Functional Connectivity of Human Premotor and Motor Cortex Explored with Repetitive Transcranial Magnetic Stimulation

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Connections between the premotor cortex and the primary motor cortex are dense and are important in the visual guidance of arm movements. We have shown previously that it is possible to engage these connections in humans and to measure the net amount of inhibition/facilitation from premotor to motor cortex using single-pulse transcranial magnetic stimulation (TMS). The aim of this study was to test whether premotor activation can affect the excitability of circuits within the primary motor cortex (M1) itself. Repetitive TMS (rTMS), which is known to produce effects that outlast the train at the site of stimulation, was given for 20 min at 1 Hz over premotor, primary motor, and sensory areas of cortex at an intensity of 80% of the active motor threshold for the motor hand area. The excitability of some corticocortical connections in M1 was probed by using paired-pulse testing of intracortical inhibition (ICI) and intracortical facilitation (ICF) with a coil placed over the motor cortex hand area. rTMS over the premotor cortex, but not other areas, changed the time course of the ICI/ICF for up to 1 hr afterward without affecting motor thresholds or motor-evoked potential recruitment. The cortical silent period was also shortened. The implication is that rTMS at a site distant from the motor cortex can change the excitability of circuits intrinsic to the motor cortex.

Key words: motor cortex; premotor cortex; repetitive transcranial magnetic stimulation; intracortical inhibition; silent period; functional connectivity

Transcranial magnetic stimulation (TMS) is now the method of choice for noninvasive stimulation of the human brain in conscious subjects. In the 15 years since its introduction, it has been used both to chart the connectivity of the cerebral cortex (e.g., the corticospinal connection from motor cortex to spinal cord, the transcallosal connection between the two motor cortices, or the cortical connection between frontal eye fields and posterior parietal cortex) (Rothwell et al., 1991; Ferbert et al., 1992; Netz et al., 1995; Paus et al., 1997) and also to produce short-term disruption ("virtual lesions") of cortical areas involved in cognitive tasks (Jahanshahi and Rothwell, 2000). More recently, repetitive TMS (rTMS) has been used to apply a series of stimuli to the same cortical area. There is good evidence that this can produce long-term changes in excitability that outlast the rTMS for ≥15 min (Chen et al., 1997; Muellbacher et al., 2000).

The question we address here is whether rTMS can produce changes in excitability not only at the site of stimulation but also at distant sites connected synaptically. If so, rTMS may be a tool to investigate the role of such networks in different behaviors, in addition to probing or even modulating pathological changes produced by disease (George et al., 1999).

In this study we sought evidence that rTMS can change the excitability of cortical networks involving the motor and premotor cortex. The reason for choosing these areas was threefold. First, such connectivity is known to be dense and highly important for tasks involving visual control of movement (Godschalk et al., 1984, 1985; Morecraft and van Hoesen, 1993; Seitz et al., 2000). Second, Civardi et al. (2001) have shown that it is possible to study connections between the premotor cortex and the motor cortex in humans using TMS. Third, Gerschlager et al. (2001) have shown recently that rTMS over premotor areas can induce long-lasting changes in motor cortex excitability, as reflected by the size of EMG responses to standard single-pulse probe stimuli. Therefore, this study was designed to give more insight into the nature of the long-lasting changes that occur in the motor cortex after rTMS over premotor areas. We assessed the excitability of both the corticospinal system [threshold and motor-evoked potential (MEP) response size] as well as neural circuits intrinsic to the motor cortex [intracortical inhibition (ICI) and intracortical facilitation (ICF)]. The results suggest that conditioning stimuli to the premotor cortex can change the manner in which the motor cortex processes data. Part of this work has been published previously in abstract format (Münchau et al., 2001).

MATERIALS AND METHODS

Subjects. We studied 13 right-handed healthy volunteers (3 women; mean age ± SD, 34.2 ± 4.7 years). All participants gave oral informed consent. The experiments were performed with the approval of the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery.

Recording system. EMG was performed with 1-cm-diameter silver chloride disk surface electrodes placed in differential pairs over the right first dorsal interosseous (FDI) muscle, using a belly-tendon montage. The EMG signals were amplified, analog filtered (32 Hz to 1 kHz) by a Digitimer D150 amplifier (Digitimer Ltd., Welwyn Garden City, Herts, UK), and acquired at a sampling rate of 5 kHz. Data were stored on a personal computer for off-line analysis (Signal software; Cambridge

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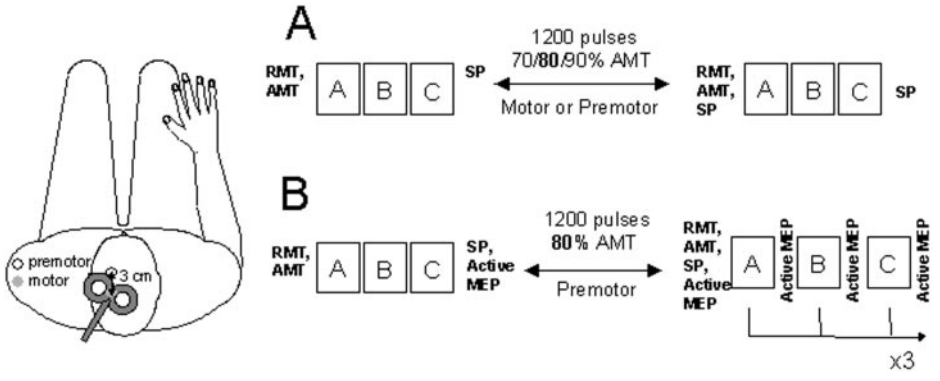

