intensity set to 70% of individual active motor threshold (AMT), corresponding to a mean intensity (±SD) of 42% (±4%) MSO. The seemingly rather high motor thresholds (in terms of MSO) in our study reflect increased impedance of the extended MR-compatible cabling running from the stimulator to the coil.

A TMS train (5 pulses at 11 Hz) was applied on all trials to left PMd (i.e., ipsilateral to the active hand for the grip task), starting 900 ms (10 echo-planar image [EPI] slice acquisitions) after presentation of the instructional visual cue. We chose this fixed interval (rather than triggering TMS from the actual movement onset on each trial) because TMS pulse application had to be synchronized and interleaved with high temporal precision in relation to each EPI slice acquisition (see below and Fig. 1B). We later verified that participants were indeed performing the grip task at the moment of TMS delivery on relevant trials (see Results), as in previous work with the same behavioral grip
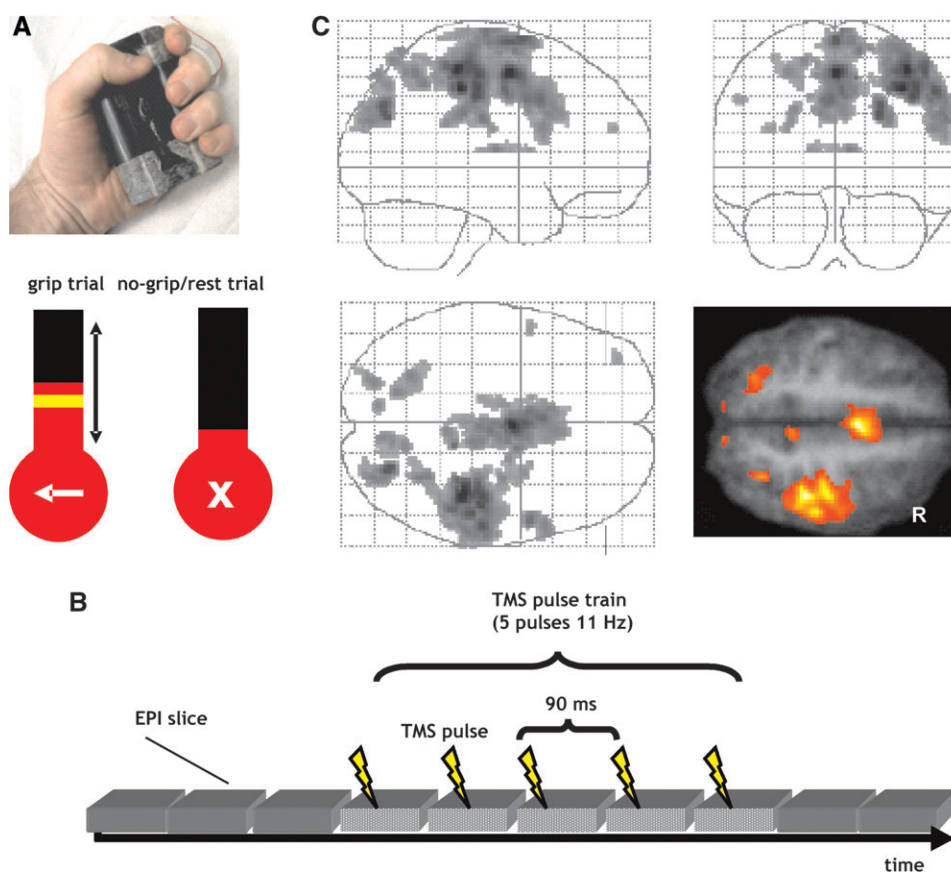
Figure 1. Experimental setup and main effects of grip task. (*A*) Grip-force manipulandum and screen display for grip (arrow to left) and no-grip (central cross) trials. During grip trials, a yellow target bar indicated the required force level of 20% MVC. The actual force exerted was indicated on line by a red column. Participants were instructed to generate a nonballistic force matching the displayed target bar using a gentle pace without major corrective movements. TMS was applied in a train, starting 900 ms after presentation of the target force bar, so as to overlap with performance of the grip. (*B*) Schematic of EPI-TMS pulse synchronization. On each trial, TMS was applied at 1 of 2 intensities (110% RMT or 70% AMT). TMS pulses were applied every 90 ms during the waiting time between EPI navigator echoes and EPI data readout. Every slice was perturbed equally often throughout a scanning session. TMS pulses were temporally separated from any slice selection gradients or excitation pulses (fat saturation pulse, trigger pulse, RF pulse, slice selection gradient, refocusing gradient, and navigator echo) and EPI data readout-gradients. (*C*) Simple fMRI main effects of grip > no grip, irrespective of TMS. Results of the group random-effects analysis are projected onto a transparent schematic of the MNI template brain and on a transverse section ($z = 54$) of the averaged normalized structural scans of all participants. The height threshold was set at $T > 4$, uncorrected for multiple comparisons across whole brain, and the extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across whole brain. Left-hand grip evoked activity in right M1, extending into adjacent PMd and ventral premotor cortex, plus primary and secondary somatosensory cortex. Additional activity increases were found in superior parietal cortex and intraparietal sulcus, reflecting the visuomotor processing involved in handgrip trials. Note that we focused imaging largely on the dorsal part of the brain. Therefore, any ventral visual activations due to visual aspects of the grip task were outside the field of view. R = right.

task (Ward and Frackowiak 2003). In total, 400 TMS pulses were applied during each scanning session, half of these at the low intensity of 70% AMT. Our stimulation protocol thus conformed to published TMS guidelines (Wassermann 1998).

