Hemispheric Asymmetries of the Premotor Cortex are Task Specific as Revealed by Disruptive TMS During Bimanual Versus Unimanual Movements

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The premotor cortex (PMC) is functionally lateralized, such that the left PMC is activated for unimanual movements of either hand, whereas the right PMC is particularly active during complex bimanual movements. Here we ask the question whether the high activation of right PMC in the bimanual context reflects either hemispheric specialization or handedness. Left- and right-handed subjects performed a bimanual antiphase tapping task at different frequencies while transcranial magnetic stimulation (TMS) was used to temporarily disrupt left versus right PMC during complex bimanual movements. For both handedness groups, more disruptions were induced when TMS was applied over the motor nondominant PMC than over the motor dominant PMC or when sham-TMS was used. In a second experiment, right-handers performed complex unimanual tapping with either hand, while TMS was applied to the PMC in both hemispheres. The novel result was that the high susceptibility of the motor nondominant PMC was specific to the bimanual context, indicating that hemispheric asymmetries of the PMC depend on the bimanual versus unimanual nature of the motor task. We hypothesize that asymmetries of PMC involvement in bimanual control reflect interhemispheric interactions, whereby the motor nondominant PMC appears to prevent motor cross talk arising from the dominant hemisphere.

Keywords: bimanual coordination, handedness, hemispheric asymmetry, premotor cortex, transcranial magnetic stimulation

Introduction

Several lines of research have suggested that the dorsal premotor cortex (PMC) is functionally lateralized. The left PMC seems to play an important role in selecting movements in response to arbitrary stimuli and in controlling complex unimanual actions. This was indicated by functional imaging studies that revealed significant left PMC activation regardless of whether movements were performed with the contralateral or ipsilateral hand (Halsband et al. 1993; Rushworth et al. 1998, 2003; Schluter et al. 2001). Interestingly, a strong tendency toward left hemispheric involvement in complex motor control was found for both right- and left-handed subjects, suggesting that this effect reflects rather hemispheric specialization than handedness (Kim et al. 1993; Verstynen et al. 2005). For response selection, the functional imaging findings were further corroborated by inducing "virtual lesions" with transcranial magnetic stimulation (TMS): Disrupting the left PMC resulted in increased reaction times, irrespective of whether the right or the left hand had to move (Schluter et al. 1998; Rushworth et al. 2003; O’Shea et al. 2007). Similarly, patients with left PMC damage are often impaired in response selection and complex motor control of both hands (Halsband et al. 1993; Rushworth et al. 1998; Haaland 2006).

In contrast, the right PMC seems to be particularly involved in controlling complex coordination, as inferred from impaired bimanual movement in patients with right PMC damage (Halsband et al. 1993). Several functional imaging studies have shown that the right PMC is strongly activated during complex bimanual coordination (Sadato et al. 1997; Wenderoth et al. 2004; Aramaki et al. 2006). In most of these studies, subjects performed either rhythmical mirror symmetric in-phase movements (simultaneous activation of homologous muscles) or antiphase movements (simultaneous activation of nonhomologous muscles). The antiphase pattern is generally more challenging, and this has become specifically apparent when the movement frequency is increased, resulting in an involuntary transition from less stable antiphase to more stable in-phase movements (Kelso 1984). The specific role of the right PMC in bimanual movements was addressed previously by Meyer-Lindenberg et al. (2002). Using positron emission tomography, it was shown that dorsal PMC, supplementary motor area (SMA), and primary motor cortices (M1) responded strongly when antiphase movements were performed at high cycling frequencies. Subsequently, disruptive TMS was applied over these key areas in both hemispheres. This seminal study showed that significantly more transitions from antiphase to in-phase were induced by disrupting the right PMC than by disrupting the left PMC, SMA, or M1, particularly, when cycling frequency was high. The authors investigated left- and right-handed individuals; however, the groups were pooled (= 10 in total) for the TMS experiment because behavioral and functional imaging analyses revealed no effect of handedness.

Here, we revisited the potential functional specialization of the right PMC in complex bimanual control to extend previous work with respect to 2 novel aspects: First, we tested whether the functional asymmetry of the PMC reflects rather hemispheric specialization than hand dominance. Second, we further tested whether potential asymmetries are specific to bimanual control when compared with well-matched unimanual movements.

Our main research question was whether the high activation of right PMC in the bimanual context reflects rather hemispheric specialization than hand dominance, which is still unanswered because most studies tested only right-handed subjects (Sadato et al. 1997; Wenderoth et al. 2004; Aramaki et al. 2006). The hemispheric specialization hypothesis describes that the area of one hemisphere is crucially involved in a task irrespective of which effector is used or the handedness of the subject. More specifically, bimanual...
movements might require increased control of spatial attention (Wenderoth et al. 2005; Procacci et al. 2009) that is possibly mediated by right lateralized brain areas, including the PMC (Corbetta et al. 2008; Capotosto et al. 2009). In contrast, the hand dominance hypothesis describes that the handedness of a subject determines which hemisphere is crucially involved in a task. It finds some intuitive support from the fact that, even in a bimanual context, controlling the nondominant hand might require more neural resources than the dominant hand.

In experiment 1, we systematically tested hemispheric asymmetries of the PMC in right-handed as well as left-handed subjects by applying disruptive TMS during bimanual antiphase tapping at different levels of difficulty. Contrary to a previous study (Meyer-Lindenberg et al. 2002), TMS intensities were relatively low such that the potential spread to other motor or frontal areas was minimized, yielding a higher anatomical specificity of the results.