Figure 1. *A*, Design of the main experiment. RMTs and AMTs, the ICI/ICF, and the cortical SP were determined before and after rTMS. The ICI/ICF was tested in three different blocks, referred to as *A–C*. Each block consisted of four (5 for the 7 subjects in whom extra ISIs were studied) different conditions: the test stimulus alone and the test plus conditioning stimuli at three (or 4) different interstimulus intervals. The order of presentation of the different conditions within a block was changed randomly. *B*, Design of the control experiment, in which the time course of effects was studied. Before rTMS, RMT, and AMT, the ICI/ICF and the SP were determined. Testing of the ICI/ICF was performed in three blocks (*A–C*), as described in *A*. In addition, the amplitude of the MEP during slight voluntary contraction of the target muscle (*Active MEP*) was measured. RMT, AMT, SP, and active MEP were determined again immediately after rTMS. Then the ICI/ICF was retested. The active MEP was measured again between blocks *A* and *B* (5 min after rTMS), between blocks *B* and *C* (10 min after rTMS), and after block *C* (15 min after rTMS). The SP was also repeated after block *C*. Finally, the ICI/ICF (blocks *A–C*) was retested 1 and 2 hr after rTMS. *Left*, The coil position during TMS measurements and during rTMS. The positions of both the motor hot spot for the FDI muscle and the premotor area are indicated by a *filled* and an *open circle*, respectively.

Electronic Devices, Cambridge, UK). During the experiments EMG activity was continuously monitored with visual (oscilloscope) and auditory (speakers) feedback. Trials in which the target muscle was not relaxed were discarded from analysis because voluntary contraction of the target muscle decreases both the ICI and the ICF (Ridding et al., 1995b).

Measurements before and after rTMS. Subjects were seated comfortably in a reclining chair and were instructed to relax but to keep their eyes open and fixed on a target directly in front of them. We determined the resting motor threshold (RMT) and active motor threshold (AMT), the MEP amplitudes at rest and during slight (10% maximum) voluntary contraction, the ICI/ICF, and the cortical silent period (SP) (Fig. 1*A*) before and after rTMS.

Measurements were performed with a High Power Magstim 200 machine and a figure-eight coil with an outer winding diameter of 90 mm (Magstim Co., Whitland, Dyfed, UK). The magnetic stimulus had a nearly monophasic pulse configuration, with a rise time of ~100 μsec, decaying back to zero over ~0.8 msec. The coil was placed tangentially to the scalp, with the handle pointing backward and laterally at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus (Fig. 1) inducing a posterior–anterior current in the brain. This orientation was chosen based on the finding that the lowest motor threshold is achieved when the induced electrical current in the brain flows approximately perpendicular to the line of the central sulcus (Brasil-Neto et al., 1992; Mills et al., 1992). The coil was held by hand in relation to marks made on the scalp.

We determined the optimal position for activation of the FDI by moving the coil in 0.5 cm steps around the presumed motor hand area of the left motor cortex. The site at which stimuli of slightly suprathreshold intensity consistently produced the largest MEPs in the target muscle was marked as the "hot spot." Baseline and post-rTMS measurements were performed over this marked area. RMT was defined as the intensity needed to evoke an MEP in relaxed muscle of >50 μV in 5 of 10 consecutive trials. AMT was defined as the intensity needed to evoke MEPs in the tonically contracting FDI of ~200 μV in 5 of 10 consecutive trials.

In addition to measuring thresholds, we also measured the amplitude of MEPs evoked by a standard suprathreshold stimulus. For subjects at rest, this was the peak-to-peak size of the unconditioned test pulse across each of the ICI/ICF blocks (Fig. 1A, *A–C*; see below). Because there was no difference in the amplitudes between blocks, the mean values across the three blocks (i.e., mean of 30 MEPs) at baseline were compared with mean values in each block after rTMS. In four subjects, MEP amplitudes were measured during slight (10% maximum) voluntary contraction before and after rTMS over the premotor area using TMS pulses with an intensity of 120% of the AMT. Tonic background contraction was continuously monitored using acoustic feedback via a loudspeaker and visually on an oscilloscope. MEPs were recorded in separate bins of 15 trials at baseline, immediately after rTMS, and then in 5 min intervals until 15 min after rTMS (Fig. 1*B*). The mean MEP size (peak-to-peak) at baseline was compared with the means of each post-rTMS bin.

The ICI/ICF was evaluated using paired magnetic pulses as described by Kujirai et al. (1993). Because we were specifically interested in changes of the ICI and ICF, we set the intensity of the first (conditioning) stimulus to a relatively low value of 80% of the AMT to avoid floor or ceiling effects. The second (test) stimulus was set at an intensity that, when given alone, would evoke an EMG response of ~1 mV peak to peak (mean ± SD intensity, 54 ± 11% of maximum stimulator output and 122 ± 10% of the RMT). All subjects received the following nine interstimulus intervals (ISIs): 2, 3, 4, 5, 6, 7, 10, 15, and 20 msec. Seven subjects received three additional ISIs of 8, 9, and 12 msec. These ISIs were applied in three different blocks (*A–C*) of 40 (50 for the seven subjects with extra ISIs) trials each, with a random interval between trials of 4–5 sec (Fig. 1*A*). Each block consisted of four (five for the seven subjects with extra ISIs) different conditions in random order: test stimulus alone and test stimulus plus conditioning stimulus at three (four) different ISIs. The order of the blocks was also randomized across subjects but was kept constant in each subject before and after rTMS. Measurements were made during each individual trial. The mean peak-to-peak amplitude of the conditioned MEP at each ISI was expressed as a percentage of the mean peak-to-peak size of the unconditioned test pulse in that block. Measurement of the ICI/ICF lasted ~10 min using 9 ISIs and ~12 min using 12 ISIs.