Prior to scanning, participants were pretrained outside the scanner until comfortable with the grip task without TMS being applied. Once inside the scanner, the TMS coil was carefully located over left PMd (see below) and the task was briefly practiced again until participants performed brief and nonballistic isometric handgrips that approximately reached the required force level on every trial. Some preliminary TMS trains were applied at 110% RMT while the participant was contracting their right-hand at around 20% MVC (i.e., with the opposite hand to that used during the scanning paradigm). This ensured that participants were comfortable with the TMS stimulation protocol and also provided a check that no twitches were induced contralaterally to TMS. No overt muscle responses were either observed in any participant or later reported by participants.

Magnetic Resonance Imaging
MRI was conducted with a 1.5-T Magnetom SONATA system (Siemens Medical Solutions, Erlangen, Germany) using the body-transmit coil and

the single-channel receive CP head array (30 cm diameter). Whole-head T1-weighted anatomical images were acquired after the experiment using a 3D MDEFT sequence with an isotropic resolution of 1 mm^3 (Deichmann et al. 2004). Each scanning session comprised functional T2*-weighted MRI transverse EPIs with blood oxygenation level–dependent (BOLD) contrast, covering the dorsal convexity of the brain down to the thalamus (848 volumes, 20 slices per volume, 64 × 96 matrix, 3 × 3 mm in-plane resolution with 50% oversampling in phase-encoding direction, 2.5 mm slice thickness plus 50% spatial gap between spatially adjacent slices, time repetition = 1800 ms; time echo = 50 ms; α = 90°; echo spacing 500 μs; 2298 Hz/pixel bandwidth; trapezoidal readout gradients with a ramp of 130 μs and a flat top of 240 μs; field of view: 192 × 192 mm; max slew rate 214.9 mT/m/ms). The oversampling in the phase-encoding direction shifted residual Nyquist ghosting (induced by the presence of the MR-compatible TMS coil inside the scanner) outside of the brain image, without compromising the spatial resolution of MR images. Physiological and technical artifacts were monitored online during scanning (Weiskopf et al. 2007). The first 5 volumes were discarded to allow for T$_1$ equilibration effects. Following the main experiment, whole-brain EPI volumes were acquired using the same orientation to facilitate spatial normalization of the spatially restricted functional image series.

Interleaved TMS-fMRI

TMS was implemented using a MagStim Rapid system (The Magstim Company, Dyfed, UK) with a custom-built MR-compatible figure of 8 stimulation coil (2 windings of 10 turns each; inner wing diameter 53 mm, distance between outer coil surface and windings of 2-3 mm [variation due to manufacturing tolerance]; coil inductance, including cable, of 20 μH; and maximal current at 100% stimulator output of ~5 kA). The stimulation unit was housed inside the scanner room in a shielded cabinet from which the stimulation coil cable was fed through a custom filter box (The Magstim Company). Residual RF transmission along the coil cable was further suppressed using ferrite sleeves. The TMS coil was connected to the stimulator in parallel to a high-voltage relay (Magstim ES9486, The Magstim Company). During EPI acquisition, the relay was in closed mode, thereby effectively preventing any residual leakage in current flow from the stimulator. The relay was opened 50 ms prior to TMS pulse discharge and closed again 8 ms after termination of the last TMS pulse of a trial. The relay and TMS were controlled with a unit developed in-house based on a BASIC Stamp 2 microcontroller (Parallax Inc, Rocklin, CA). TMS pulses were applied during the dead time between the EPI navigator echoes and the EPI data readout and separated from RF slice excitation pulses (Gaussian-like symmetric sync, 2560-ms duration, Bestmann, Baudewig, Frahm 2003). Throughout scanning, each slice coincided equally often with TMS pulses to avoid any systematic influences on slice-by-slice variance.

The stimulation site over the left PMd was determined as the scalp point 2 cm anterior and 1 cm medial to the so-called motor "hot spot" for evoking single muscle twitches in the contralateral first dorsal interosseous (Schluter et al. 1998; Johansen-Berg et al. 2002; O'Shea et al., 2007). Inside the scanner, the stimulation coil was placed over the marked location using an MR-compatible, custom-built coil holder, allowing stable positioning of the TMS coil with several degrees of freedom. The coil was oriented tangential to the scalp, at approximately 45° from the mid-line, inducing a biphasic current with an initial anteroposterior-induced direction. Foam-padded cushions were used to restrict head movements.

All visual stimulation, grip-force data acquisition, TMS triggering and intensity regulation, and relay settings were controlled using the toolbox Cogent 2000 (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/cogent) running under Matlab (The Mathworks, Natick, MA). Participants wore earplugs (single number ratings = 36 dB) to reduce acoustic noise from the scanner and the TMS discharge sound.