The hemispheric specialization hypothesis will be confirmed if the results in both handedness groups show that TMS over the right PMC induce more disruptions of the bimanual movements than TMS over the left PMC. In contrast, the hand dominance hypothesis will be confirmed if more disruptions are evoked by stimulating the motor nondominant PMC as compared with the motor dominant PMC. Thus, in right-handed subjects, bimanual movements will be more frequently disrupted when TMS is applied to the right (nondominant) hemisphere, whereas, in left-handed subjects, disruptions will be evoked more frequently when stimulating the left (nondominant) hemisphere.

Experiment 1 revealed that TMS over the motor nondominant PMC induced more disruptions than TMS over the motor dominant PMC, irrespective of subject’s handedness.

We then performed a second experiment using a well-matched unimanual coordination task to test whether the observed hemispheric asymmetry simply reflected higher liability of the motor nondominant hemisphere (NDH) to disruptions or whether it was specific to the bimanual context as opposed to the unimanual control condition. Context specificity was confirmed, as experiment 2 revealed that TMS over the motor nondominant PMC did not lead to more disruptions of unimanual movements (even though they were executed with the nondominant hand) than TMS over the motor dominant PMC.

Materials and Methods

Experiment 1

Subjects
In the first experiment, participants were 8 strongly left-handed volunteers (3 males and 5 females, Oldfield score range: [–75, –100], age: 25 ± 0.9 years) and 8 strongly right-handed volunteers (4 males and 4 females, Oldfield score range: [89, 100], age: 23 ± 1.4 years) (Oldfield 1971). None of them suffered from overt sensorimotor deficits, and all were naive with respect to the task. Informed written consent was obtained from all subjects. The informed consent and the study were approved by the local Ethics Committee of Biomedical Research at K.U. Leuven in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Electromyography and Kinematics
Surface electromyography (EMG) was recorded from the right and left abductor pollicis brevis (APB). Disposable Ag–AgCl surface electrodes (Blue sensor SP) were placed over the middle portion of the muscle belly and aligned with the longitudinal axis of the muscles. The EMG signal was sampled at 5000 Hz (CED Power 1401; Cambridge Electronic Design), amplified, band-pass filtered (5–1500 Hz), and stored on a PC for online display and offline analysis.

A 3D tracking system (Optotrak 3020; NDI) was used to register the movement of the fingers. Active markers (infrared-emitting diodes) were placed on the nails of the thumbs, index, middle, and ring fingers, and tapping movements were registered at 100 Hz (Fig. 1).

TMS Preparation
A 70-mm figure-8 coil connected to a Magstim 200 stimulator through the Bistim Module (Magstim) was used. To elicit motor evoked potentials (MEPs), the coil was positioned over the hemisphere contralateral to the target muscle, with the handle pointing backwards and 45° away from the midsagittal line. This produced a posterior–anterior current, which was approximately perpendicular to the central sulcus. First, the hot spot of the APB was determined, which is the optimal coil position for eliciting MEPs with maximal amplitude. Subsequently, the rest motor threshold (RMT) was determined, defined as the minimal stimulus intensity to evoke MEPs of >50 μV in at least 5 of 10 consecutive trials (Rossini et al. 1994). This was done for both the motor NDH and the motor dominant hemisphere (DH).

In this experiment we aimed to disrupt PMC activity. A neuro-navigation system (ANT) was used to confirm that the coil was positioned over subject’s PMC and that the same area was stimulated in all experimental sessions. Therefore, the subject’s skull was coregistered to an individual T1-weighted high-resolution anatomical image (3T Philips Intera repetition time/echo time = 968/4.6 ms, inversion time = 1100 ms, field of view = 250 mm, matrix = 256 × 256, slice thickness = 1.3 mm, 182 slices), and the coil position was displayed online relative to subject’s anatomy. To minimize spread of the TMS pulses to M1, the coil was positioned over the anterior portion of the PMC, approximately 3 cm anteriorly from the APB hot spot as measured at the skull (Munchau et al. 2002) (see Supplementary Figure 1, group mean MNI coordinates for left PMC: x = –33 ± 2, y = 3 ± 2, z = 68 ± 1; right PMC: x = 37 ± 2, y = 7 ± 2, z = 63 ± 1; left APB hot spot: x = –32 ± 2, y = –18 ± 2, z = 73 ± 1; and right APB hot spot: x = 38 ± 2, y = –18 ± 2, z = 69 ± 1, as estimated at the cortical surface). The coil was held tangentially to the scalp with the handle pointing 90° laterally from the nasion-inion line to induce a medially directed current. Coil position and stimulation intensity were chosen such that overflow to M1 was minimized. This was additionally controlled by the EMG signal, which confirmed that PMC stimulation did not evoke MEPs in rest or during active movements. Two pulses at 110% of the RMT were applied with an interstimulus interval (ISI) of 10 ms. We aimed to apply the disruptive TMS pulses within a short time interval, to simulate an
event-like disturbance similar to resetting experiments (de Poel et al. 2007). This specific ISI was selected based on pilot work using our bimanual task and is in agreement with recent findings showing that the 10 ms ISI does not directly influence interhemispheric interactions between PMC and contralateral M1 (as opposed to shorter ISIs; Koch et al. 2007). Two extra control conditions were included in the experiment. First, we applied sham-TMS to the PMC of both hemispheres, which does not result in cortical stimulation but maintains the ancillary aspects of TMS. For sham-TMS the coil was rotated 90° around its longitudinal axis, such that cortical stimulation was absent (Lisanby et al. 2001). Second, we stimulated both left and right M1 at the hot spot of the APB at 110% RMT to test whether the observed results are anatomically specific for the PMC.

