Distinct Temporospatial Interhemispheric Interactions in the Human Primary and Premotor Cortex during Movement Preparation

The preparation of a voluntary unimanual action requires sequential processing in bihemispheric motor areas. In both animals and humans, activity in the dorsal premotor cortex (PMd) ipsilateral to the moving hand has been demonstrated to precede ipsilateral primary motor cortex (M1) activity. We investigated with double-pulse transcranial magnetic stimulation how right-hemispheric motor areas (rM1, rPMd) modulate left M1 (IM1) during the preparatory period of a finger movement with the dominant right hand. We tested the hypothesis that the influence of higher order motor areas such as rPMd on IM1 (rPMd-IM1) precedes interhemispheric interactions between homologue primary motor areas (rM1-IM1). rPMd-IM1 showed modulation in the early and late phase of movement preparation, whereas the intrinsic state of inhibition between rM1-IM1 was only modulated in the late phase. The present results complement existing hierarchical models of cortical movement control by demonstrating temporospatially distinct involvement of interhemispheric interactions from PMd and M1 during movement preparation.

Keywords: interhemispheric interaction, movement preparation, premotor cortex, primary motor cortex, transcranial magnetic stimulation

Introduction

Task-related modulation of connectivity is regarded as a core principle governing brain function at single-cell, local network, and systems level (Swinnen, 2002; Fries, 2005). At the systems level of the human motor cortex, several intra- and interhemispheric interactions between motor areas have been identified in the control of unimanual and bimanual motor behavior in humans (Andres et al. 1999; Cardoso de Oliveira et al. 2001; Gerloff and Andres 2002; Meyer-Lindenberg et al. 2002; Swinnen 2002; Swinnen and Wenderoth 2004; Serrien et al. 2006; Greffkes, Eickhoff, et al. 2008; Serrien 2008). Topography and timing of bihemispheric motor activity during the initiation of a simple voluntary movement have been well characterized in animal and human models. The primary (M1) and the dorsal premotor cortex (PMd) are considered key motor structures in the control of unimanual and bimanual movements (Godschalk et al. 1981; Tanji et al. 1988). Single-cell recordings in animals and imaging studies in humans, which revealed bihemispheric activity, are suggestive of interhemispheric interplay, but do not provide information on event-related interhemispheric connectivity changes. Hence, it remains an open question whether the dynamics of interhemispheric interactions originating in ipsilateral PMd and M1 correspond to the temporospatial patterns observed in monkey neurons. Using double-pulse transcranial magnetic stimulation (dpTMS), we tested the hypothesis that the pattern of movement-related interhemispheric interactions corresponds to the sequential pattern of activity in the ipsilateral monkey motor cortex (Godschalk et al. 1981; Weinrich and Wise 1982; Weinrich et al. 1984; Tanji et al. 1988). dpTMS has proven a powerful tool in investigating directional modulation of facilitatory and inhibitory circuits (effective connectivity) with excellent time resolution (Hallett 2000; Pascual-Leone et al. 2000; Walsh and Cowey 2000; Reis et al. 2008). Moreover, the use of dpTMS is well established for the event-related assessment of interhemispheric interactions between premotor and motor cortical areas (Murase et al. 2004; Duque, Hummel, et al. 2005; Duque, Mazzucchelli, et al. 2005; Koch et al. 2006; Koch et al. 2007; O'Shea, Sebastián, et al. 2007; Ni et al. 2008; Perez and Cohen 2008). However, little is known about whether interhemispheric interactions in secondary and primary motor areas show differential temporal modulations reflecting hierarchical models of motor control. We thus directly compared the modulatory patterns of interhemispheric PMd-M1 versus M1-M1 modulation during the whole preparatory period of a simple, unimanual movement, which has not been elucidated so far. PMd-M1 modulation has been
investigated during action selection (Koch et al. 2006; O’Shea, Sebastian, et al. 2007); however, PMd is also involved during the preparatory period of simple voluntary movements that do not require decision making (Ball et al. 1999; Huang et al. 2004; Greffkes, Eickhoff, et al. 2008). Hence, we opted for a simple motor reaction time (RT) task because we wanted to reveal sequential interhemispheric interactions in primary and premotor regions without the involvement of decision processes that besides premotor regions also engage numerous additional frontal areas (Cavina-Pratesi et al. 2006).

Materials and Methods

The study consists of a “main experiment,” an “intensity experiment,” and a “control experiment.” The scope of the main experiment was to investigate 1) the “timing” and 2) motor cortical “topography” of interhemispheric interactions during the preparatory period of a movement with the dominant right hand. In the intensity experiment, we investigated different TMS intensities over PMd, because the effect of stimulation intensity over secondary motor areas is rarely addressed in this type of movement-related dpTMS experiments (Koch et al. 2006). In the control experiment, we investigated early components of movement preparation before and after an instruction to move the hand to confirm the rapid modulatory effect of rPMd-lM1 in the main experiment.