Behavioral and fMRI Data Analysis

For each grip trial, we measured the movement onset, grip duration, and peak force. Movement "onset" was defined as the latency between the onset of the visual cue indicating a grip trial and the point when grip force exceeded 20% of baseline value. This, however, means that the cortical activity related to grip-force production probably started several tens or hundreds of milliseconds before this level was reached. Moreover, the extended nature of the TMS train (360-ms duration) ensured that it would overlap with grip-related brain states during the grip task. Grip duration was defined between the 2 successive time points at which grip force exceeded the 20% of baseline boundary, and the maximum force was determined during this period. Paired 2-tailed t-tests were used for comparison of these parameters across participants and stimulation conditions.

Following trajectory-based online reconstruction of functional images (Josephs et al. 2000), EPI slices coinciding with TMS pulses were replaced by interpolation between the previous and subsequent acquisition of the same slice (Ruff et al. 2006). Additionally, any remaining slices whose mean signal deviated from the mean signal of the slice time series by more than 1.5 SD were replaced in the same way (only 0.19% in total).

Imaging data were analyzed using Statistical Parametric Mapping (SPM5, http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 6. The first 5 volumes were discarded, and all subsequent volumes were realigned to the first volume in order to correct for interscan movement. Additional unwanted variance related to interactions of head motion and geometric distortions were removed using the "unwarp" toolbox as implemented in SPM5 (Andersson et al. 2001).

The whole-brain EPI images were normalized to a standard EPI template based on the Montreal Neurological Institute (MNI) reference brain in Talairach space, using 4th degree b-spline interpolation, and resampled to $2 \times 2 \times 2$ mm^3 voxels. The resulting transformation parameters were applied to the image volumes of the main experiment. Preprocessing included detrending of time series in each voxel with a linear model of the global signal (Macey et al. 2004); grand mean scaling of 100 over voxels and scans, AR(1) model to account for serial autocorrelations of the data; and spatial smoothing of normalized images with an isotropic 8-mm full-width at half-maximum Gaussian kernel to allow for valid statistical inference according to Gaussian random field theory.

Statistical analysis followed a 2-stage procedure. First, a single-subject fixed-effects model was computed for each participant by multiple regression of the voxelwise time series onto a composite model containing the covariates of interest. Each of the 4 event-related trial types (TMS$_{high}$-grip, TMS$_{low}$-grip, TMS$_{high}$-no grip, TMS$_{low}$-no grip) were modeled as delta functions, with onsets defined as the first TMS pulse, and were included as separate covariates. To account for any additional variance induced by any slight trial-by-trial and or intersubject variations in grip force and grip duration, additional covariates comprising a delta function scaled by the actual peak force and duration exerted for each handgrip were included in the model. All covariates were convolved with a canonical synthetic hemodynamic response function in a general linear model (Friston et al. 1995, 1998) together with a single covariate representing the mean (constant) term over scans. Thus, for each participant, voxelwise parameter estimates for each covariate were calculated, resulting from the weighted least squares fit of the model to the data. The statistical parametric maps (SPMs) of the t statistic resulting from linear contrasts of each covariate were then generated and stored as separate images for each participant.

At a second level of analysis, contrast images for each participant and covariate were entered into a 1-sample t-test for each covariate of interest in a random-effects analysis across participants. Any age-related changes in cortical connectivity (Della-Maggiore et al. 2000; Rowe et al. 2006) or PMd function (Ward and Frackowiak 2003) were considered by including the age of each participant as a covariate in this second-level model. This revealed no age-related influences for the critical findings, namely for the main effect of TMS; the main effect of grip; or their interaction, in any grip-task-related areas for the present dataset. The height threshold for SPM{t} maps was set at $T > 4$, uncorrected for multiple comparisons across the whole brain, and the extent (cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across the whole brain.

The site of stimulation (left PMd) around the left precentral sulcus (x, y, $z = -26$, -14, 62; see Amiez et al. 2006) was a priori defined as a region of interest (ROI) for our fMRI analysis. This region corresponds well to previously reported activity changes at the putative site of stimulation following premotor repetitive TMS (Rowe et al. 2006). Small volume correction for this ROI was applied by using a spherical mask around this location with a diameter of 15 mm. Activity within this ROI is reported at $P < 0.05$, cluster level corrected for multiple comparisons within the spherical ROI.

Analysis of Condition-Dependent Functional Coupling between Stimulated Left PMd and Other Brain Regions

In addition to the standard event-related analysis of fMRI activity by condition described above, we also ran a supplementary analysis testing for any changes in "functional coupling" or effective connectivity between areas as a function of condition (Friston et al. 1997; Gitelman et al. 2003). Specifically, we tested for any regions showing higher functional coupling with the targeted left PMd site when stimulated with high versus low TMS under the active grip versus no-grip conditions. To do so, we used the well-established "psychophysiological interaction" or PPI approach (e.g., Friston et al. 1997; Gitelman et al. 2003; Lee et al. 2003; Egner and Hirsch 2005; Wolbers et al. 2006), a data-driven analysis that makes relatively minimal assumptions. We tested for areas showing stronger functional coupling with left PMd (i.e., higher covariation in residual activity patterns with the time course of left PMd, once the mean condition-specific activations identified by the main SPM analysis, as above, had been factored out from all regions).