**Tapping Task**

Subjects performed a bimanual finger-tapping task using index fingers and thumbs, which were moved in alternation to the beat of a metronome. The in-phase pattern required simultaneous taps of homologous effectors of the left and right hand, such that simultaneous taps of both thumbs (T1_T2) alternated with simultaneous taps of both index fingers (I1_I2), that is, I1_T1–I2_T2–I1_T2–I2_T1 and so on. During antiphase tapping, subjects had to perform nonhomologous finger movements, i.e., tapping the index finger with the thumb of the other hand, that is, I1_T2–I2_T1–I1_T2–I2_T1. As this antiphase tapping task is not part of the natural movement repertoire, subjects practiced this tapping mode unsupervised 1 week prior to the experiment to reduce learning effects during the main experiment. They practiced with eyes closed and were paced by a metronome such that the tapping frequency increased every 2 s by 0.125 Hz. Subjects were also informed that they could only participate in the main experiment when they were able to produce the bimanual antiphase pattern consistently. In the main experiment, subjects had to perform the same task as during training with each trial lasting 20 s. Before TMS stimulation was applied, the spontaneous transition frequency (STFpre) was determined for each subject by increasing the frequency of the metronome (every 2 s by 0.125 Hz) until the subject could not comply with the imposed metronome rhythm any more such that a spontaneous transition from the antiphase to the in-phase coordination pattern occurred. STFpre was determined by averaging the STFs measured in a series of 10 trials. Typically, the transition frequencies varied unsystematically across trials but differed not more than ±0.125 Hz from the mean value. Subsequent TMS stimulation was based on the individual STFpre. In this way, we aimed to further optimize previous protocols, by using a relatively complex bimanual task and adjusting the tapping frequencies to the subject’s personal capabilities.

**Experimental Procedure**

Real- or sham-TMS stimulation was applied to PMC or M1 while subjects performed the antiphase tapping task at increasing frequencies. Analogous to the classical experiment introduced by Kelso (Kelso 1984), subjects were instructed not to resist a transition. It is well documented that antiphase coordination becomes less stable when tapping frequency increases. Therefore, TMS stimulation was delivered at 5 different frequencies, namely STFpre – 0.375 Hz, STFpre – 0.25 Hz, STFpre – 0.125 Hz, STFpre, and STFpre + 0.125 Hz. The 2 highest frequencies were included because small learning effects usually occurred during the experiment. By this procedure, movement difficulty was matched across subjects. Additionally, we aimed to ensure that the antiphase task was performed fast enough to challenge the motor system and to reveal sensitive results with respect to the TMS stimulation. It is commonly observed that antiphase coordination becomes instable at the end of long trials. To minimize this potential confound, the stimulation was randomly provided between 8 and 10 s, therefore the start frequency was adjusted such that stimulation was still given within the targeted frequency interval. For example, when the target frequency was 2 Hz and TMS was applied at 8 s, the start frequency was set to 1.5 Hz (note that the metronome frequency increased every 2 s by 0.125 Hz). Importantly, when TMS was applied within the same target frequency but only after 10 s, the start frequency was 0.125 Hz lower, that is, 1.375 Hz. The motor DH and motor NDH were stimulated alternately, in blocks consisting of 5 PMC stimulations (one for each frequency), 2 sham stimulations over PMC (all 5 tapping frequencies were tested but were randomized over the blocks), and 1 M1 stimulation (only at STFpre – 0.125Hz) in randomized order. M1 stimulation was only applied at one frequency because more trials would lead to a longer duration of the experiment and potential fatigue or decrease of attention to the task. In half of the subjects, TMS stimulation started in the left hemisphere, in the other half in the right hemisphere. In total, there were 5 blocks per hemisphere that were spread over 2 sessions with a separation of maximally 3 days.

**Data Analysis**

Transitions from antiphase to in-phase tapping. EMG recordings and kinematics were analyzed offline to determine 1) whether TMS induced a transition from antiphase to in-phase tapping, 2) the transition time, 3) the corresponding transition frequency, and 4) the laterality index. Finger taps were derived from the kinematic recordings. A peak-picking algorithm yielded the minima in z-direction for each finger as well as the time points for each tap. To determine which fingers moved simultaneously, we determined for each finger tap of one hand the nearest finger tap of the other hand. The transition time for switching from antiphase to in-phase was defined as the time point when the tap of a finger of one hand would be closer to the tap of the homologous finger than to the nonhomologous finger of the other hand (Fig. 2). Based on this criterion, the transition time and frequency were confirmed by visual inspection of the taps and the EMG signal whereby the experimenters were blind to the stimulation condition of the trial and subject’s handedness. When the transition occurred within 2 s after TMS or sham-TMS, it was defined as TMS-induced transition, otherwise it was categorized as a spontaneous transition. (Note that a transition from antiphase to in-phase tapping is a rather dramatic change of behavior that might not occur immediately after the stimulation because it is often preceded by an increasing variability of tapping performance.) For each condition, the percentage TMS-induced transitions were calculated as the ratio of the induced transitions over the number of TMS stimulations administered to the same area.