Subjects

Ten healthy right-handed subjects (8 females; mean age: 23.5 ± 0.5 years, age range 22–27 years) participated in the study. According to the Edinburgh Inventory of Handedness (Oldfield 1971) all subjects were right-handed (mean score: 0.97 ± 0.02). They were naive to the experimental purpose of the study. None of the subjects had ever engaged in regular piano playing or any other regular musical training involving the hands. None of the subjects had a history of serious medical, neurological, or psychiatric illnesses, as probed by a standardized questionnaire. All subjects gave written informed consent to participate in the experiment according to The Code of Ethics of the World Medical Association (Declaration of Helsinki; http://www.wma.net/e/policy/b3.htm) prior to all experimental procedures. The study was approved by the local ethics committee at the University of Hamburg and the University of Tuebingen. All experiments were performed at the Department of Neurology, Brain Imaging and Neurostimulation Laboratory, University Medical Center Hamburg-Eppendorf, Germany.

Interhemispheric Interactions during Movement Preparation—Main Experiment

Behavioral Paradigm

Interhemispheric interactions were assessed during the preparatory period of a simple right, unimanual movement. Subjects were seated comfortably in an armchair with hands and forearms resting on a table. They were instructed in a standardized fashion to perform a simple RT task. They fixated a crosshair presented centrally on a computer screen 50 cm in front of the table. As soon as the crosshair turned to “Go!,” subjects performed as quickly as possible a brisk right index finger abduction (Fig. 1A). The intertrial interval varied between 6 and 8 s. Before the measurements of interhemispheric interactions, 30 trials were recorded in order to determine the RT. RT was defined as the time between the Go-signal and EMG onset (Fig. 1A). The first 5 trials

Figure 1. (A) Behavioral paradigm and experimental setup. Double-pulse TMS was applied in a simple RT task before brisk abduction of the right index finger in order to evaluate interhemispheric interactions during movement preparation from the right (gray coil, conditioning stimulus [CS]) to the left (black coil, test stimulus [TS]) hemisphere. Subjects were instructed to abduct the right index finger as fast as possible after appearance of “GO!”. The EMG signal from the FDI was recorded and used to determine the individual RT. (B) Stimulation locations. Interhemispheric interactions during movement preparation were investigated by delivering a CS (gray coil) over the right hemisphere 10 ms preceding a TS over the left hemisphere (TS, black coil). The TS was applied over the representation of the FDI in the primary motor cortex. In order to assess interhemispheric interactions between homologue areas of the primary motor cortex (rM1-lM1), the CS was given over the right primary motor representation of the FDI. For evaluation of interhemispheric interactions between the dorsal premotor cortex and the contralateral primary motor cortex (rPMd-lM1), the CS-coil was moved 2 cm anterior and 0.5 cm medial from the right primary motor representation of the FDI. (C) Stimulation timing. MEPS were recorded from the right FDI contralateral to the TS. As soon as the instruction was given by determining the ratio of conditioned MEP peak-to-peak amplitudes in the double-pulse trials [CS+TS] by unconditioned MEPS in the single-pulse trials (only TS): CS+TS/TS. Stimulation intensities were determined before assessment of interhemispheric interactions. Stimulation intensity of TS was adjusted so as to obtain unconditioned 1-mV MEPS at 50% (t2) of the individual RT (unconditioned MEP: thin line in the inset on top). Stimulation intensity of CS was adjusted so as to obtain 1-mV MEPS at 50–70% of unconditioned value) at t2 in the conditioned trials (rM1-lM1, conditioned MEP: bold line in the inset on top). In order to determine the time course of interhemispheric interactions during movement preparation, 4 different time points at which TMS was delivered after the Go-signal were chosen: 20% (t1), 50% (t2), 80% (t3) and 95% (t4) of the RT.
served as practice trials in order to familiarize subjects with the task, environment, and procedures. They were discarded from RT calculation. The last 25 trials were used to characterize the individual mean reaction time (iRT) of each subject. Outliers (±2 standard deviations) were discarded from the calculation of the iRT that was used to define the time points for the measurement of interhemispheric interactions as reported below and employed in several previous studies (Murase et al. 2004; Duque, Hummel, et al. 2005; Harris-Love et al. 2007; Hummel et al. 2009).

Electrophysiological Recordings
Pairs of Ag/AgCl surface electrodes were used in a belly-to-tendon montage for surface EMG recordings with the active electrode oriented parallel to the vertical axis of the muscle and centered over the muscle belly of the right and left first dorsal interosseous muscle (FDI) and the reference electrode over the proximal interphalangeal joint of the respective left or right index finger. The EMG signal was recorded from both FDI comitantly with 2 separate channels, digitized (sampling rate: 5 kHz, bandpass filter: 50 Hz to 1 kHz, CED 1902 amplifier; Cambridge Electronics, Cambridge, UK), and stored on a personal computer for offline analysis using a data analysis script written in Signal Software (Cambridge Electronics). EMG recordings served as the assessment of RT at the beginning of the experiment and motor-evoked potentials (MEPs) during the measurement of interhemispheric interactions.