Specifically, we extracted the first eigenvariate of the fMRI signal from an 8-mm ROI sphere, centered around the group peak voxel for the main effect of high- versus low-intensity TMS in the basic SPM analysis (x, y, z = −18, −16, 62). We then tested for a PPI interaction, namely a stronger covariation of left PMd with other areas related to the motor task that was specific to high versus low TMS, during the active grip-task versus the no-grip condition. To focus on the task-related areas of interest, we restricted this PPI analysis to those regions which had shown an overall activation for grip versus no grip (inclusive masking at $P < 0.05$, uncorrected, but with the PPI itself thresholded at our conventional threshold of $T > 4$ and extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across the whole brain). At the second-level model, we included the age of each participant as a covariate. Again, no age-related influences for the critical contrast were observed.

For all fMRI analyses (except for the ROI consideration of left PMd), allocation of BOLD signal changes to anatomical regions was made according to anatomical landmarks identified from the mean T1-weighted structural image of all participants with the aid of the atlas of Duvernoy (Duvernoy 1991) as well as by using a 3-dimensional probabilistic computerized cytoarchitectonic atlas (Eickhoff et al. 2005).

Results

Behavioral Results

None of the participants reported any adverse side effects. Participants reached the desired target force level accurately on grip trials (mean ± SD) 20.3 ± 1.5% of MVC, with left-hand grip movements exceeding our criterion for movement onset (>20% of baseline) on average 1.13 ± 0.21 s after the instructional cue and lasting for 1.1 ± 0.5 s. Note that here we used a conservative measure for movement onset (>20% of baseline). Accordingly, neural changes related to grip probably started several tens or hundreds of milliseconds prior to this, and given the extended train of TMS (360-ms duration), some of this train must overlap with neural states associated with the active grip. Force level, movement onset, and movement duration within participants were highly correlated across the 2 TMS stimulation conditions within single subjects (all $r > 0.7$, $P < 0.05$), indicating that performance was comparable across conditions. There were no behavioral differences between the high and low TMS condition in peak force (TMS$_{low}$ mean ± standard error of the mean: 20.3 ± 1.5% MVC, TMS$_{high}$: 20.3 ± 1.6% MVC, t_{11} = −0.24, P = 0.81), grip duration (TMS$_{low}$: 1.12 ± 0.45 s, TMS$_{high}$: 1.09 ± 0.43 s, t_{11} = 1.97, P = 0.08), or grip-onset time (TMS$_{low}$: 1.13 ± 0.16 s, TMS$_{high}$: 1.15 ± 0.15 s, t_{11} = −1.02, P = 0.33). This was important for our design, as our aim was that the motor task here should serve to manipulate the "motor state" of the cortical network when high versus low TMS was applied, rather than high TMS serving to disrupt performance of the motor task (which might then have complicated interpretation of any fMRI changes). Instead, the approach here was to apply TMS as a physiological "perturbation" during 1 motor state (active grip) versus another (rest) and then measure the impact of this on remote but potentially interconnected brain regions with fMRI. No mirror movements of the right-hand occurred during TMS, as confirmed by the right-hand grip-force recordings, in addition to participants' self-report.

Functional Imaging Results: Activations by Condition

Figure 1C shows the main effect of the grip-force motor task as compared with the no-grip condition, when pooling across the 2 TMS intensities: (TMS$_{high}$-grip + TMS$_{low}$-grip) − (TMS$_{high}$-no grip + TMS$_{low}$-no grip). In accord with prior fMRI work with this grip task (Ward and Frackowiak 2003), left-hand grip induced largely right lateralized activity in primary sensorimotor cortex, right PMd, supplementary and cingulate motor area, ventral premotor cortex, secondary somatosensory cortex, and intraparietal sulcus (see Fig. 1C; see also Table 1, grip > no grip). Additional activity increases were observed in left PMd (peak: x, y, z = −30, −8, 60), albeit at a lower threshold of $P < 0.001$, uncorrected for multiple comparisons across the brain.

By contrast, when participants had to withhold movements (i.e., following appearance of an "×" symbol, rather than a leftward arrow, at the start of no-grip trials, see Fig. 1A), activity was significantly larger in left and right dorsolateral prefrontal cortex, as compared with grip trials (see Table 1, no grip > grip), possibly reflecting response inhibition in these no-grip trials.

Turning to the impact of high- versus low-intensity TMS [i.e., (TMS$_{high}$-grip + TMS$_{high}$-no grip) − (TMS$_{low}$-grip + TMS$_{low}$-no grip)], given that the TMS probe was located over left PMd, we considered an a priori ROI for inspection of local TMS effects (see Materials and Methods above). As shown in Figure 2, the ROI analysis of left PMd revealed a significant activity increase for TMS$_{high}$ (vs. TMS$_{low}$), which was attributed to premotor area 6, with 80% probability, by a computerized cytoarchitectonic atlas designed for use with SPM (Eickhoff et al. 2005). This assignment would thus correspond to the PMd (Picard and Strick 2001; Chouinard and Paus 2006; Mayka et al. 2006) (see Table 1). Therefore, TMS at high (vs. low)-intensity elicited a small yet significant relative activity increase at the putative site of stimulation.