During the experiment, the transition frequency was also determined for all trials with spontaneous switches (STFexp) and the mean was calculated for each block. This allowed us to quantify whether subjects improved antiphase tapping performance across blocks, as indicated by an increase in STFexp.

In addition, the produced tapping frequency was calculated for each trial to verify whether subjects complied with the rhythm of the metronome. Moreover, we determined whether the switch toward in-phase was caused by the left or the right hand. Therefore, we calculated the laterality index as described by Aramaki et al. (2006). In brief, a transition requires that taps of either the left or right index finger/thumb slow down or speed up relative to the metronome, such that the produced intertap interval changes during the transition. The deviation of the intertap interval of each finger from the metronome intertap interval was calculated for 2 time points before, 1 time point after, and the transition time itself. The laterality index for the index fingers was then calculated as follows:

\[
\text{Laterality index} = \frac{\left( \sum \text{dev}(I_L) \right)^2 - \left( \sum \text{dev}(I_R) \right)^2}{\left( \sum \text{dev}(I_L) \right)^2 + \left( \sum \text{dev}(I_R) \right)^2}.
\]

The value can range between -1 and 1, a positive value indicates that the phase transition is mainly based on fluctuations of the right hand. The laterality index was also calculated for the thumb movements, and the maximal value was subjected to further analyses.

**Statistical Analysis**

Differences between transitions evoked by TMS over the motor dominant versus motor nondominant PMC (D-PMC vs. ND-PMC) were tested with an analysis of variance for repeated measurements (repeated measures ANOVA), with the within-subject factors stim (D-PMC, ND-PMC, and sham) and freq (STFpre – 0.375, STFpre – 0.25, STFpre – 0.125, STFpre, and STFpre + 0.125) and the between subject factor group (left-handers and right-handers).
The differences in percentage induced transitions between M1 and PMC were tested by repeated measures ANOVA with hemi (DH and NDH) and area (PMC and M1).

Differences in RMT were analyzed by repeated measures ANOVA with hemi (DH and NDH) and group (left-handers and right-handers). The difference between left- and right-handers in STF at the start of the experiment (STFpre) was analyzed with an independent \( t \)-test. Changes in STF during the experiment (STFexp) were analyzed with a repeated measures ANOVA with blocks (10 levels) and group (left-handers and right-handers).

The laterality index for transitions induced by TMS of the PMC was analyzed by an ANOVA with the factors hemi (DH and NDH) and group (left-handers and right-handers). In addition, a one-sample \( t \)-test was performed for each handedness group to test whether the laterality index differed significantly from 0.

Level of significance was set to \( \alpha = 0.05 \), and the effect size (ES) is reported for significant results. Post hoc tests were performed by planned comparisons with Bonferroni correction. Group results are reported by mean and standard errors.

Figure 2. Representative examples of finger tapping and phase transition from antiphase to in-phase. (A and B) Upper panel: Taps of the left (gray, upper hand) and right (black, lower hand) thumb and index finger are represented by squares with different texture (thumb = dashed and index finger = dotted symbol) and shown relative to the beep of the metronome, as indicated by the thin vertical lines. Quasi-synchronous taps are connected by vertical lines. The thick black line indicates the TMS stimulation. Lower panel: EMG signal of the left (gray) and right thumb APB (black). (C and D) show the same data as (A) and (B), respectively. The required tapping frequency, as indicated by the metronome, is represented by the stepwise increasing solid line. The actual tapping frequencies are shown separately for the left thumb (\( T_L \), dashed gray), right thumb (\( T_R \), dashed black), left index finger (\( I_L \), dotted gray), and right index finger (\( I_R \), dotted black). The TMS stimulation is marked with the thick black line. (A) and (C) show a spontaneous transition via the left hand approximately 3 s after the TMS stimulation. (A) Initially, the left thumb/right index finger tap alternately with the left index finger/right thumb in an antiphase pattern, that is, dashed squares and dotted squares are nearly synchronous and the EMG shows clearly alternating bursts of the thumbs. However, between the 12th and 14th second, a transition occurs to an in-phase pattern, such that the thumbs (dashed squares) tap nearly synchronous and in alternation with the index fingers (dotted squares). This can be clearly seen in the EMG, which shows a change from alternating to synchronous bursting activity of the left and right APB. (C) shows a clear irregularity in the tapping frequencies of the left thumb and index finger between the 12th and 14th second, such that the left thumb (dashed gray line) is speeding up and the left index finger (dotted gray line) is slowing down. Importantly, the transition occurs approximately 3 s after the TMS stimulation and was classified as a spontaneous transition (i.e. not induced by the TMS pulses). (B) and (D) show a TMS-induced transition as the right thumb and index finger switch to a synchronous tapping mode immediately after TMS was applied.
Experiment 2