Assessment of Interhemispheric Interactions
Interhemispheric interactions were assessed between the right and left primary motor representation of the FDI (rM1-lM1; Fig. 1B) and between the right dorsal premotor cortex and the left M1 of the FDI (rPMd-lM1; Fig. 1B) using dpTMS. In essence, the principle of dpTMS for the measurement of interhemispheric interactions is based on the effect of a conditioning pulse on a subsequent pulse (test stimulus [TS]) over the primary motor cortex of the opposite hemisphere (Ferbert et al. 1992; Gerloff, Cohen, et al. 1998). In order to assess interhemispheric interactions, dpTMS requires single-pulse and double-pulse trials. In a single-pulse trial, a TS was given over the left M1, and the resulting MEP was recorded from the right FDI. The peak-to-peak amplitude of the MEPs recorded in the single-pulse trials served as reference measurements for double-pulse trials. In a double-pulse trial, a conditioning stimulus (CS) followed by a TS (CS + TS; interstimulus interval [ISI]: 10 ms) was given. The CS was delivered either over right M1 (rM1-1M1) or right PMd (rPMd-1M1). MEPS from the single-pulse trials (TS) were contrasted to MEPS from the double-pulse trials (CS + TS). The interhemispheric interactions were defined as the ratio of (CS + TS)/TS × 100 - 100. Values above 0 indicated interhemispheric facilitation of the left M1 by the CS, values below 0 indicated interhemispheric inhibition (IHI).

dpTMS was delivered at 4 different time points between "Go!" and EMG onset: t1 (20% of iRT), t2 (50% of iRT), t3 (80% of iRT), and t4 (95% of iRT; see Fig. 1C). Additionally, we determined IHI at rest (no motor preparation) according to well-established IHI paradigms for M1-M1 (Ferbert et al. 1992) and PMd-M1 (Mochizuki et al. 2004). Similar to previously described event-related dpTMS paradigms that evaluated interhemispheric interactions between homologous primary motor areas (Murase et al. 2004; Duque, Hummel, et al. 2005; Duque et al. 2007), we delivered a conditioning stimulus over the right M1 (CSrM1) and then a TS over the left M1 separated by an ISI of 10 ms (location: rM1-lM1). We adapted this paradigm for the assessment of the interhemispheric interactions between rPMd and IM1 (location: rPMd-1M1). The CS was given over the right PMd (CSrPMd) and the TS over the left M1, here as well separated by an ISI of 10 ms (Mochizuki et al. 2004). For each time point (t1, t2, t3, t4) 18 TS alone, 18 CSrM1 + TS, and the interhemispheric RT at the beginning of the experiment and the respective left M1 hotspot. For determination of the rPMd location, we shifted the coil 2 cm anterior and 0.5 cm medial relative to M1, a procedure well established in a number of previous studies and validated with coregistration of TMS coil position on subjects’ individual magnetic resonance imaging scans of the brain (Schluter et al. 1998; Johansen-Berg et al. 2002; Mochizuki et al. 2004; O’Shea, Sebastian, et al. 2007). We then determined the stimulation intensities (expressed in % of maximum stimulator output) for TS, CSrPMd, and CSrM1. TS was adjusted at rest and during movement preparation because motor excitability differs substantially between rest and when cued for a reaction (Chen et al. 1998). Hence, we collected 10 trials of TS alone at 50% of iRT (adjustment run at t2) and repeated the adjustment run to obtain unconditioned MEPS of stable average peak-to-peak amplitude around 1 mV. MEP amplitudes evoked by TS at rest were also adjusted to 1 mV. This is an optimal excitability level to induce IHI as demonstrated in a previous study that tested IHI at different intensities of TS (Daskalakis et al. 2002). It has been shown in 2 previous studies that rM1-1M1 is inhibited during early phases of movement preparation (Murase et al. 2004; Duque, Hummel, et al. 2005). We thus chose a stimulation intensity of CSrM1 to reach an inhibition of 30-50% (conditioned MEP = 50-70% of unconditioned value). We collected 10 trials of CSrM1 + TS at 50% of iRT and repeated the adjustment run, so to obtain MEPS of stable average peak-to-peak amplitude around approximately 0.5-0.7 mV. We adopted the same stimulation intensity of CSrM1 for CSrPMd in order to rule out stimulation intensity as a potential confounder.