In an additional control experiment, the ICI/ICF was measured three times after premotor rTMS (immediately after rTMS and 1 and 2 hr later) to assess the time course of the effects seen in the first experiments (Fig. 1*B*). Four subjects were studied.

The duration of the SP was determined during isometric voluntary contraction (~50% maximum) of the right FDI muscle, which was monitored using acoustic feedback via a loudspeaker and visually on an oscilloscope. Fifteen single suprathreshold TMS pulses (intensity, 150% of the AMT, or ~75% of the maximum stimulator output on average) were applied with an ISI of 10 sec to avoid fatigue. EMG traces were rectified but not averaged. The mean length of the SP was determined on the basis of measurements from each individual trial. The SP was measured from the onset of the MEP elicited by the suprathreshold TMS pulse to the onset of continuous EMG activity after the period of EMG suppression.

rTMS conditioning. Focal 1 Hz rTMS was applied using a figure-eight coil connected to a Magstim Rapid stimulator. The magnetic stimulus had a biphasic waveform with a pulse width of ~300 μsec. During the first phase, the stimulator induced a posterior–anterior current flow in the brain. The coil was held in an identical manner as described above for the TMS measurements (Fig. 1). The intensity of rTMS was referenced to each individual's AMT of the motor cortex hand area as assessed using the Magstim Rapid stimulator. AMT and RMT were measured before and after rTMS using Magstim Rapid stimulator pulses. We also determined the AMT when the coil was held over the premotor area in a subgroup of eight subjects before rTMS. In the first three subjects, we also attempted to determine the RMT over the premotor area. However, considerably higher intensities were needed, which subjects found difficult to tolerate at this scalp location. Therefore, the RMT over the premotor area was not studied in the remaining subjects.

All 13 subjects received left premotor rTMS. Eight of them also

received left motor cortex rTMS, separated by an interval of at least 5 d from premotor rTMS. The sequence of motor and premotor stimulation was randomly altered across these eight subjects. Single trains of 20 min duration (i.e., 1200 pulses) were applied in each session. Because we were interested in determining whether there are differential effects of motor versus premotor rTMS on motor cortex excitability, we used low-intensity stimulation to avoid the spread of activity from the motor cortex to the premotor cortex during motor cortex stimulation, and vice versa. Therefore, rTMS stimulus intensities were set at 80% of the AMT, as determined over the motor cortex for all subjects. In a subgroup of four subjects, we also studied the effects of rTMS of 70 and 90% of the AMT over the premotor cortex, separated by an interval of at least 5 d from premotor rTMS of 80% of the AMT. Stimulation variables were in accordance with published safety recommendations (Wassermann, 1998). We monitored EMG activity by acoustic feedback throughout the rTMS sessions to ensure that stimulation intensities were below the motor threshold.

The coil position for premotor rTMS was defined relative to the position of the motor hot spot for the FDI. A positron emission tomographic (PET) study showed that the dorsal premotor cortex is located ~2 cm anterior to the motor cortex hand area (Fink et al., 1997). To minimize motor cortex activation during premotor rTMS, we calculated for each subject 8% of the distance between the nasion and inion (typically ~3 cm) and defined the premotor area as this distance anterior to the hot spot of the motor cortex hand area (Fig. 1).

It is possible that effects of rTMS applied over the premotor area are the result of direct low-intensity stimulation from the posterior bifurcation of the figure-eight coil that was positioned over the motor cortex area during premotor stimulation. Because the stimulus intensity at this part of the coil equals that in the anterior bifurcation, we performed a control experiment in which the coil was moved 3 cm posterior from the motor cortex hand area so that the anterior bifurcation was held over the motor cortex and the site of maximal stimulation at the coil center was positioned over the sensory cortex. Four subjects received such stimulation (1 Hz rTMS; intensity, 80% of AMT). If changes in motor cortex excitability had been caused by low-intensity direct stimulation from the posterior bifurcation of the coil during the premotor rTMS condition, one would expect similar changes to occur after stimulation from the anterior bifurcation in the sensory rTMS condition.

Statistical analysis. The paired-samples *t* test was used to compare the RMT and the AMT before and after rTMS. The effects of rTMS on the duration of SP and MEP size (both under resting conditions and during slight voluntary muscle contraction) were evaluated by one-way repeated-measures ANOVA. The effects of rTMS on paired-pulse curves were studied separately for each stimulation site (motor vs premotor area) using a two-factor repeated-measures ANOVA with time before and time after rTMS and ISI as within-subject factors. A direct statistical comparison was made between the two stimulation sites using a three-factor repeated-measures ANOVA that compared pre-rTMS and post-rTMS paired-pulse curves with time, ISI, and site (motor and premotor) as within-subject factors. In this three-way study, we increased the power by reducing the number of ISIs entered into the analysis by averaging data from adjacent intervals: the inhibition period (ISIs of 2, 3, and 4 msec), the facilitation period (ISIs of 10, 15, and 20 msec), and the intermediate period (ISIs of 5, 6, and 7 msec). The Greenhouse–Geisser correction was used when necessary to correct for nonsphericity. Conditional on a significant *F* value, *post hoc* paired-samples *t* tests were performed. A p value of < 0.05 was considered significant for all statistical analyses.

RESULTS

With the parameters of stimulation used in these experiments, none of the subjects reported adverse effects after rTMS.