Subjects
In the second experiment, 12 strongly right-handed volunteers (3 males and 9 females, Oldfield score range: [83, 100], age: 24.3 ± 1.3 years) participated in the experiment (Oldfield 1971). No subject had overt sensorimotor deficits, and all were naive with respect to the task. Informed written consent was obtained from all subjects. The informed consent and the study were approved by the local Ethics Committee of Biomedical Research at K.U. Leuven in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Tapping Task
Subjects performed a unimanual tapping task using their thumb (T), index (I), middle (M), and ring (R) finger that had a similar characteristic as the bimanual paradigm. Subjects used either their left or their right hand and performed complex finger movements by tapping with the thumb together with the middle finger alternating with a tap of the index together with ring finger, that is, TM–IR–TM–IR. Subjects practiced this unimanual tapping task separately with each hand in an unsupervised manner, 1 week prior to the experiment. The same instructions were given as in experiment 1, and subjects practiced under the same conditions as during the main experiment. In the main experiment, subjects closed their eyes while performing the unimanual tapping task at the rhythm imposed by a metronome. Prior to the main experiment, the STF was determined for each subject and separately for the left hand (STFpreL) and right hand (STFpreR) using the same procedure as in experiment 1. In short, during a series of 10 trials for each hand, the frequency of the metronome increased every 2 s by 0.125 Hz until the subject could not keep up with the metronome frequency and was not able to maintain the coordination pattern anymore.

Experimental Procedure
TMS, EMG, and optotrak preparation were identical to experiment 1. Real- or sham-TMS stimulation (110% RMT) was applied to the motor dominant or motor nondominant PMC that was either ipsilateral or contralateral to the unimanual tapping hand. TMS stimulation was also applied to the motor dominant or motor nondominant M1 ipsilateral to the unimanual tapping hand. (Stimulation of M1 during contralateral movements was not tested because it induces a strong muscle twitch, which results in nearly 100% disruptions due to the peripheral disturbance.) Again, subjects were instructed not to resist a disruption. During the main experiment, subjects tapped at a constant frequency (STFpreL-0.125Hz and STFpreR-0.125Hz), which ensured that the task was challenging but could be maintained for a whole trial lasting 11 s. Moreover, this procedure allowed us to match difficulty between the left and the right limb task.

Stimulation was given randomly between 5 and 10 s following trial initiation. The motor dominant and motor NDH were stimulated alternately, in blocks consisting of 4 PMC stimulations (2 for contralateral and 2 for the ipsilateral condition), 2 sham stimulations over PMC, and 2 ipsilateral M1 stimulations in randomized order. In half of the subjects TMS stimulation started in the left hemisphere, in the other half in the right hemisphere.

Data Analysis
EMG recordings and kinematics were analyzed to determine whether TMS induced a disruption in the complex unimanual pattern, in the same way as in experiment 1. The transition time for switching from the complex pattern to another pattern or phase wandering was defined as the time point when the tap of a finger (i.e., thumb) would be closer to the tap of the alternating (i.e., index/ring) finger than to the synchronized (i.e., middle) finger (Fig. 3).

Statistical Analysis
Differences in STFpre between the right and left hand were analyzed with a paired t-test. A paired t-test was also used to analyze the differences in RMT. The percentage of TMS-induced transitions was subjected to a repeated measures ANOVA, with the within-subject factors hemi (DH and NDH) and area (PMC-ipsi, PMC-contra, and PMC-contral).

Results

Experiment 1

Stimulation Intensities and Tapping Frequencies
Mean RMT was significantly higher in the right-handed group (48 ± 1.5% of the maximal stimulator output) than in the left-handed group (42.9 ± 1.0%) (F1,14 = 5.59; P < 0.05; ES = 0.285) because 3 right-handed subjects had a relative high RMT (>50% maximal stimulator output). Importantly, no significant differences were found between the RMT for motor NDH (46.4 ± 1.4%) and motor DH (44.5 ± 1.6%) (F1,14 = 2.37; P = 0.146). Stimulation was given at 110% RMT, corresponding to 56 ± 2.1% for right-handers and 50 ± 1.6% for left-handers.

The mean STF (STFpre) was 1.99 Hz (±0.05). STFpre did not differ significantly between right-handers (2.06 ± 0.14 Hz) and left-handers (1.92 ± 0.11 Hz) (F1,14 = 0.189; P = 0.67). The STFexp increased over the blocks of the experiment (F5,126 = 3.72; P < 0.05; ES = 0.199), and post hoc tests revealed significant differences between the 1st block (1.87 Hz) and the 7th (2.09 Hz), 8th (2.05 Hz) and 10th (2.09 Hz) block, suggesting that subjects had to get used to performing the task in the specific experimental setting during the first block. Accordingly, no significant changes of STFexp were observed after the second block.

Percentage TMS-Induced Transitions
Our main result is that TMS induced more disruptions when applied over the motor nondominant PMC than over the motor dominant PMC or when sham-TMS was used.

Accordingly, the ANOVA revealed a main effect of stim (F2,28 = 15.9; P < 0.01; ES = 0.532) such that TMS provoked significantly more transitions when applied over the ND-PMC (40 ± 3.3%) than over the D-PMC (31 ± 3.0%) (post hoc test P < 0.05) or sham (21 ± 2.2%) (post hoc test P < 0.05) (Fig. 4). This effect did not differ between left- and right-handed subjects as indicated by the absence of a significant group effect (F1,28 = 0.08; P = 0.786) or a significant stim × group interaction (F2,28 = 0.278; P = 0.760). As no strong inference can be drawn on the basis of nonsignificant effects, an additional ANOVA was calculated after data were reordered by stimulation side (i.e., left vs. right PMC). This analysis confirmed that in

Figure 3. Individual example of finger tapping with the left hand, the pattern is disrupted by TMS over M1. Same conventions as in Figure 2.
right-handers more transitions were induced by applying TMS to the right hemisphere, whereas left-handers exhibited more transitions when stimulated over the left hemisphere (see Supplementary Material for statistical details). In summary, these data indicate that more disruptions were found when TMS was applied over the hemisphere controlling the non-dominant hand, irrespective of hand dominance.