Intensity Experiment
Stimulation intensity over secondary motor areas is an important modulator of interhemispheric interactions (Koch et al. 2006). Therefore, we additionally investigated the effect of different increasing stimulation intensities over PMd on the pattern of interhemispheric interactions. In a pilot study, we used a fixed CS of 120% above rMT1M1. However, with this intensity we observed MEPS in the left FDI evoked by the CS when positioned over rPMd. Using this high intensity for the CS, the pattern of rM1-lM1 and rPMd-lM1 did not significantly differ. As reported for the main experiment (see Results) adjusting the intensity to an individual level as described above allowed lower intensities for the CS and led to a substantially different pattern in the interhemispheric modulation of rM1-1M1 compared with rPMd-1M1 supporting the hypothesis. In order to map effects on the rPMd-lM1 interaction each subject was moved to a different resting motor threshold (rMT1M1) and its respective location on the electromagnetic output which induced an MEP in the contralateral FDI and maximum peak-to-peak amplitudes of the MEP. Resting motor threshold was defined as the minimal stimulator output which induced an MEP >50 μV in 5 of 10 consecutive trials (Rossini et al. 1994). Based on imaging studies (Fink et al. 1997) and in line with previous studies (Mochizuki et al. 2004; O’Shea, Sebastian, et al. 2007), the location of CSrPMd was determined relative to the right M1 hotspot. For determination of the rPMd location, we shifted the coil 2 cm anterior and 0.5 cm medial relative to M1, a procedure well established in a number of previous studies and validated with coregistration of TMS coil position on subjects’ individual magnetic resonance imaging scans of the brain (Schluter et al. 1998; Johansen-Berg et al. 2002; Mochizuki et al. 2004; O’Shea, Sebastian, et al. 2007). We then determined the stimulation intensities (expressed in % of maximum stimulator output) for TS, CSrPMd, and CSrM1. TS was adjusted at rest and during movement preparation because motor excitability differs substantially between rest and when cued for a reaction (Chen et al. 1998). Hence, we collected 10 trials of TS alone at 50% of iRT (adjustment run at t2) and repeated the adjustment run to obtain unconditioned MEPS of stable average peak-to-peak amplitude around 1 mV. MEP amplitudes evoked by TS at rest were also adjusted to 1 mV. This is an optimal excitability level to induce IHI as demonstrated in a previous study that tested IHI at different intensities of TS (Daskalakis et al. 2002). It has been shown in 2 previous studies that rM1-1M1 is inhibited during early phases of movement preparation (Murase et al. 2004; Duque, Hummel, et al. 2005). We thus chose a stimulation intensity of CSrM1 to reach an inhibition of 30-50% (conditioned MEP = 50-70% of unconditioned value). We collected 10 trials of CSrM1 + TS at 50% of iRT and repeated the adjustment run, so to obtain MEPS of stable average peak-to-peak amplitude around approximately 0.5-0.7 mV. We adopted the same stimulation intensity of CSrM1 for CSrPMd in order to rule out stimulation intensity as a potential confounder.
rPMd location. Here again, subjects were instructed to abduct the right index finger after the Go-signal. We increased the stimulation intensity until we observed MEPs above 50 µV from the left FDI in more than 5 of 10 trials. We defined this intensity as the motor threshold for rM1 with the CS coil held over rPMd (MT\textsubscript{rPMd}). Although PMd has proper direct corticospinal projections (Dum and Strick 1991), animal studies suggest that induction of visible movements with stimulation of PMd needs 20-fold higher intensities compared with low-threshold sites in primary motor regions (Weinrich and Wise 1982; Graziano et al. 2002). Thus, the stimulation procedure up to about 0.5-fold of the resting motor threshold suggests that MEPs observed when PMd was stimulated are most likely produced by M1.

In order to differentiate "sub-" from "suprathreshold" effects, we ran the intensity experiment at 4 different CS\textsubscript{rPMd} intensities: 1) 110% of rMT\textsubscript{rM1} (Sub 1, an intensity clearly below MT\textsubscript{rPMd} corresponding to 83.3% of intensity at individual MT\textsubscript{rPMd}, see Table 1), 2) MT\textsubscript{rPMd} minus 2% (Peri), 3) MT\textsubscript{rPMd} plus 3% (Supra 1), and (d) MT\textsubscript{rPMd} plus 10% of maximum stimulator output (Supra 2). Additionally, we included the results obtained for rPMd-1M1 at t1 in the main experiment (Main exp t1).

The interhemispheric interactions were expressed as the ratio (CS \textsubscript{PMd} + TS)/TS and, as a reference, 18 TS trials at 20% of iRT (t1).

Control Experiment
It is known that PMd-M1 modulation may occur in very early time frames (Koch et al. 2006; O'Shea, Sebastian, et al. 2007), but the modulation of interhemispheric connectivity in time windows immediately before an instructional cue remains an open question. To provide further evidence that modulation of PMd-M1 connectivity is rapidly changed, we compared the pattern of connectivity change immediately before and after the instructional cue within this control experiment. To this end, we studied the interhemispheric interactions between rM1-1M1 and rPMd-1M1 in 4 subjects (3 females, age 23.5 ± 1.1 years, age range 22–27 years) 40 ms before the Go-signal and at t1 (20% of individual iRT; see Main Experiment). For each time point (t1, 20% of iRT; see Table 1), and location (rM1-1M1, rPMd-1M1) we collected 18 TS + TS trials at —40 ms and t1. In analogy to the main experiment, we first determined the hotspots of the right and left FDI, the respective rMT\textsubscript{rM1} of both hemispheres, and the right PMd location. We then adjusted the stimulation intensity of the TS to obtain 1-mV unconditioned MEP amplitudes at t1. CS intensity was adjusted until 30–50% inhibition was yielded in the rM1-1M1 at t1 in short “adjustment runs,” as outlined above for the main experiment.

Statistical Analysis
The interhemispheric interactions were expressed as the ratio (CS + TS)/TS × 100 – 100 for all analyses. Kolmogorov–Smirnov tests for normal distribution were calculated before statistical parametric testing was applied. A 2-factorial repeated-measures analysis of variance (RmANOVA) with the factors time (5 levels: rest, t1, t2, t3, t4) and location (2 levels: rM1-1M1, rPMd-1M1) was calculated in order to evaluate task-related interhemispheric interactions. Greenhouse-Geisser epsilon determination was used to correct for nonsphericity. Post hoc analysis was performed using the Scheffé test. A paired 2-tailed t-test was used to compare the rMT\textsubscript{rM1} between the right and left hemispheres.