Motor threshold and MEP size

The mean ± SD AMT for stimulation over the motor cortex was 40.4 ± 7.7% of the maximum stimulator output. When the coil was placed 3 cm anterior to the premotor cortex, the AMT rose to 53 ± 9%, a mean increase of 33 ± 17% ($p < 0.001$; paired-samples *t* test). rTMS over the motor or premotor cortex at 80% of the AMT had no effect on the RMT or the AMT (Fig. 2*A*). Furthermore, the amplitude of unconditioned MEPs evoked by stimulation over the motor cortex in the ICI/ICF paradigm was not affected by rTMS over the motor cortex at 80% of the AMT, whereas a small but nonsignificant decrease was noted after rTMS over the premotor cortex at 80% of the AMT (Fig. 2*B*). The results also give an indication of the reproducibility of the population baseline measures, because data from motor and premotor rTMS were obtained from the same eight subjects on different days. The amplitude of MEPs evoked during active contraction was unaffected by rTMS in the four subjects in whom it was tested (Fig. 2*C*).

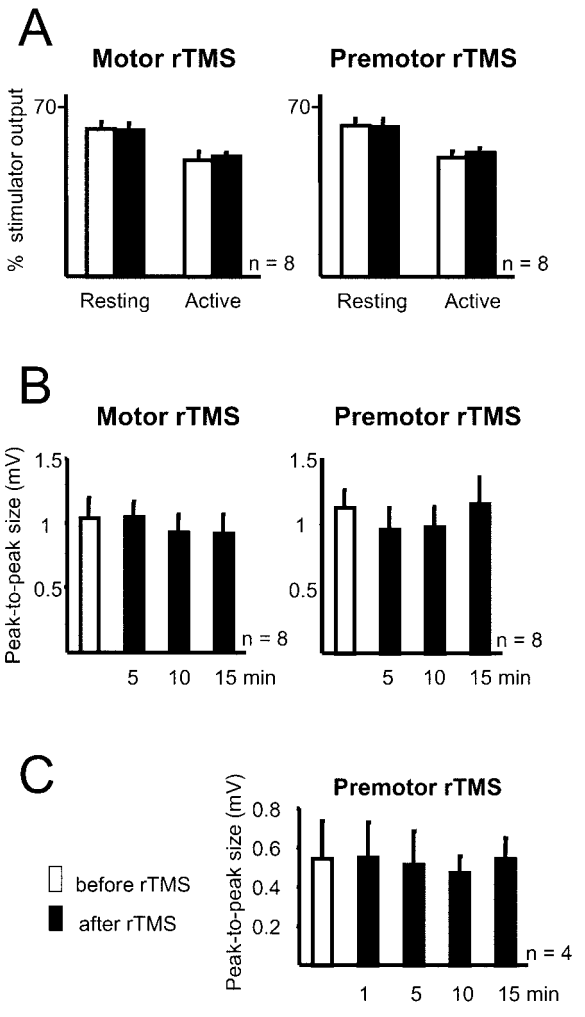

Figure 2. *A*, RMTs and AMTs before and immediately after rTMS in the eight subjects who had received both motor and premotor rTMS. There was no significant change after motor or premotor rTMS. Error bars indicate SEM. *B*, MEP size of relaxed FDI muscle before and after motor rTMS and premotor rTMS in the same eight subjects. Measurements were repeated three times after rTMS, at 5, 10, and 15 min. Resting MEP amplitudes were slightly smaller 5 and 10 min after premotor rTMS, but this difference was not significant. *C*, MEP size during slight voluntary contraction of FDI muscle before and after premotor rTMS in four subjects. MEP size was determined 1, 5, 10, and 15 min after rTMS. There was no significant difference from baseline.

ICI/ICF

Figure 3*A* illustrates the effect of rTMS over the motor cortex and the premotor cortex on the motor cortex ICI/ICF. The same eight subjects participated in conditioning experiments at both sites. A separate two-way analysis of the motor and premotor stimulation sites showed that rTMS had no effect on the time course of the