Comparing both types of TMS event (high and low together, 50% of which contained grip trials at each TMS intensity) jointly against null events confirmed that presence versus absence of any TMS stimulation activated bilateral auditory cortex, as would be expected, due to the click sound inevitably associated with TMS application (see Supplementary Fig. S1 and Supplementary Table S1). This is consistent with all prior combined TMS neuroimaging studies (e.g., Siebner et al. 1999). However, such considerations are tangential to the hypothesis-driven focus on motor-related regions here and cannot explain our other fMRI findings.

The critical question in the present study was how the high > low TMS manipulation (which activated the target site in left PMd, see Fig. 2) would interact with the current motor state, that is, (TMS$_{high}$-grip − TMS$_{low}$-grip) − (TMS$_{high}$-no grip − TMS$_{low}$-no grip). Critically, we found (Fig. 3A) a significant crossover interaction between motor state and TMS intensity to left PMd, arising in right M1 hand region (BA4a) and the caudal aspect of right PMd (BA6). Figure 3B plots the parameter estimates (proportional to percent signal change) for the peak voxels within each of these areas for each of the 4 main conditions. This reveals that during the grip task that activated the motor network, TMS$_{high}$ (versus TMS$_{low}$) to left PMd led to relative increases in contralateral right PMd and M1. By contrast, when TMS$_{high}$ was applied to left PMd during no-grip rest, this led to relative decreases instead in contralateral motor and premotor activity, as compared with TMS$_{low}$ (see Fig. 3B). Note that due to its 'crossover' nature, this interaction cannot simply be explained away by scaling effects, changes in baseline activity per se; nor can it be explained by age-dependent

Table 1

Imaging results

Anatomical/functional region	MNI coordinates			Peak voxel		Cluster
	x	y	z	Z score	T score	P value
Simple effect grip > no grip (irrespective of TMS)						
R M1/PMd	38	−22	54	4.85	10.29	<0.001
R postcentral sulcus	40	−34	54	5.56	15.37	<0.001
Supplementary motor area/cingulate motor area	2	−4	54	5.28	13.05	<0.001
BL supplementary motor area	0	−4	70	5.07	11.62	<0.001
BL cingulate cortex	0	−22	48	4.42	8.17	<0.001
R posterior superior parietal cortex	8	−78	52	3.79	5.90	0.002
R posterior intraparietal sulcus	24	−74	30	5.02	11.32	<0.001
R ventral premotor cortex	54	6	38	4.35	7.87	<0.001
L superior occipital gyrus	−24	−86	26	3.79	5.91	<0.02
L superior parietal cortex	−26	−60	54	4.33	7.79	<0.001
R thalamus	8	−12	10	3.92	6.31	<0.02
Simple effect no grip > grip (irrespective of TMS)						
L medial frontal gyrus	−32	28	52	4.93	10.75	<0.001
R medial frontal gyrus	32	36	50	4.32	7.74	<0.01
Simple effect TMS$_{high}$ > TMS$_{low}$ (irrespective of grip)						
R inferior temporal gyrus	46	−20	8	3.80	5.93	<0.05
L precentral sulcus	−18	−16	62	3.21	4.40	<0.05*
Simple effect TMS$_{low}$ > TMS$_{high}$ (irrespective of grip)						
R anterior inferior parietal cortex	54	−58	42	4.48	8.44	<0.005
L superior parietal cortex	−26	−46	66	4.35	7.87	<0.05
Interaction (TMS$_{high}$-no grip–TMS$_{low}$no grip) − (TMS$_{high}$-grip–TMS$_{low}$-grip)						
R precentral gyrus	34	−16	58	4.58	8.88	<0.05
R central sulcus	36	−26	62	4.11	6.93	<0.05

Note: Height threshold of $T > 4$, uncorrected for multiple comparisons across whole brain, and extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across whole brain. R, right; BL, bilateral; L, left.
*$P < 0.05$ cluster level corrected for multiple comparisons across whole across small volume of interest in *a priori* defined area of stimulation.
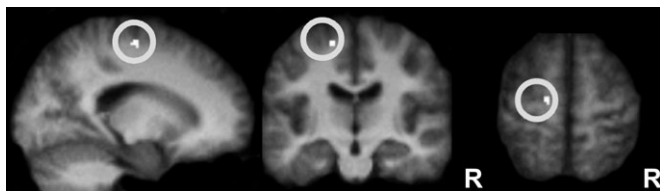
Figure 2. Local effect of TMS stimulation on BOLD signal from left PMd ROI analysis for TMS$_{high}$ > TMS$_{low}$, irrespective of grip, in an a priori defined ROI (sphere of 15 mm diameter, illustrated by the white circle) centered around the site of stimulation in the left PMd (x, y, z: −24, −14, 62). TMS$_{high}$ versus TMS$_{low}$ induced more activity in this area ($P < 0.05$, corrected for multiple comparisons at the cluster level within ROI). Thus, TMS at 110% of RMT evoked a local activity increase, relative to stimulation at the low intensity (70% AMT). Activations are projected onto the average normalized structural scans of all participants. R = right.

changes in PMd function, as we had assessed any such influences in our analysis. The critical interaction remained significant after taking into account these factors.