The percentage of TMS-induced transitions increased significantly when TMS was applied at higher movement frequencies (Fig. 5) (freq main effect: $F_{4,56} = 59.4; \ P < 0.01; \ ES = 0.804$). Moreover, there was a significant freq $\times$ stim interaction ($F_{4,11} = 3.8; \ P < 0.01; \ ES = 0.215$) as increasingly more transitions were induced by real-TMS than by sham-TMS when applied at high movement frequencies. This is confirmed by post hoc tests ($P < 0.05$) that showed significant differences between sham-TMS and TMS of either the ND-PMC and D-PMC at the highest frequency (STFpre + 0.25).

The ANOVA hemi $\times$ area revealed that, stimulation of M1 (78 $\pm$ 9%) induced significantly (main effect area: $F_{1,14} = 79.2; \ P < 0.05; \ ES = 0.850$) more transitions than stimulation of PMC (30 $\pm$ 4%) at the same tapping frequency (STFpre - 0.25), but, importantly, no differences were found between hemispheres or groups (hemi: $F_{1,14} = 0.816; \ P = 3.82$; group: $F_{1,14} = 1.76; \ P = 0.206$).

### Laterality Index

Mean values of the laterality index were calculated for transitions provoked by TMS of the PMC. These data were highly variable within as well as between subjects, but the repeated measures ANOVA revealed that the laterality index was positive for left-handers (0.12 $\pm$ 0.14) and negative for right-handers (-0.27 $\pm$ 0.08). A one-sample $t$-test for each handedness group showed that the laterality index for right-handers differed significantly from zero ($t = -3.28; \ P < 0.05$), whereas significance was not reached for left-handers ($t = 0.93; \ P = 0.368$). This suggests that, for both groups, the non-dominant hand tended to be more susceptible to transitions from antiphase to in-phase than the dominant hand independent of which hemisphere was stimulated. However, this effect was more outspoken for right-handed subjects. Exemplary data of a representative subject are shown in Figure 2. As can be seen, the dominant fingers maintain the imposed tapping frequency, while the nondominant fingers change the tapping speed, which causes the phase transition.

### Experiment 2

#### Stimulation Intensities and Tapping Frequencies

RMT was very similar in the left hemisphere (53.2%) and right hemisphere (53.9%) ($t = -0.63; \ P = 0.55$). STFpre was significantly higher for the right hand (STFpreR: 1.71 $\pm$ 0.06 Hz) than for the left hand (STFpreL: 1.64 $\pm$ 0.07 Hz) ($t = -3.9; \ P < 0.05$).

#### Percentage TMS-Induced Transitions

For complex unimanual movements, TMS induced significantly more disruptions than sham stimulation (real-TMS: 22.6 $\pm$ 0.4%, sham: 7.3 $\pm$ 0.1%; stimulation main effect $F_{1,11} = 16; \ P < 0.05; \ ES = 0.602$). Furthermore, more disruptions were evoked when TMS was applied over ipsilateral M1 than over contralateral or ipsilateral PMC as indicated by a main effect of area ($F_{2,22} = 8.2; \ P < 0.01; \ ES = 0.428$) and confirmed by post hoc test ($P < 0.05$) (Fig. 6). Importantly, TMS over the motor nondominant PMC revealed slightly less disruptions than TMS over the motor dominant PMC during ipsilateral movements. However, this was not significant as indicated by an additional planned comparison testing the difference between DH and NDH stimulation over the PMC ($t = 1.20; \ P = 0.25$).

### Discussion

The goal of this experiment was to evaluate hemispheric asymmetries of PMC during complex bimanual control. In particular, we asked the question whether differences between the left and right PMC depend on hemispheric specialization (i.e., more behavioral disruptions when the right than the left
hemisphere is stimulated with TMS irrespective of subjects’ handedness) or hand dominance (i.e., more disruptions when the NDH as compared with the DH is stimulated). A novel result was that TMS induced more transitions from bimanual antiphasic to in-phase when applied over the “motor nondominant” PMC than over the motor dominant PMC, in both right-handed and left-handed subjects. This finding strongly supports the hand dominance hypothesis, that is, the motor NDH is more susceptible to TMS disruption in both handedness groups. Furthermore, the higher susceptibility of the motor nondominant PMC to TMS was only found for complex bimanual but not for complex unimanual coordination performance, indicating that hemispheric asymmetries of PMC are task dependend.

**Reliability of Behavioral Performance Measurement**

In this experiment we aimed to evoke a radical change in behavior, that is, a transition from antiphasic to in-phase movements by stimulating the PMC at moderate intensities (110% RMT). Therefore, we ensured that subjects performed the antiphasic tapping task at medium to high levels of difficulty. Subjects practiced the bimanual antiphasic tapping task a week prior to testing such that they were able to perform the pattern stably at the pre-session when the individual STF was determined (STFpre). Despite this prior training, we observed an increase of the transition frequency during the experiment (STFexp) indicating further task improvement. To counter this effect, the highest tapping frequency, tested during the experiment, was located slightly above the average STF of the pre-session. Thus, the tapping task provided a sensitive measurement of TMS-induced effects over the PMC at later stages of the experiment such that we were able to induce transitions, suggesting that task performance was still fragile enough to be disrupted. However, using the highest tapping frequency puts particular emphasis on the sham-TMS condition. At high tapping frequencies, subjects approach the limit of their antiphasic tapping capabilities such that even small external distractions, like the click of the coil, can induce a transition to in-phase tapping. The sham-TMS condition controlled for these unspecific effects. Subjects were not informed that real- and sham-TMS were intermixed, and the majority of them reported during the de-briefing that they were unaware that different TMS protocols were used because they strongly focused on the tapping tasks. Importantly, real-TMS induced significantly more transitions than sham-TMS, indicating that the quantified changes in behavior resulted rather from disrupting neural activity of the PMC than other unspecific effects.

