

Slow frequency repetitive transcranial magnetic stimulation affects reaction times, but not priming effects, in a masked prime task

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Abstract

Objective: Slow frequency repetitive transcranial magnetic stimulation (rTMS) reduces motor cortex excitability, but it is unclear whether this has behavioural consequences in healthy subjects.

Methods: We examined the effects of 1 Hz rTMS (train of 20 min; stimulus intensity 80% of active motor threshold) over left motor or left premotor cortex on performance in a visually cued choice reaction time task, using a ‘masked prime’ paradigm to assess whether rTMS might affect more automatic motor processes. Twelve healthy volunteers participated.

Results: Motor cortex rTMS and, to a lesser extent, premotor cortex rTMS resulted in a slowing of right (stimulated) hand responses, but not of left (unstimulated) hand responses. In a control experiment, rTMS of the left somatosensory cortex did not lead to slower right hand responses.

Discussion: We conclude that long trains of low intensity 1 Hz rTMS over the motor or premotor cortex can have subtle behavioural consequences outlasting the stimulation. rTMS did not affect the modulation of reaction times by subliminal primes, suggesting that priming effects triggered by subliminal primes are not generated at the level of motor or pre-motor cortex.

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1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) has become a useful tool to non-invasively study human motor cortex excitability because it can induce excitability changes that outlast the period of stimulation. For instance, the size of motor evoked potentials (MEPs) elicited by suprathreshold stimulation is reduced after 5 min of slow frequency, high intensity motor cortex rTMS (Chen et al., 1997). In patients with writer’s cramp, 1 Hz rTMS for 20 min normalises deficient intracortical inhibition and improves motor performance (Siebner et al., 1999). Interestingly, there is as yet no clear evidence for behavioural correlates of reduced motor cortex excitability in healthy

subjects. For example, no effects of motor cortex rTMS were found on finger tapping speed (Chen et al., 1997), or on peak force or peak acceleration in the corresponding muscles (Muellbacher et al., 2000). However, in these studies tasks were self-paced and required responses with one hand only, presumably resulting in low levels of response readiness and a constant response bias. Thus, conceivably these tests were not sensitive enough to capture subtle changes in motor performance following rTMS in healthy subjects. Alternatively, rTMS-induced reduction of cortical excitability might be insufficient to affect voluntary movements, but could affect more automatic motor responses.

To address both possibilities, we examined the effects of rTMS on reaction times (RTs) in a ‘masked prime task’ (Schlaghecken and Eimer, 1997, 2000; Eimer and Schlaghecken, 1998). In this paradigm, participants have to decide quickly and accurately, on a trial-by-trial basis,

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whether to respond with the left or the right hand to a simple visual stimulus. This presumably leads to high levels of response readiness and to an unstable balance between left and right motor cortex activations, possibly allowing even subtle changes in cortical excitability to become manifest in overt voluntary behaviour. Moreover, this paradigm allows to study effects on automatic motor processes. On each trial, the target stimulus is preceded by a ‘prime’, which is a stimulus either mapped to the same response as the subsequent target (compatible trial), or to the alternative response (incompatible trial). Primes are presented below identification threshold due to a masking procedure (see Fig. 1). Nevertheless, they systematically affect responses to the subsequent target (e.g. Schlaghecken and Eimer, 1997, 2000; Eimer and Schlaghecken, 1998). As evidenced by the lateralised readiness potential (LRP; an electrophysiological index of unimanual response preparation generated in the motor/premotor cortex, Neshige et al., 1988), an initial pre-activation of the response assigned to the prime is subsequently inhibited and turned into a pre-activation of the opposite response. Correspondingly, a ‘positive compatibility effect’ (PCE; faster and more accurate responses on compatible than on incompatible trials) is obtained with short prime-target intervals. With longer intervals, the PCE reverses into a ‘negative compatibility effect’ (NCE). To the extent that these processes triggered by subliminal primes are automatic rather than voluntary, they might be more susceptible to rTMS-induced changes in cortical excitability than overall RTs.

To investigate whether stimulation of cortical motor areas affects overt responses – either directly by influencing

overall reaction times, or indirectly, by influencing priming effects – 1 Hz rTMS was applied over left motor and (in a separate session) left premotor cortex before and after a masked prime task. In a control experiment, rTMS was applied over left somatosensory cortex.

2. Methods

Participants were 12 volunteers, aged 21–45 years (mean: 29.9), for the main experiment, and 7 volunteers, aged 25–45 years (mean: 33.0), for the control experiment. Participants gave informed consent. All participants were healthy, right-handed (handedness quotient (Oldfield, 1971): mean = 90.6, standard deviation, SD = 11.4), and had normal or corrected-to-normal vision.