### Functional Imaging Results: Condition-Specific Functional Coupling with Left PMd

In addition to assessing the overall activations per condition, as analyzed above, we also implemented a PPI analysis for any condition-specific functional coupling between TMS-stimulated left PMd (which provided the "seed" area) and other brain regions, with a particular interest in those related to the grip task. The resulting SPM for the PPI seeded with left PMd (i.e., testing for covariations in any other brain regions, with the residual time course of left PMd) confirmed that TMS-stimulated left PMd showed significantly higher effective

connectivity with contralateral PMd (x, y, z = 36, −14, 56) and contralateral M1 (x, y, z = 36, −24, 64) during grip than for the no-grip condition (see Fig. 3C). Thus, these right hemispheric motor regions showed stronger functional coupling with the stimulated left PMd during voluntary control of left-hand grip than in the no-grip condition.

Discussion

Using short bursts of TMS to left PMd during fMRI, we showed state-dependent causal influences of left PMd on activity levels in remote anatomically interconnected regions (right PMd and M1). Whereas previous work had shown that more extended TMS (for 10 s or more) to PMd can produce some remote activity increases in cortical motor structures distant to the stimulation site (Chouinard et al. 2003; Bestmann et al. 2005), the new finding here was that such remote influences can vary depending on the current motor state. Moreover, they can do so in an event-related fashion, when the motor state changes unpredictably from trial to trial, and TMS is applied in short bursts.

This state dependence arose predominantly for motor structures engaged in the current motor behavior (i.e., within the network activated by the left-hand grip task), rather than being broadly distributed across the entire range of potentially interconnected brain regions (including ipsilateral M1). Although, TMS is known to produce some unspecific secondary activity changes (see Supplementary Fig. S1), as, for example, in auditory cortex (Siebner et al. 1999), the present findings cannot be readily explained away or attributed merely to such undesired stimulation effects. In particular, the crossover
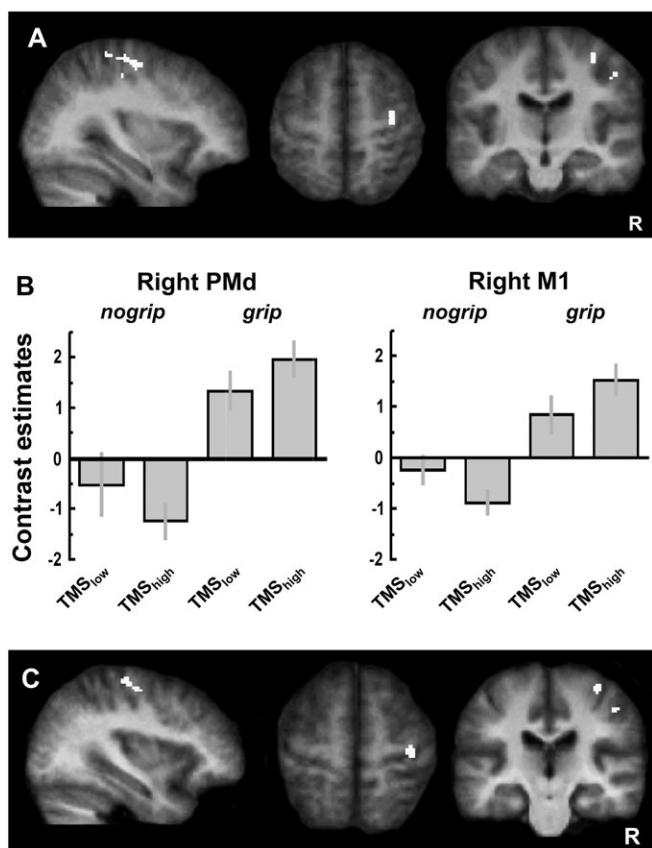
Figure 3. Interaction between TMS intensity and current motor state for BOLD signals. (A) SPMs showing a significant interaction [(TMS$_{high}$-no grip − TMS$_{low}$-no grip) − (TMS$_{high}$-grip−TMS$_{low}$-grip)] in right PMd contralateral to TMS stimulation (x, y, z: 34, −16, 58; assigned to area 6 with a 50% (30–70%) probability, [Eickhoff et al. 2005]) and right M1 (36, −26, 62; assigned to area 4a with a 60% probability). These same regions were also modulated by the grip task (i.e., were active during left-hand grip), see Table 1, Figure 1C. (B) SPM parameter estimates showing that TMS$_{high}$ to left PMd at rest led to a relative activity decrease in contralateral PMd and M1 (BA4a), as compared with TMS$_{low}$. By contrast, when applied during active left-hand grip, TMS$_{high}$ to left PMd now led instead to a relative increase in activity in these regions. This illustrates the state-dependent influence of left PMd TMS on contralateral motor areas, which are implicated in hand movement control. (C) Regions implicated in the grip task that also show changes in functional coupling (as revealed by an independent PPI analysis) with the site of TMS stimulation (left PMd) as a function of event-related condition. A significant context-dependent covariation with left (stimulated) PMd was revealed in 2 similar clusters as for the SPM interaction analysis located in contralateral right PMd (x, y, z = 36, −14, 56) and right M1 (x, y, z = 36, −24, 64). Thus, the functional coupling between left PMd and right PMd and right M1 was stronger for TMS$_{high}$ versus TMS$_{low}$ during grip than during no grip. Results are projected onto the average normalized structural scans of all participants (random-effects analysis, corrected for multiple comparisons across the whole brain at $T > 4$, and extent [cluster] threshold of $P < 0.05$). R = right.