**Anatomical Specificity**

Our main anatomical target was the dorsal PMC. Additionally, we aimed to avoid activation spread to M1. Therefore, stimulation intensity was much lower than in previous work (Meyer-Lindenberg et al. 2002) and the coil was positioned approximately 3 cm anteriorly of the APB hot spot, oriented such that the induced current flow proceeded from lateral to medial direction. This procedure reduced the probability of stimulating M1 or prefrontal areas. The question emerges whether TMS might have affected SMA. Previous research has indicated that SMA is an important structure for rhythmical bimanual control, and its temporal disruption can result in involuntary transitions from antiphasic to in-phase movements, similar to the results reported here (Meyer-Lindenberg et al. 2002; Serrien et al. 2002; Steyvers et al. 2003). Even though we cannot firmly exclude that SMA was affected by TMS, it seems rather unlikely because TMS applied with a figure-8 coil at 120% RMT induces an electric field high enough to depolarize neurons (i.e., larger than 100 V/m) only within a distance of 1.5 cm underneath the coil (Roth et al. 2007). Moreover, the field strength was shown to be reduced by half at a distance of 2 cm from the coil midpoint along the medial-lateral or posterior-anterior orientation (Roth et al. 2007). Given that we stimulated at an even lower intensity (110% RMT) and that the coil was positioned approximately 3 cm laterally from the head’s midline, it is unlikely that we induced strong effects in SMA. Therefore, we believe that the observed effects concerning hemispheric asymmetries were predominantly mediated by the lateral aspect of the dorsal PMC. As an additional control condition for the anatomical specificity of our stimulation, TMS was also applied over M1. In contrast with Meyer-Lindenberg et al. (2002), M1 stimulation resulted in a large number of transitions, most likely because TMS provoked a simultaneous muscle twitch in thumb and index finger of the contralateral hand that acted like a resetting signal in our slightly more difficult task than used previously (Meyer-Lindenberg et al. 2002). As a result, subjects were not able to maintain the antiphasic pattern and switched to the in-phase pattern. Similar results were also found by Chen et al. (2005) showing that TMS over M1 provoked a phase transition from antiphasic to in-phase movements. Importantly, in our study, the effect of M1 stimulation on transitions did not differ between hemispheres or groups. Thus, it is unlikely that the hemispheric asymmetries reported for PMC stimulation arose from spread to M1. In summary, we believe that the applied methodology was suitable to test the causal relationship between the dorsal PMC...
and complex bimanual control. Our results are largely consistent with previous findings. However, contrary to the study of Meyer-Lindenberg et al. (2002), our data indicate that handedness predicts functional asymmetries of PMC during bimanual control.

**Potential Physiological Mechanisms Underlying Hemispheric Asymmetries of PMC Lateralization During Bimanual Movements**

Experiment 1 suggested that the PMC controlling the nondominant hand limits performance during a nonmirror symmetric bimanual coordination task. This was inferred not only from the occurrence of TMS-induced transitions that were most frequent when the PMC of the nondominant hand was stimulated but also by the laterality index suggesting that most transitions occurred via the nondominant hand, irrespective of the stimulation site. Moreover, the findings from experiment 2 confirmed that this functional lateralization is specific to bimanual movements, whereas complex unimanual control tended to reveal the opposite lateralization pattern in a control group of right-handed subjects. This indicates that functional asymmetries of the PMC depend on the bimanual versus unimanual nature of the motor task.

This finding can be interpreted within the broader framework of experimental and modeling studies on bimanual coordination and of neurophysiological studies identifying PMC as a key area mediating interhemispheric inhibition and facilitation.