For the intensity experiment, we calculated a Friedman test across CS\textsubscript{rPMd} intensity levels (within-subject factor CS\textsubscript{rPMd} intensity, 5 levels: Main exp t1, Sub 1, Peri, Supra 1, Supra 2). For the control experiment, we calculated a Friedman test across timings (—40 ms, t1) and locations (rM1-1M1, rPMd-1M1). For post hoc comparisons, we performed Wilcoxon tests.

The significance level was set at P < 0.05 for all tests. All data are expressed as mean ± standard error. SPSS software (version 15.0.1 for Windows, SPSS Inc, Chicago, IL) was used for all statistical analyses.

Results

Main Experiment

Basic Parameters
The mean iRT was 185.3 ± 3.7 ms. The rMT\textsubscript{rM1} was comparable between hemispheres (35.7 ± 1.5% for the left hemisphere and 37.0 ± 1.5% for the right hemisphere, z = 1.65; P = 0.15). The stimulation intensities for the assessment of interhemispheric interactions were 43.2 ± 1.6 for the TS (corresponding to 121.4 ± 2.1% of the intensity at the individual rMT\textsubscript{rM1}) and 43.0 ± 1.9% for CS (116.1 ± 1.6% of the intensity at the individual rMT\textsubscript{rM1}). The mean MEP values for TS and CS + TS are displayed in Table 1.

Interhemispheric Interactions—Main Experiment

RmANOVA revealed a main effect of location (F\textsubscript{1,9} = 29.77; P < 0.001) and time (F\textsubscript{4,36} = 8.61; P = 0.001) on interhemispheric interactions. More importantly, the temporal pattern of interhemispheric interactions clearly differed between rM1-1M1 and rPMd-1M1, as confirmed by a significant time × location interaction (F\textsubscript{4,36} = 4.58; P = 0.008; Fig. 2). In line with the hypothesis and monkey data, modulation of the intrinsic state of interhemispheric interactions between PMd-1M1 already occurred very early during movement preparation at 20% of RT (t1). On the contrary, the earliest modulation shown by rM1-1M1 was observed late in the process of initiating a voluntary movement when compared with the rest value (80% of RT: t3).

Table 1

<table>
<thead>
<tr>
<th>Time</th>
<th>MEPs right FDI</th>
<th>MEPs left FDI</th>
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<tbody>
<tr>
<td>Rest</td>
<td>1.24 ± 0.10</td>
<td>1.86 ± 0.31</td>
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<tr>
<td>t1</td>
<td>2.26 ± 0.38</td>
<td>2.82 ± 0.42</td>
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<tr>
<td>t2</td>
<td>1.32 ± 0.15</td>
<td>1.66 ± 0.24</td>
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<tr>
<td>t3</td>
<td>1.24 ± 0.17</td>
<td>1.95 ± 0.24</td>
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<tr>
<td>t4</td>
<td>1.32 ± 0.18</td>
<td>1.86 ± 0.31</td>
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*In the rest condition TS alone were collected for rM1-1M1 and for rPMd-1M1 separately. No MEPs were evoked in the left FDI during rPMd-1M1 measurements.

Figure 2. Group analysis with factors time (rest, t1, t2, t3, t4) and location (rM1-1M1, rPMd-1M1). The significant interaction of time × location (P = 0.008) was explained by early modulation of rPMd-1M1 compared with late modulation in rM1-1M1. Asterisks indicate significant differences compared with the respective rest condition (Scheffé tests all P < 0.05). Of note, rPMd-1M1 shows an early and late facilitatory peak, whereas rM1-1M1 shows continuous release of inhibition in the late phase of movement preparation. Group mean values ± standard error are demonstrated. Individual predetermined RT (mean ± standard error): 185 ± 4 ms. TMS timings based on predetermined RTs (in ms): t1 = 36 ± 1; t2 = 90 ± 3; t3 = 144 ± 5; t4 = 171 ± 5.
Of note, rPMd-lM1 shows a facilitatory peak early (t1) and late (t4: 95% of RT), whereas rM1-lM1 showed a reduction of inhibition only in the late phase, but did not turn into facilitation (Fig. 2).

Intensity Experiment
This experiment was performed to confirm the results observed in the main experiment and to demonstrate that the “early facilitation at t1” observed for rPMd-lM1 is abolished when stimulation over rPMd produces EMG activity in the left hand. Basic parameters were comparable to the main experiment: The mean iRT was 180.1 ± 6.8 s. The rMTM1 was 34.0 ± 1.4% for the left hemisphere and 35.4 ± 1.2% for the right hemisphere. The stimulation intensity for the TS was 41.8 ± 2.2 (116.1 ± 2.8%), expressed as a percentage of the intensity at the individual rMTM1.

In order to differentiate between effects originating in rPMd and rM1, we increased the CS intensity with the coil held over rPMd from sub- to suprathreshold values. Motor threshold (MT(rPMd)), here, was defined as the intensity of maximum stimulator output at which MEPs in the left FDI were evoked during movement preparation (for details see Intensity Experiment in Materials and Methods). Of note, the CS coil was always held over rPMd and thus MEPs in the left FDI are most likely explained by a spread of the magnetic field toward lM1. The values for MT(rPMd) and the different intensities employed for CS(rPMd) are illustrated in Table 2. As expected, we did not observe any MEPs from the left FDI (contralateral to the application of the CS) for CS(rPMd) at subthreshold intensities, between 1 and 8 MEPs of 18 trials at perithreshold intensity and—as intended—we saw MEPs in all trials at suprathreshold intensities.