interaction (see Fig. 3B) from our random-effects analysis shows that the effect of TMS$_{high}$ versus TMS$_{low}$ to left PMd on right PMd and right M1 depends on the current motor state. This is also unlikely to be accounted for by any scaling effects, changes in baseline activity per se, or any conceivable artifacts generated by the TMS pulse.

We argue below that the present findings most likely reflect event-related such changes in the effective connectivity of left PMd with contralateral (right) motor areas. Note also that our TMS-fMRI study demonstrated state-dependent interactions not only between left PMd and right M1 (potentially consistent with Koch et al. 2006, 2007 work with conditioning-test double-coil approaches) but also for left PMd influences on

right PMd. This goes beyond what could be achieved with conditioning-test double-coil TMS techniques alone, as those must always rely on an induced electromyography (EMG) response and thus are ultimately confined to considering possible effects on M1.

The contralateral activity decrease resulting from PMd stimulation reported here, under no-grip rest, seems broadly compatible with previously described transcallosal inhibition between M1 in both hemispheres during rest (Ferbert et al. 1992). For example, recent conditioning-test TMS studies show that PMd stimulation at rest can decrease contralateral corticospinal excitability, as measured by the EMG response to a subsequent TMS pulse over M1 of the other hemisphere (Mochizuki, Huang, Rothwell 2004; Mochizuki, Terao, et al. 2004; Baumer et al. 2006; Koch et al. 2006, 2007). The specific latencies at which PMd stimulation influences contralateral M1 excitability favor a direct transcallosal mechanism (Mochizuki et al. 2004a, 2004b; Baumer et al. 2006; Koch et al. 2006, 2007). Whereas callosal fibers are predominantly excitatory (Innocenti 1986; Bloom and Hynd 2005), transcallosal inhibition is thought to be mediated by these excitatory fibers projecting onto γ-aminobutyric acidergic inhibitory neurons (Ferbert et al. 1992).

In our particular case, this would suggest that the contralateral reduction in BOLD fMRI signal observed for TMS$_{high}$ versus TMS$_{low}$ during the no-grip condition may reflect a transcallosally mediated net activity decrease contralateral to the site of stimulation. This finding is similar to the relative contralateral BOLD signal reductions previously observed at rest when using more prolonged TMS to M1 (Bestmann, Baudewig, Siebner, et al. 2003; Bestmann et al. 2004). However, here we demonstrate that this can arise in both right PMd and right M1 due to left PMd TMS, even for short stimulation periods of just a few 100 ms applied in an event-related manner.

By contrast, when participants were performing a single isometric left-hand grip we found instead a relative activity increase (rather than decrease, i.e., the opposite BOLD effect) in PMd and M1 contralateral to the site of stimulation, for TMS$_{high}$ versus TMS$_{low}$ to left PMd. Thus, during a left-hand grip task, left PMd TMS$_{high}$ now led to a boost in activity in contralateral motor regions. Our data thus lend credence to the concept of transcallosal signaling as a dynamic process, such that at a functional level either (or both) interhemispheric transcallosal inhibition and excitation may arise, with the relative dominance of these depending on the current state (Bloom and Hynd 2005). In our case, interhemispheric facilitatory effects of PMd stimulation become more influential during voluntary left-hand action. Facilitatory interneurons in M1/PMd may then have a lowered threshold for being activated by transcallosal pathways, as targeted by our PMd stimulation. Recent findings from conditioning-test double-coil studies corroborate this idea: inhibitory and excitatory interactions from left PMd to contralateral right M1 interact differentially with motor state during movement of the left-hand (Koch et al. 2006) as opposed to rest. Notably, here we found analogous BOLD consequences for right PMd as well as right M1. We suggest that here either left PMd stimulation may have direct effects on both right M1 and PMd or that the stimulation effect may first be transmitted to transcallosally to right PMd and then propagated to right M1. These different possibilities might be differentiated by applying the present new paradigm to patients with right PMd lesions that spare right M1. We should note, however, that relating BOLD signal increases or decreases

to actual inhibitory or excitatory neural activity is a complex issue that cannot be resolved directly by fMRI alone but inevitably requires combination of more invasive recordings of neural activity with fMRI (Logothetis et al. 2001; Logothetis and Wandell 2004). Although the present study found that remote BOLD effects of left PMd TMS could reverse in direction with current motor state, in apparent analogy to recent twin-coil findings (Koch et al. 2006), the relation between BOLD changes and neural firing must always be considered with care. We note also that TMS to left PMd did not alter grip behavior, although we did observe a clear effect on BOLD signal in right PMd and right M1 that reversed during rest. Our design has in fact aimed to avoid any behavioral changes due to TMS, as otherwise those might have further complicated interpretation of the BOLD effects. We therefore conclude that the observed BOLD changes were caused by the physiological TMS intervention here, rather than reflecting differences in behavior.