EMG was recorded with AgCl disc electrodes (1 cm diameter) placed in differential pairs over the right first dorsal interosseous (FDI) muscle, using a belly-tendon montage. Signals were sampled at 5 kHz, amplified and analog filtered (32 Hz–1 kHz), and displayed online on a computer.

TMS was administered using a hand-held figure-of-eight coil (outer winding diameter: 70 mm) connected to a Magstim Rapid stimulator, while participants sat in a comfortable chair. Magnetic pulses were biphasic with a pulse width of about 300 μ s. The coil was placed tangentially to the scalp with the handle pointing backwards and laterally at a 45° angle away from the midline, approximately perpendicular to the central sulcus, inducing a posterior-anterior current in the brain. Motor cortex hand

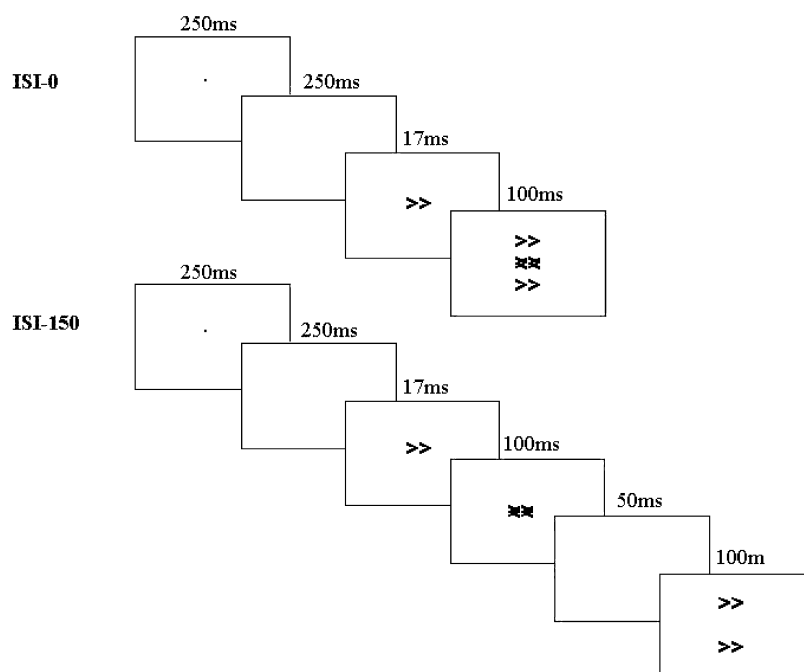


Fig. 1. Trial structure and timing in ISI-0 (top) and ISI-150 (bottom) blocks. Examples show compatible trials only.

area was determined by moving the coil in 0.5 cm steps around the presumed ‘hot spot’ to the site where slightly suprathreshold stimuli consistently produced the largest MEPs with the steepest negative slope. Pre-motor rTMS was applied to a position 3 cm anterior to this site (Münchau et al., 2002). Somatosensory rTMS was applied 3 cm posterior to the motor ‘hot spot’

Before and after rTMS, motor thresholds were measured by reducing stimulus intensity in 1% steps from supra-threshold levels. Active threshold was the lowest stimulus intensity triggering MEPs of 200 μ V in the tonically contracting FDI in 5 out of 10 consecutive trials. Resting threshold was the first stimulus intensity failing to produce MEPs of more than 50 μ V in 5 out of 10 subsequent trials.

Single trains of 20 min duration (1200 pulses) were applied in each session. Intensity was set at 80% of active motor threshold for each participant, to avoid spread of activity from the target area to adjacent areas (Münchau et al., 2002). Stimulation variables were in accordance with published safety recommendations (Wassermann, 1998). Motor and pre-motor rTMS sessions took place on 2 separate days with an interval of at least 5 days, the sequence being randomised across participants.

2.1. RT task

Prime and targets were left- and right-pointing double arrows (‘<<’ and ‘>>’), subtending a visual angle of approximately $1.7^\circ \times 0.6^\circ$. Superimposed left- and right-pointing double arrows served as mask. Stimuli were presented in black on a white background of a 9" computer screen. Participants sat in a dimly lit, quiet room in front of a laptop computer (viewing distance approximately 50 cm). They responded as fast and accurately as possible to left- or right-pointing target arrows by pressing the left or right SHIFT key, respectively.

Experimental blocks consisted of 40 trials each. Trials started with a 250 ms fixation dot, followed by a 250 ms empty interval. Primes were presented centrally for 17 ms, immediately followed by a 100 ms central mask. Targets were slightly displaced, with two identical targets appearing 1.7° above and below fixation (i.e. just above and below the outer contours of the mask) for 100 ms. In half of the blocks, mask and targets appeared simultaneously (prime-target inter-stimulus interval (ISI) 0 ms; ISI-0 blocks). In the other half, the mask appeared alone, followed by a 50 ms empty screen, followed by the targets (prime-target ISI 150 ms; ISI-150 blocks). See Fig. 1 for details. Trials were termed ‘compatible’ when prime and target were identical, and ‘incompatible’ otherwise. Compatibility conditions and target directions were equiprobable and randomised within each block.