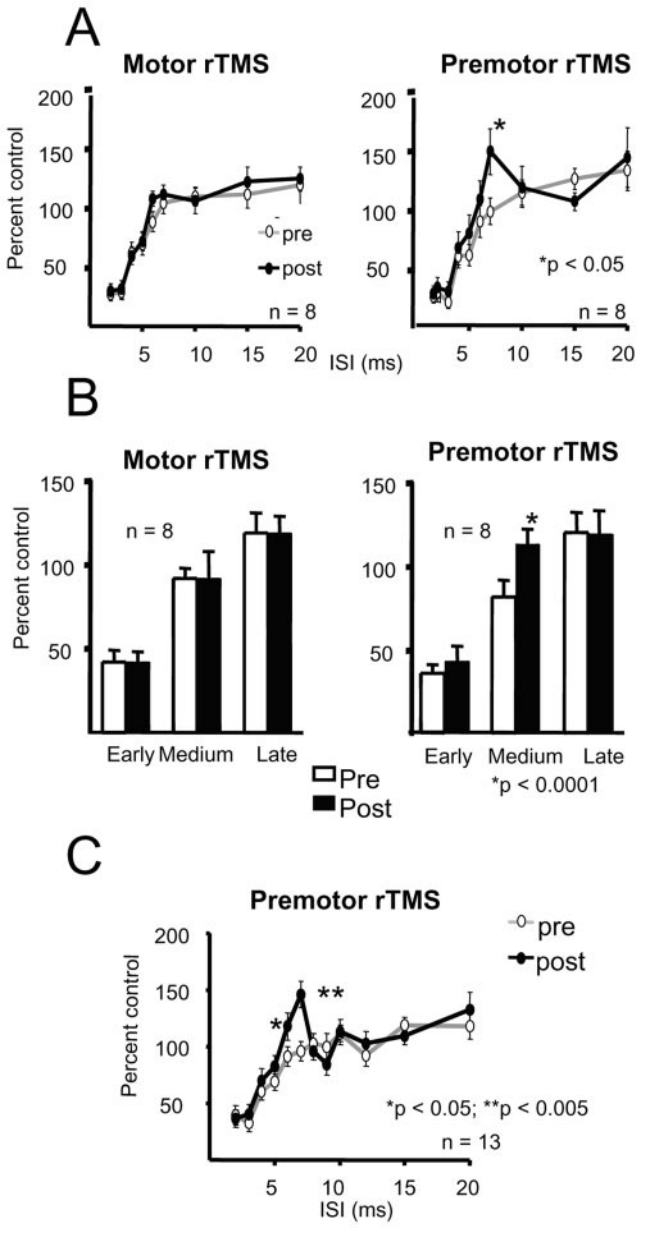

Figure 3. *A*, ICI/ICF curves before and after rTMS at 80% of the AMT over motor and premotor areas. The mean (± SEM) time course of the conditioned test MEP after rTMS is superimposed on the time course at baseline. The size of the conditioned test response is expressed as a percentage of the unconditioned test size. Data from the eight subjects who had both motor and premotor rTMS are shown. Nine different ISIs were studied. After motor rTMS there was no significant change from baseline. In contrast, after premotor rTMS there was significantly increased facilitation at an ISI of 7 msec ($t_{(7)} = -2.5$; $p = 0.041$; *post hoc* paired-samples *t* test). *B*, Comparison of the averaged size of the conditioned test response of adjacent time points, separated into early ISIs (2, 3, and 4 msec), medium ISIs (5, 6, and 7 msec), and later ISIs (10, 15, and 20 msec). rTMS had no effect on the ICI/ICF when applied over the motor cortex. In contrast, after premotor rTMS the conditioned MEP size was significantly increased compared with baseline at medium ISIs ($p < 0.0001$; *post hoc* paired-samples *t* tests) but not at the other intervals. *C*, ICI/ICF curves before and after rTMS at 80% of the AMT over the premotor area. Data from all 13 subjects who had premotor rTMS are shown. In this larger group there is increased facilitation at ISIs of 6 msec ($t_{(12)} = -2.3$; $p < 0.05$) and 7 msec ($t_{(12)} = -3.5$; $p < 0.005$). In 7 of these 13 subjects, additional ISIs (8, 9, and 12 msec) were studied. There was no significant difference from baseline at any of these ISIs.

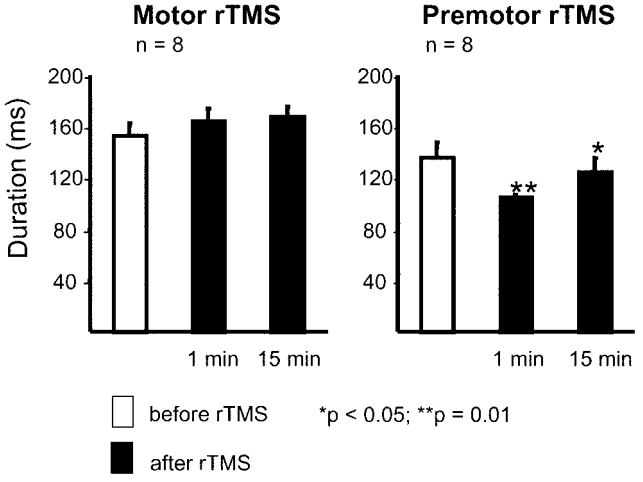

Figure 4. Effects of rTMS on the duration of the SP. There was a significant reduction in the duration of the SP immediately after premotor rTMS ($t_{(7)} = 5.6$; $p = 0.01$; paired-samples *t* test) but not after motor rTMS. The effect after premotor rTMS was still significant 15 min later ($t_{(7)} = 2.3$; $p < 0.05$).

ICI/ICF when applied over the motor cortex. However, the data revealed a significant interaction between ISI and time ($F_{(2,14)} = 10.9$; $p = 0.001$) after rTMS over the premotor cortex (Fig. 3*A*). *Post hoc* paired-samples *t* tests showed that this interaction effect was caused by a significantly increased facilitation at an ISI of 7 msec ($t_{(7)} = -2.5$; $p = 0.041$).

A three-factor repeated-measures ANOVA, with time (before and after rTMS), ISI, and site (motor or premotor conditioning) was performed to study whether rTMS had a differential effect on the motor cortex and the premotor cortex. As outlined in Materials and Methods, we averaged adjacent time points over the period of inhibition (ISIs of 2, 3, and 4 msec), the period of facilitation (ISIs of 10, 15, and 20 msec), and the intermediate period (ISIs of 5, 6, and 7 msec) to increase the power of this analysis. There was a significant three-way interaction of time, site, and ISI ($F_{(2,14)} = 3.8$; $p < 0.05$), indicating that rTMS over one of the sites had an effect on part of the ICI/ICF curve (Fig. 3*B*). *Post hoc* paired-samples *t* tests revealed an increase in the conditioned MEP size after premotor rTMS compared with baseline at the intermediate ISIs (5, 6, and 7 msec) ($p < 0.0001$) but not at the other ISIs (Fig. 3*B*).

A total of 13 subjects, including all 8 of those shown in Figure 3*A*, had premotor rTMS (Fig. 3*C*). Seven of these subjects were studied at additional intervals of 8, 9, and 12 msec (Fig. 3*C*) to determine whether we had missed any effect of premotor stimulation at these intervals. There was no significant difference in pre-rTMS and post-rTMS at any of these timings. A two-factor repeated-measures ANOVA (omitting the additional intervals) demonstrated a significant interaction between time and ISI ($F_{(3.4,40.3)} = 3.4$; $p < 0.05$). *Post hoc* paired-samples *t* tests revealed an increase in the conditioned MEP size after premotor rTMS compared with baseline at ISIs of 6 msec ($t_{(12)} = -2.3$; $p < 0.05$) and 7 msec ($t_{(12)} = -3.5$; $p < 0.005$).