The Friedman test demonstrated a significant effect of intensity on interhemispheric interaction (df = 4, $\chi^2 = 17.7$, $P = 0.001$). As observed in the main experiment, all subjects showed facilitation in the rPMd-lM1 interaction when CS(rPMd) was delivered at subthreshold intensities that were clearly below MT(rPMd) (Fig. 3A). However, at perithreshold intensity, facilitation was abolished. At suprathreshold intensities—when MEPs in the left FDI were visible throughout all trials—inhibition similar to rM1-lM1 at t1 appeared. Paired analysis using Wilcoxon tests revealed the following significant differences: Sub 1 > Supra 1, Supra 2; Main exp t1 > Peri, Supra 1, Supra 2; Peri > Supra 1, Supra 2 (all $P = 0.043$, Fig. 3A).

Control Experiment
Basic parameters were comparable to the main experiment: The mean iRT was 178.1 ± 9.1. The rMTM1 was 36.5 ± 3.0% for the left hemisphere and 36.0 ± 2.4% for the right hemisphere. The stimulation intensity for the TS was 42.5 ± 4.3 (116.5 ± 2.8%, expressed as a percentage of the intensity at the individual rMTM1) and for the CS 41.5 ± 2.5 (112.1 ± 2.3%, expressed as a percentage of the intensity at the individual rMTM1).

The Friedman test across both stimulation locations (rM1-lM1, rPMd-lM1) and time points (−40 ms, t1) yielded significance (df = 3, $\chi^2 = 8.4$, $P = 0.04$; Fig. 3B). Post hoc comparisons indicated a trend toward significance for rPMd-lM1 −40 ms > rPMd-lM1 t1 and rPMd-lM1 t1 > rM1-lM1 t1 (both Wilcoxon tests $P = 0.07$). At −40 ms before the Go-signal

### Table 2

All relative data are expressed as percentages of respective motor thresholds +/− standard error.

<table>
<thead>
<tr>
<th></th>
<th>MT(rPMd)</th>
<th>Main exp</th>
<th>Sub 1</th>
<th>Peri</th>
<th>Supra 1</th>
<th>Supra 2</th>
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<tr>
<td>In % of SO</td>
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<tr>
<td>47.2 ± 2.6</td>
<td>42.6 ± 2.3</td>
<td>23.9 ± 2.4</td>
<td>45.2 ± 2.6</td>
<td>50.2 ± 2.6</td>
<td>57.2 ± 2.6</td>
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<tr>
<td>In % of rMTM1</td>
<td>133.6 ± 7.0</td>
<td>71.016 ± 1.6</td>
<td>110.0 ± 0.0127</td>
<td>7.01421 ± 7.11620 ± 7.4</td>
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<tr>
<td>In % of MTrPMd</td>
<td>100.0</td>
<td>90.6 ± 4.6</td>
<td>a 83.3 ± 4.2</td>
<td>95.7 ± 0.2</td>
<td>106.4 ± 0.3</td>
<td>121.4 ± 1.1</td>
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Note: MT(rPMd), intensity at which MEPs from left FDI above 50 μV in 5 of 10 trials could be elicited at T1, with the coil held over the right PMd location (see Materials and Methods for details); Main exp T1: values from main experiment at T1, for rPMd-lM1; Sub 1: 110% of intensity at the individual rMTM1, but clearly below MT(rPMd); Peri: rMTM1 minus 2% of SO; Supra 1: rMTM1 plus 3% of SO; Supra 2: MT(rPMd) plus 10% of SO; SO: maximum stimulator output; me: main experiment.

*For determination of rPMd-lM1 T1 relative to rMTM1 from main experiment MT(rPMd) as determined in the intensity experiment was used.

### Figure 3

(A) Intensity experiment (at 20% of iRT: t1). With increasing stimulus intensity over rPMd from sub- to suprathreshold (i.e., below and above MT(rPMd)) the facilitatory effect of rPMd on IM1 turns into inhibition (Friedman test: df = 4, $\chi^2 = 17.7$, $P = 0.001$). MT(rPMd) is defined as the intensity at which MEPs above 50 μV from the left FDI in more than 5 of 10 trials were observed. Main exp t1: rPMd-lM1 data obtained in the main experiment at t1; Sub 1: T1 = MT(rPMd) + 110% of rMTM1; Peri: rMTM1 minus 2% of maximum stimulator output (SO); Supra 1: rMTM1 + 3% of SO; Supra 2: MT(rPMd) + 10% of SO; for exact stimulation intensities please consider Table 2. All measurements including MT(rPMd) determination were performed during movement preparation at 20% of individual RT (t1). For detailed information on the methods and employed stimulation intensities see Materials and Methods and Table 1. Group mean values ± standard error are demonstrated. Asterisks above dotted lines indicate significant differences as determined by Wilcoxon tests ($P < 0.05$).

(B) Control experiment. Interhemispheric interactions were determined 40 ms before and 20% of iRT (t1) after the Go-signal. Only rPMd-lM1 demonstrated an early modulation after the Go-signal (Friedman test: df = 3, $\chi^2 = 8.4$, $P = 0.04$; Wilcoxon test: rPMd-lM1 −40 ms > rPMd-lM1 t1 $P = 0.07$). Group median values ± standard error are demonstrated.
rPMd-IM1 and rM1-IM1 did not differ. Taken together, only rPMd-IM1 showed an early modulation after the Go-signal, whereas rM1-IM1 remained unchanged.