Previous work on bimanual coordination has suggested that cortical cross talk, that is, the unwanted overflow of motor related information from one hemisphere to the other, might be the main mechanism causing involuntary transitions from antiphase to in-phase coordination patterns (Cardoso de et al. 2001; Cardoso de 2002). Moreover, it was argued that the amount of cross talk is asymmetric, such that the motor DH exerts a stronger influence on the NDH than vice versa (Peper et al. 2004; de Poel et al. 2006). Currently, interhemispheric interactions represent a candidate mechanism for mediating cross talk during motor actions (Carson 2005) and, particularly, the dorsal PMC was shown to have strong reciprocal connections with ipsilateral (Dum and Strick 2005) as well as contralateral motor areas, most prominently with medial and lateral dorsal PMC and M1 (Marconi et al. 2003; Boussaoud et al. 2005). Recently, it was shown that functional connectivity between the left dorsal PMC to the right dorsal PMC and M1 is state dependent, such that its influence on the other hemisphere is modulated as a function of motor task demands (Bestmann et al. 2008). Moreover, several studies performed in right-handed subjects have indicated that the PMC plays an important role in regulating interhemispheric communication (Giovannelli et al. 2006; Koch et al. 2006). Giovannelli et al. (2006) applied low-frequency repetitive TMS over the right PMC that caused an increase in mirror EMG activity in the right hand when the left hand executed unimanual movements. This suggests that the PMC is involved in a “nonmirror transformation” network, probably via inhibitory projections to the contralateral M1. Koch et al. (2006) applied a double-pulse TMS paradigm that is a more direct method to investigate functional connectivity between different areas. They confirmed that the interaction between the left PMC and the right M1 is mainly inhibitory during the preparation of unimanual movements with the right hand. The same results were found for the interhemispheric interaction between the right PMC and the left M1 when the left hand was preparing to move. However, additionally they demonstrated a facilitatory influence that was only found from the left PMC to right M1 when left-handed movements were prepared. Based on the above results it is tempting to speculate that the PMC plays an important role in regulating neural cross talk, such that interhemispheric interactions are dynamically modulated, depending on whether bimanual or unimanual movements are performed (Duque et al. 2005). As most transitions occurred via the nondominant hand irrespective of the stimulation condition and more transitions were induced by TMS over the nondominant PMC, it appears that the nondominant PMC plays a crucial role in controlling which input reaches nondominant M1 during bimanual movements. Important pathways that most likely interact during this process are either direct PMC-M1 projections within the NDH or transcallosal connections originating from PMC or M1 of the DH. Moreover, several studies have indicated that transcallosal interactions are asymmetric such that, in right-handers, the dominant left hemisphere exerts both inhibitory and facilitatory influences on the nondominant right hemisphere, whereas the nondominant right hemisphere has only inhibitory influences on the dominant left hemisphere (Koch et al. 2006).

Thus, focusing on our right-handed subjects first, we hypothesize that during antiphase bimanual movements, the PMC of either hemisphere inhibits interfering neural cross talk deriving from the other hemisphere. Note that this is necessary to prevent a transition to the mirror symmetric in-phase pattern. Accordingly, when the PMC of either hemisphere was temporarily disrupted by TMS, inhibitory mechanisms might have been impaired, resulting in increased cross talk that leads to a transition to symmetric in-phase movements. Importantly, in right-handers, the dominant left hemisphere exerts task-specific facilitatory influences on the nondominant right hemisphere (but not vice versa) (Koch et al. 2006). We hypothesize that the nondominant right PMC might play a crucial role in interacting with this transcallosal input because disrupting nondominant PMC with TMS produced frequent transitions. For example, it is possible that non-dominant M1 relies on strong input from ipsilateral PMC to counteract influences from the other hemisphere that would facilitate mirror-symmetric movements. This is in line with previous research suggesting that right PMC plays an important role in preventing unwanted mirror activity (Giovannelli et al. 2006). Indirectly these results are also in agreement with previous imaging studies reporting that the right PMC showed more activation during antiphase than during in-phase bimanual movement, whereas the left PMC was equally activated during both tasks (Sadato et al. 1997; Aramaki et al. 2006). Interestingly, transition-related activation was also only seen in the right PMC (Aramaki et al. 2006).

An alternative explanation for the PMC lateralization could be that the nondominant PMC is just more easily disrupted during complex task performance. However, the unimanual results of experiment 2 in right-handed subjects contradicted this explanation. Note that the experimental procedure and data analysis were closely matched to the bimanual tapping task. However, we found no asymmetries between hemispheres for unimanual tapping. As relative difficulty was matched between the left and right hand, this result does not reflect differential dexterity but rather differential control.
mechanisms. Only for unimanual movements, there was a tendency that more disruptions were induced by TMS over the left than the right PMC. Even though this result did not reach significance it is consistent with the functional imaging literature describing that the left PMC is strongly involved in unimanual movements irrespective of whether they are executed with the left or right hand (Haaland et al. 2004; Serrien et al. 2006; Pollok et al. 2008) and the model of interhemispheric interactions based on the results of Koch et al. (2006).

The aforementioned results on interhemispheric communication were revealed in right-handed subjects only. However, it was argued previously that asymmetries in interhemispheric inhibition between the primary motor cortices might depend on handedness, such that inhibition is stronger from the motor DH to the motor NDH (Baumer et al. 2007). Accordingly, behavioral experiments testing bimanual in- and antiphasic movements in right-handers and left-handers have shown that the motor DH has a stronger influence on the motor NDH than vice versa. Our data suggest that hand dominance might be reflected in a similar way by transcallosal connections of the PMC. Thus, our model predicts that, interhemispheric interactions should exhibit the opposite lateralization in left-handed subjects, such that facilitatory effects between hemispheres should be most prominent from the right to the left hemisphere.

**Summary and Conclusions**

The present study demonstrated that TMS over the motor nondominant PMC leads to more frequent disruptions of bimanual antiphasic movements than TMS over the motor dominant PMC in both left- and right-handed subjects. A second experiment indicated that this functional asymmetry was specific to the bimanual context. We suggest that this result reflects asymmetries of interhemispheric interactions, whereby the motor dominant PMC exerts a strong facilitatory influence on the other hemisphere such that the motor nondominant PMC plays an important role in preventing mirror symmetric movements.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

**Funding**

Fund For Scientific Research-Flanders (FWO-Flanders); K.U. Leuven Research Council (CREA/07/037) (G.0577.06, G.0749.09)

**Notes**

*Conflict of Interest*: None declared.

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