The silent period

Figure 4 illustrates that after premotor rTMS (but not after motor rTMS) there was a significant reduction in the duration of the SP. A two-factor ANOVA showed a significant interaction between time and site ($F_{(2,6)} = 8.9$; $p = 0.016$). Single-factor ANOVAs on

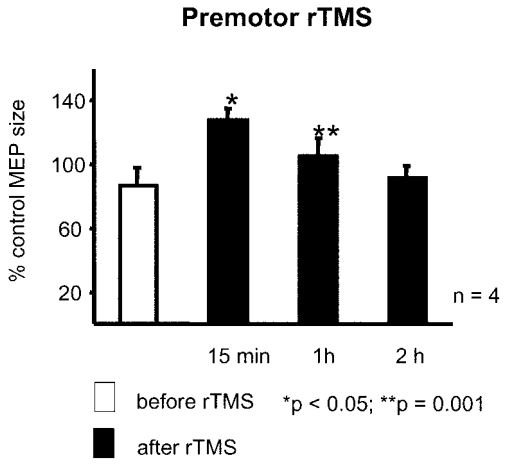

Figure 5. Time course of the increased ICF at ISIs of 6 and 7 msec after rTMS over the premotor area in four subjects. The conditioned MEPs at 6 and 7 msec were significantly larger immediately after rTMS ($t_{(3)} = -3.2$; $p < 0.05$) and 1 hr later ($t_{(3)} = -13,2$; $p = 0.001$; paired-samples t tests). They were still slightly increased compared with baseline at 2 hr, but this was no longer statistically significant.

the data for the two sites separately showed a significant influence of time after premotor rTMS ($F_{(2,6)} = 17.6$; $p = 0.003$) but no effect after motor rTMS ($F_{(2,10)} = 1.4$; $p = 0.29$). *Post hoc* paired-samples t tests demonstrated a significant shortening of the SP immediately ($t_{(7)} = 5.6$; $p = 0.01$) and 15 min after rTMS ($t_{(7)} = 2.3$; $p < 0.05$).

ICI/ICF: time course of rTMS effect

In four subjects, we studied how long rTMS over the premotor area affected the ICI/ICF (Fig. 5). The paired-pulse paradigm was repeated three times, immediately after rTMS and 1 and 2 hr later. Because significant changes from baseline were found at ISIs of 6 and 7 msec in the main experiment, we focused our analysis on these ISIs, and combined the data from the two intervals into a single mean. A repeated-measures ANOVA on this mean showed a significant effect of time before and after rTMS on the size of conditioned MEPs ($F_{(3,9)} = 8.2$; $p = 0.006$). *Post hoc* analysis demonstrated that the conditioned MEP at 6 and 7 msec was significantly larger immediately after rTMS ($t_{(3)} = -3.2$; $p < 0.05$) and 1 hr later ($t_{(3)} = -13,2$; $p = 0.001$; paired-samples t tests). After 2 hr, conditioned MEPs no longer differed from baseline.

Influence of rTMS stimulus intensity on the ICI/ICF

In four subjects, we examined whether changes in the ICI/ICF after premotor rTMS depended on rTMS stimulus intensity. Premotor rTMS was given at 70, 80, or 90% of the AMT in separate blocks of trials performed on different days. As shown in Figure 6*A*, rTMS at 70 or 90% of the AMT had no effect on the ICI/ICF curves. However, after rTMS at 80% of the AMT, as in the previous data from the entire group of 13 subjects, there was a significant difference before and after rTMS at an ISI of 7 msec ($t_{(3)} = -3.1$; $p < 0.05$). The conditioning stimulations had no significant effect on the size of the control MEP. Even after rTMS at 90% of the AMT, the control MEP size was smaller immediately after the conditioning train in two of the four subjects but was unaffected in the other two. In a previous study, Gerschlager et al. (2001) found that rTMS over premotor areas at 90% of the AMT decreased the amplitude of MEPs from the primary motor cortex. However, they defined the location of the premotor stimulus as 2.5 cm anterior to the motor hand area, whereas our location was, on average, 0.5 cm anterior to this. As noted by Gerschlager et al. (2001), the threshold for producing an effect on MEP amplitude is slightly higher for more anterior conditioning sites.

Effect of rTMS over the sensory cortex on ICI/ICF

In four subjects, we tested whether a 20 min train of 1 Hz rTMS at 80% of the AMT over the sensory cortex (with the anterior bifurcation of the coil positioned over the motor cortex) would produce changes similar to those seen after premotor rTMS at 80% of the AMT (when the posterior bifurcation of the coil was over the motor cortex). Figure 6*B* illustrates that there was no change in the ICI/ICF curves after stimulation of the sensory cortex. Nevertheless, for these subjects, as in the entire group, there was a significant effect of premotor rTMS at an ISI of 7 msec ($t_{(3)} = -3.8$; $p < 0.05$; paired-samples t test). We conclude that the changes in motor cortex excitability seen after premotor stimulation cannot be explained by low-intensity direct stimulation of the motor cortex from the posterior bifurcation of the coil.

DISCUSSION

Our study shows that a 20 min submotor threshold train of 1 Hz rTMS over the premotor area can affect the time course of the ICI/ICF for a period of up to 1 hr without any significant effect on motor thresholds or MEP amplitudes. In addition, the cortical SP was shortened. No effects were seen when the same rTMS train was given directly over the motor cortex or the sensory cortex.