Discussion

The main finding of the present study was that human interhemispheric interactions in the human primary and premotor cortex are distinctly modulated during movement preparation in keeping with timings observed in monkeys (Tanji et al. 1988). During the preparation of a movement with the dominant right hand, the right dorsal premotor cortex exerted an early and less pronounced late facilitatory influence on the contralateral primary motor cortex (rPMd-IM1). IHI between homologue representations of the primary motor cortex (rM1-IM1) was not modulated early during movement preparation, but was continuously reduced before movement onset (Fig. 2). Interhemispheric facilitation of rPMd-IM1 early during movement preparation could only be elicited when subthreshold intensities for CS were used (no MEPs evoked by the CS applied to rPMd at t1, for details on methods and employed stimulation intensities see Materials and Methods and Fig. 3 A). On the contrary, perithreshold stimulation abated interhemispheric facilitation and suprathreshold stimulation exerted IHI in analogy to rM1-IM1 results of the main experiment. We could further add evidence that rPMd-IM1 modulation occurs in a very early time frame after presentation of an instructional cue to move the hand, but less likely before as an anticipatory process (Fig. 3B).

The interplay of motor areas of the right and left brain during motor performance has recently gained major attention and complemented the understanding of motor control processes in health (Bestmann et al. 2008; Grefkes, Eickhoff, et al. 2008) and disease, especially after stroke (Johansen-Berg et al. 2002; Hummel and Cohen 2006; O’Shea, Johansen-Berg, et al. 2007; Grefkes, Nowak, et al. 2008). Novel approaches in brain imaging such as dynamic causal modeling have advanced the knowledge of movement-related adjustments in interhemispheric balance (Johansen-Berg et al. 2002; Grefkes, Eickhoff, et al. 2008). Movement-dependent changes of the interhemispheric interplay have been probed with TMS over the PMd, which influenced the blood oxygen level–dependent response of the contralateral PMd and M1 in a state-related manner during the performance of an isometric grip task (Bestmann et al. 2008). The results of the present study fit well into the framework of movement-related modulation of interhemispheric interactions that originate in ipsilateral motor areas. As a new finding, our data delineate the temporal pattern of movement-dependent interhemispheric interactions between M1 and PMd according to proposed hierarchical models of motor preparation (Tanji et al. 1988; Hoshi and Tanji 2007) and specify the modulation of interhemispheric connectivity in terms of facilitation and inhibition. The results lend evidence to the proposition that sequential bihemispheric motor activation during movement preparation may reflect multiple stages needed to prepare and perform a movement (Tanji et al. 1988; Grafton and Hamilton 2007; Hoshi and Tanji 2007) and that interhemispheric interactions are organized in a temporally analogous way. Moreover, we found that during movement preparation, rPMd-IM1 connections show a predominantly facilitatory effect with an early and late peak, whereas rM1-IM1 connections engage in movement preparation by continuously reducing the intrinsic state of inhibition toward movement onset.

Distinct Temporospatial Organization of Interhemispheric Movement Control

We observed both the right PMd and right M1 to be involved in the modulation of contralateral M1 activity. The differential temporal and topographic pattern of interhemispheric interactions suggests distinct timing and distinct pathways of successively operating functional motor units to engage in interhemispheric motor control. According to physiological investigations in humans, M1-M1 interaction between motor areas as assessed with double-pulse TMS is considered to predominantly employ colossal pathways (Boroojerdi et al. 1996; Boroojerdi et al. 1998; Gerloff, Cohen, et al. 1998; Meyer et al. 1998; Wahl et al. 2007; Reis et al. 2008; Rizzo et al. 2008). Nevertheless, it cannot be completely excluded that subcortical mechanisms may have contributed to M1-M1 (Gerloff, Cohen, et al. 1998) and to PMd-M1 interactions (not yet determined in experimental designs). M1 and PMd have direct corticospinal projections (Dum and Strick 1991) which—both uncrossed and double crossed—target the ipsilateral anterior horn (Bucy 1933; Jankowska and Stecina 2007; Stecina et al. 2008). However, recordings from epidural electrodes implanted in humans for pain relief demonstrated that a preceding CS over M1 reduced the size of the spinal volley evoked by the TS applied to the opposite M1, indicating a supraspinal interaction (Di Lazzaro et al. 1999). It remains the possibility, although unlikely, that PMd might have influenced ipsilateral corticospinal output at the spinal level.