Our further PPI analysis revealed significantly enhanced functional coupling (covariation in BOLD signal) between left PMd and right PMd/M1, specifically for TMS$_{high}$ versus TMS$_{low}$ in the grip versus no-grip conditions. That is, functional coupling between these regions was strongest when high-intensity TMS was applied to left PMd during the active grip condition, consistent with variations in activity for this stimulated region then propagating in a corresponding manner to right PMd and right M1, in the context of the left-hand grip task. This provides further evidence that a voluntary motor task with the left-hand leads to facilitatory interactions between left PMd and right PMd/M1, rather than just transcallosal inhibition as is typically found at rest. Moreover, our data suggest that the topography of these interregional influences is specific to brain regions engaged in the current task. This implies that while the effects of TMS are not restricted to the site of stimulation, they are also not broadcasted across the entire range of putatively interconnected brain regions, as, for example, ipsilateral M1. In addition, TMS here predominantly influenced currently task-relevant brain regions that are intimately connected to the stimulation site and concerned with the generation of movement (Ehrsson et al. 2000, 2001; Ward et al. 2007), whereas other regions activated during the task such as contralateral, parietal, and somatosensory cortex were not influenced by TMS in a context-dependent way.

The present study demonstrates how concurrent TMS-fMRI can be used to characterize context- or state-dependent interactions between remote but interconnected regions of the motor system. TMS-fMRI may have some advantages over classical TMS approaches relying on EMG measures after stimulation of M1, and of other sites as in conditioning-test approaches, in addition to allowing measurement of activity levels in numerous brain regions concurrently. Quantification of peripheral muscle responses during contraction can become ambiguous because different I-waves (Amassian and Stewart 2003) are recruited during motor behavior than at rest. During voluntary action, it can thus remain unclear whether any changes in peripheral muscle responses (e.g., due to M1 TMS, in the context of a conditioning pulse elsewhere or not) reflect different I-wave recruitment or the stimulation of different intracortical pathways. Whereas, the BOLD signal itself has to be interpreted with some caution (Logothetis and Wandell 2004), it can nevertheless provide a very different, potentially complementary measure of the cortical areas affected during stimulation of primary and secondary motor areas, and of their

functional interplay, without having to rely on peripheral muscle responses or direct M1 stimulation.

We had chosen to target left PMd with TMS here, while varying the requirements for an ipsilateral handgrip task, precisely because we did not seek to disrupt performance via TMS but rather to "inject" a well-controlled input into PMd and then measure its remote causal influences with fMRI, while varying context in an event-related manner. The present approach thus extends previous repetitive TMS (Plewnia et al. 2003; Schambra et al. 2003) or "off-line" TMS studies that were combined with neuroimaging measures (Paus 1999; Chouinard et al. 2003; Siebner et al. 2003; Tegenthoff et al. 2005), which had disclosed some network activity changes following extended stimulation of sensorimotor areas. The more "online" combination of fMRI with interleaved short bursts of TMS in the scanner, as used here, can evidently disclose rapid event-related effects and their spatial topography within the sensorimotor network. Although, we had specifically chosen to stimulate left PMd here because of its suggested dominance for the selection and preparation of hand movements (Schluter et al. 2001; Astafiev et al. 2003; Rushworth et al. 2003; Davare et al. 2006), we would not make any strong claims for hemispheric specialization based on the current data. It may be useful to extend the same approach as introduced here to stimulation of right PMd instead in future work and indeed to stimulation of numerous different motor sites.

Other approaches in fMRI research without TMS (e.g., Worsley et al. 1998; Friston et al. 2003; Roebroeck et al. 2005; Patel et al. 2006) have also emphasized that "functional coupling" or "effective connectivity" between interconnected brain regions may vary with the current state. This may dynamically reconfigure how much influence a given brain region has upon certain others. The present combined TMS-fMRI approach may provide a particularly direct empirical approach to such theoretical issues concerning interregional influences. We thus think our study establishes and corroborates a methodological approach that may be of more general use in studying how motor networks may be reconfigured as a function of the current state (Massimini et al. 2005; Paus 2005).

In conclusion, by obtaining fMRI measures of brain activity during event-related TMS, rather than relying on peripheral muscle activity (as required in the standard conditioning-test TMS method), we were able not only to confirm previous hypotheses of interhemispheric causal influences between (left) PMd and contralateral (right) M1 but also to reveal these for contralateral right PMd. Moreover, we showed that controlling hand movements engages interhemispheric interplay specifically within a network of movement-related cortical motor structures. Finally, we found that the nature of this functional interplay between interconnected motor areas depended critically on the current motor state. For future studies, the current approach might be extended to clinical applications to assess how the observed interplay between interconnected brain regions might be affected in disease states; how this reacts to damage of other parts of the brain, after stroke; and how the observed remote effects of PMd TMS might change in patients with recovery, either spontaneously over time since brain injury or after specific interventions.

Supplementary Material

Supplementary figure S1 and table S1 can be found at: http://www.cercor.oxfordjournals.org/.

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