Civardi et al. (2001) showed that single-pulse TMS over premotor areas at similar intensities could reduce the amplitude of the MEPs evoked by stimulation of the motor cortex 4–6 msec later. Control experiments suggested that this was attributable to an effect on the motor cortex rather than to activation of direct spinal projections from the premotor cortex. However, the effect of a single stimulus lasted only 5–10 msec. In a different study, Gerschlager et al. (2001) used rTMS (90% of the AMT) over the premotor cortex and observed a long-lasting decrease in the excitability of corticospinal MEPs evoked by single-pulse TMS. A similar but much less robust effect was noted in this study, presumably because we used lower-intensity (80% of the AMT) stimulation over the premotor cortex. The novel feature of these results is that premotor rTMS at 80% of the AMT can affect the excitability of primary motor cortex circuits tested in the ICI/ICF paradigm for up to 1 hr.

Site of action of the conditioning rTMS

rTMS was applied at 8% of the nasion–inion distance (~3 cm) anterior to the hand area of the motor cortex as defined by single-pulse mapping of MEPs. The latter lies directly over the "hand knob" of the precentral cortex as defined on magnetic resonance imaging and corresponds to a position of maximal activation in PET scans during voluntary finger movements (Wassermann et al., 1996). The dorsal premotor cortex is thought to be 2 cm anterior to this (Fink et al., 1997), so we can be relatively certain that the center of the coil was over premotor areas in these experiments. The question is whether we were stimulating elements under the center of the coil or whether we were stimulating the motor cortex directly because of current spread away from the coil.

The latter possibility seems unlikely. First, the AMT of the premotor area itself was approximately one-third higher than that of the motor cortex, indicating that the premotor stimuli of 80%

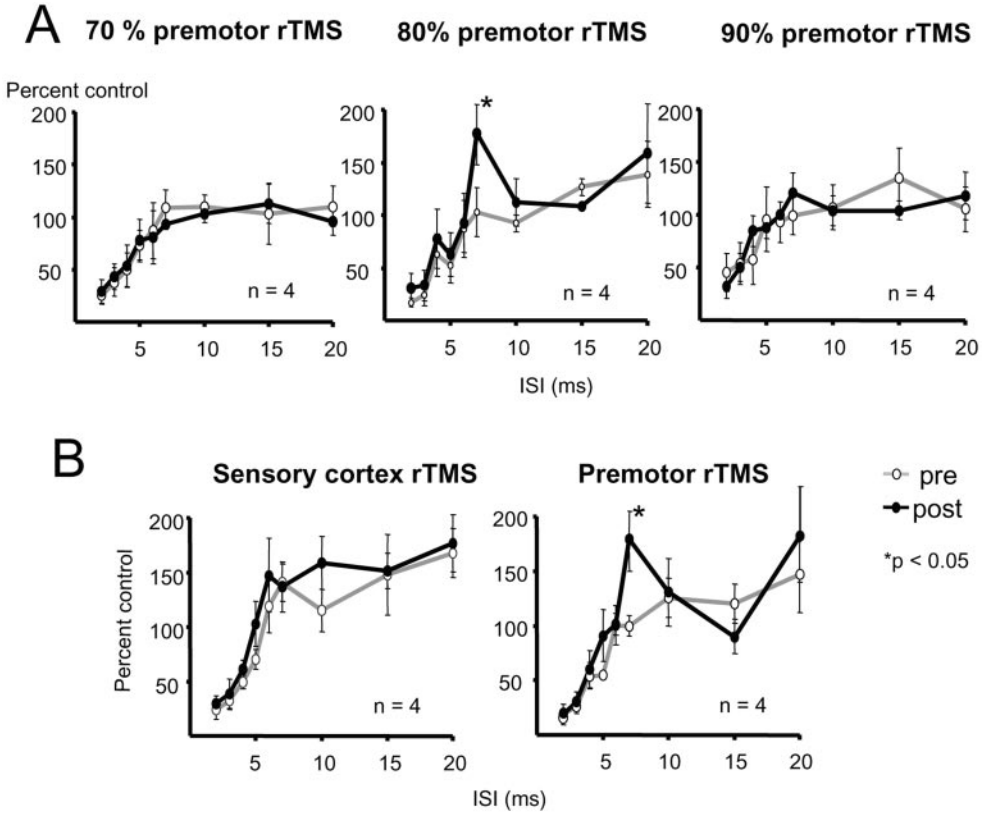

Figure 6. *A*, ICI/ICF curves after premotor rTMS using different stimulus intensities in four subjects. There was no change after rTMS at 70 or 90% of the AMT. For comparison, the time course before and after rTMS at 80% of the AMT for the same four subjects is also shown. After rTMS at 80% of the AMT there was a significant facilitation at an ISI of 7 msec ($t_{(3)} = -3.1$; $p < 0.05$; paired-samples t test). *B*, ICI/ICF curve after sensory rTMS in four subjects. There was no significant change from baseline. For comparison, the time course before and after rTMS at 80% of the AMT premotor cortex for the same four subjects is also shown. Significant facilitation is present at an ISI of 7 msec ($t_{(3)} = -3.8$, $p < 0.05$; paired-samples t test).

of the AMT would have an effective intensity of only 60% of the AMT at the motor hand area. No effects on motor cortical excitability have been reported after direct stimulation at this intensity. Second, if we moved the conditioning coil (80% of the AMT) so that its center was over the motor hand area, or more posterior, over the sensory cortex, there was no effect on the ICI/ICF. We conclude that the premotor rTMS was not activating elements in the motor cortex.