Neuroanatomical data have shown a strong preponderance of homotopic (ipsilateral PMd – contralateral PMd) compared with heterotopic (ipsilateral PMd – contralateral M1) interhemispheric connections from PMd (Rouiller et al. 1994; Marconi et al. 2003; Boussaoud et al. 2005; Fang et al. 2008). Based on anatomical evidence, the preferred pathway to mediate interhemispheric facilitation appears to be a homotopic pathway, but heterotopic connections may also have mediated the effects observed in the present study (Fig. 4). Intrahemispheric rPMd-rM1 connections (Dum and Strick 2005) might also be involved, but this is unlikely in the light of differential temporal patterns of rPMd and rM1 activity early during movement preparation. Given the biphasic early and late modulation, the PMd appears as a suitable candidate for top-down processing of movement control not only within (Wise 1985; Wise et al. 1997) but also across hemispheres (Fig. 4A). On the contrary, it has been proposed that M1 operates in a predominantly movement-related manner (Godschalk et al. 1981; Tanji et al. 1988) (Fig. 4B). The increase of contralateral M1 excitability occurring between 0 and 100 ms, that is, in the early phase of movement preparation (Chen et al. 1998), appears to be accompanied by persistent inhibition between homologue M1 areas (rM1-IM1) until the appropriate motor program is shaped by higher order motor areas such as PMd (Fig. 4A,B). The sequential organization of interhemispheric interactions as observed in our study is in line with the proposition that motor commands are organized in a hierarchical fashion (for reviews, see Grafton and Hamilton 2007; Hoshi and Tanji 2007). The dorsal premotor cortex of monkeys and humans has been demonstrated to engage in early and late activity during movement preparation (Godschalk et al. 1981;
Our results show immediate modulation of rPMd-IM1 interactions not only right after the instructional cue to move the hand but also in the late phase before movement onset. The employed paradigm does not allow firm conclusions regarding the exact functional relevance of the different interhemispheric patterns of rPMd-IM1 and rM1-IM1, but, as hypothesized, the results share temporospatial similarities with the activity observed in the monkey motor cortex ipsilateral to the moving hand (Godschalk et al. 1981; Weinrich and Wise 1982; Weinrich et al. 1984; Tanji et al. 1988). In keeping with animal data, imaging studies have demonstrated ipsilateral activation before and during the generation of a simple movement in humans (Huang et al. 2004; Bestmann et al. 2008; Sterr and Dean 2008) that is organized into sequential activation stages from secondary toward primary motor areas (Ball et al. 1999; Huang et al. 2004). The proposed model (Fig. 4) is a simplification because it is restricted to the regions and potential pathways tested in the present study. Moreover, dpTMS required measurement of the corticospinal output as a common final pathway. Hence, modulation of connectivity as result of a conditioning pulse over a distant brain area is at least 1 synapse away from the corticospinal axis, but can potentially involve several more synapses and connectivity nodes in between. Furthermore, it is well known that the preparation of a simple voluntary movement involves several additional regions such as cingulate (CMA) and supplementary (SMA) motor areas (Picard and Strick 1996; Picard and Strick 2001). Data obtained with MEG and high-resolution EEG suggest that activity starts in CMA and is then subsequently shifted to SMA, PMd, and, finally, to M1 (Ball et al. 1999; Huang et al. 2004). The sequential activation from higher-order motor areas (PMd and SMA) to M1 has also been observed in the hemisphere ipsilateral to the moving hand (Huang et al. 2004) and, in the light of our results, may reflect sequential organization of interhemispheric exchange engaged in preparing a simple unimanual movement. The results are limited to right-to-left interhemispheric interactions during movements of the dominant right hand. An interesting question arising from the present study is whether the sequential pattern of interhemispheric interactions differs for dominant compared with nondominant hand movements. In a previous study, facilitation of interhemispheric PMd-M1 connectivity at only 50 ms after the Go-signal has been found similar to modulation at the earliest examined time point in our study. However, no effect of left- versus right-hand movements could be revealed (O’Shea, Sebastian, et al. 2007). On the contrary, intermanual differences have been reported for M1-M1 interaction during movement preparation (Duque et al. 2007). During movement selection, the left PMd is particularly involved (Schluter et al. 1998), but inconsistent results regarding intermanual differences with respect to interhemispheric interactions have been reported (Koch et al. 2006; O’Shea, Sebastian, et al. 2007). Thus, the influence of hand dominance during simple and choice reaction remains an important open question for future investigations.

**Figure 4.** Model of dynamic modulation of interhemispheric interactions during (A) early and (B) late stage of movement preparation. (A) The rPMd has an early facilitatory effect on the IM1. Potential pathways could be either dense homotopic connections (rPMd-IPMdl) or less likely the sparser rPMd to IM1 connections (Rouiller et al. 1994; Marconi et al. 2003; Boussaoud et al. 2005; Fang et al. 2008). Interneurons might be involved in rPMd-IM1 interactions, but this alternative has not been experimentally tested yet and thus is not implemented in the present model. The structural organization of rM1-1M1 connections has been shown in humans using fiber tracking (Wahl et al. 2007). Based on our and previous physiological investigations, rM1 exerts an inhibitory effect on the IM1, most likely (GABA-ergic) (di-/(poly-)synaptically mediated along glutamatergic to γ-aminobutyric acidergic pathways (Chen 2004; Perez and Cohen 2008). (B) The decrease in IHI directed toward the right dominant hand at the end of movement preparation as demonstrated in the present and previous studies is most likely explained by a release of excitatory to inhibitory influence (Murase et al. 2004; Duque, Hummel, et al. 2005; Duque et al. 2007). The sequential organization of interhemispheric interaction with early premotor and late concerted premotor/primary motor activity supports proposed hierarchical models of cortical movement control (Grafton and Hamilton 2007; Hoshi and Tanji 2007).

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**Notes**
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References