Similar arguments can be made that the ICI/ICF paradigm is testing circuits that are intrinsic to the motor cortex. At an intensity of only 80% of the AMT, the conditioning stimulus is unlikely to spread to other areas of the cortex. In addition, direct stimulation of the exposed cortex through subdural electrodes causes the ICI between adjacent electrodes spaced 1 cm apart on the motor strip, but not over larger distances (Ashby et al., 1999), suggesting that the circuitry being tested is relatively local.

The conclusion is that rTMS acts on premotor areas, and that this produces a long-lasting effect on circuitry in the primary motor cortex. There was no effect on the excitability of pyramidal-tract neurons, at least as tested by single-pulse MEP measurements. Indeed, because (1) the intensity of rTMS was the same as the intensity of the first pulse in the ICI/ICF paradigm (80% of the AMT), and (2) rTMS over the motor cortex had no effect on the ICI/ICF, we can presume that premotor rTMS did not have a direct effect on the intracortical elements activated in the ICI/ICF paradigm. Thus, premotor rTMS was influencing interneurons in the motor cortex through corticocortical connections.

This circuitry would be compatible with the electrophysiology of connections between the premotor cortex and the primary motor cortex as studied in monkeys (Ghosh and Porter, 1988; Tokuno and Nambu, 2000). Stimulation of the premotor cortex results predominantly in short-latency inhibition of pyramidal-tract neurons that may involve excitatory inputs to superficial inhibitory interneurons in the motor cortex (Tokuno and Nambu, 2000). If the latter contribute to the ICI/ICF, this pathway may account for some of the effects we observed. In a behavioral test of this connection, Strafella and Paus (2000) instructed resting healthy subjects to observe other people while they were writing. During observation of this action, there was a decrease in the level of the ICI/ICF in muscles involved in handwriting, similar to what would happen if subjects had voluntarily activated their own muscles (Ridding et al., 1995b). Given the importance of the premotor cortex in selecting movements that are guided by visual cues (Schluter et al., 1998), the authors argued that activation of the premotor cortex during action observation could lead to inhibitory, shaping effects on motor cortex excitability, perhaps via the same connections as those involved in these experiments.

Mechanism of the premotor effect

When applied to the motor cortex, rTMS at the same frequency and duration reduces resting cortical excitability for ≥15 min (Chen et al., 1997; Maeda et al., 2000). If the same happens to the premotor cortex after rTMS in these experiments, it may reduce activity in the connection between the premotor cortex and the motor cortex and result in changes in the motor cortex ICI/ICF curve. The intensity for premotor cortex effects is lower than that used for the motor cortex (95% of the RMT vs 80% of the AMT), but this may be because the premotor cortex, on the crown of the precentral gyrus, is nearer the stimulating coil than the motor cortex, the majority of which is buried in the central sulcus (Gerschlager et al., 2001). Interestingly, increasing the intensity

of premotor rTMS from 80 to 90% of the AMT reduced the effect on the ICI/ICF. We suggest that this is because the connections between the premotor cortex and the motor cortex are both facilitatory and inhibitory (Ghosh and Porter, 1988; Tokuno and Nambu, 2000). Stimulation at a higher intensity might be more likely to evoke a mixture of effects that cancel out the changes observed at 80% of the AMT.

Why should the main effect on the ICI/ICF occur at ISIs of 6 and 7 msec? Previous studies in Parkinson's disease have shown that pathology can affect particular ISIs of the ICI/ICF time course (at intervals of 2 and 5 msec) (Ridding et al., 1995a), so the specificity of the effect is not unprecedented. We suggest that the usual time course of the ICI/ICF is a composite of inhibitory and excitatory processes that are recruited with different time courses and strengths by the conditioning pulse (Hanajima et al., 1998). Because ISIs of ~6–7 msec lie at the boundary between net inhibitory and net excitatory effects, changes in the balance of premotor input to these systems might show up most clearly at this time. Alternatively, premotor stimulation may access a particular subset of interneurons that have a maximum contribution to the ICI/ICF time course at these particular ISIs.

A final question is whether the effect on the SP was linked to the effect on the ICI/ICF. Interactions occur between these two effects (Chen et al., 1997), but it is generally thought that they use different subsets of inhibitory neurons (Werhahn et al., 1999). The high intensity of stimuli that are used to produce the SP (150% of the AMT) could spread to recruit neurons from outside the motor cortex. This means that we cannot conclude for certain that the effect of premotor rTMS on the SP was attributable to an effect on motor cortical circuits. It is conceivable, for example, that premotor rTMS had a direct effect on the excitability of inhibitory projections from the premotor cortex that are normally activated in the SP.

Implications for previous work

In previous studies of the effect of rTMS on the ICI/ICF (Ziemann et al., 1998; Siebner et al., 1999; Peinemann et al., 2000; Wu et al., 2000), rTMS was applied directly over the motor cortex, and at a higher intensity and/or frequency than we used in our experiments (90–120% of the RMT). Therefore, it is possible that some of the effects on intracortical inhibition were attributable to spread of the current to premotor areas.

We conclude that 1 Hz submotor threshold rTMS at a site distant from the motor cortex can interfere specifically with some intrinsic circuits of the motor cortex. These changes outlast the rTMS by up to 1 hr and are likely to be mediated by corticocortical neurons projecting from the premotor cortex to the motor cortex. This is consistent with the concept that the premotor area has a shaping or focusing role in the execution of movements by modulating activity of motor cortex interneurons. In a broader context, our findings also indicate that it might be important to account for effects at a distance when interpreting any functional consequences of rTMS, for instance when rTMS is used as a treatment.

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