Each ISI condition was delivered in 3 blocks before stimulation and in 3 blocks afterwards, resulting in a total of 12 experimental blocks, each taking approximately 1.5 min. Before stimulation, each condition was delivered

in 3 successive blocks, each series starting with a short practice block (20 trials). After stimulation, both conditions were delivered in an alternating sequence. Order of blocks was balanced across participants.

2.2. Statistical analysis

Two participants in the main experiment produced more than 40% errors in one or more conditions and were excluded from analysis. For the remaining 10 participants, repeated measures analyses of variance (ANOVA) was performed on correct response RTs for the factors Compatibility (compatible, incompatible), ISI (0 ms, 150 ms), Stimulation (before, after), Site (motor, premotor), and Hand (left, right). Significant main effects in the ANOVA were followed by direct post-hoc comparisons of the two conditions using paired *t* tests. Resting and active motor thresholds before and after rTMS were compared using paired *t* tests. For the control experiment, an ANOVA was performed on correct response RTs for the factors Compatibility, ISI, Stimulation, and Hand.

3. Results

3.1. Motor thresholds

As expected (Münchau et al., 2002), thresholds were not affected by rTMS (all $t(9) < 1.6$, all $P \geq 0.2$). Resting thresholds in the pre-motor and motor sessions were 58.2% (SD = 6.9) and 58.7% (SD = 7.9) before rTMS, and 57.6% (6.7) and 58.0% (8.9), respectively, after rTMS. Active thresholds were 47.4% (4.0) and 47.8% (5.4) before rTMS, and 46.8% (3.5) and 47.8% (5.6) after rTMS.

3.2. Overall RTs (see Fig. 2)

No significant main effect of Stimulation was found in either the main experiment ($F(1, 9) < 3.7$, $P > 0.08$) or the control experiment ($F(1, 6) < 3.1$, $P > 0.13$). Instead, an interaction of Stimulation \times Hand was observed in the main experiment ($F(1, 9) = 9.3$, $P = 0.014$), as right-hand responses, but not left-hand responses, were slower after stimulation. This was confirmed by subsequent *t* tests, conducted for each hand separately and collapsed across stimulation sites (right hand: $t(9) = 2.7$, $P = 0.024$; left hand: $t < 0.4$, $P > 0.76$). Importantly, no such selective slowing was observed in the control experiment (interaction Hand \times Stimulation: $F(1, 6) < 0.3$, $P > 0.64$). Fig. 2 does indeed indicate that both left- and right-hand responses – if anything – tended to be faster after somatosensory rTMS. In the main experiment, motor cortex stimulation tended to increase RTs more than premotor cortex stimulation (right hand: motor cortex +15 ms, premotor cortex +8 ms; left hand: –4 and +6 ms, respectively), although neither the Site \times Stimulation nor the Site \times Stimulation \times Hand

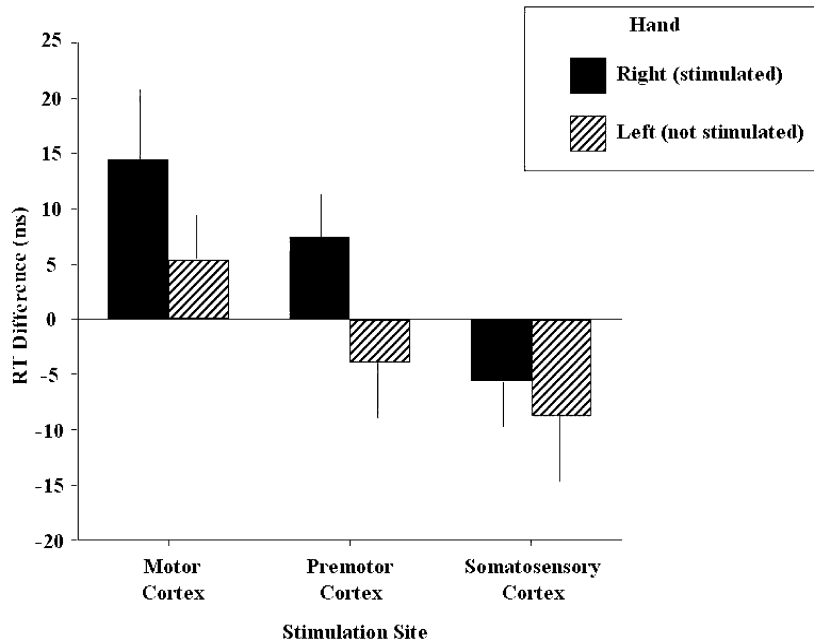


Fig. 2. Effects of rTMS – Reaction time difference (in ms) between pre-rTMS blocks and post-rTMS blocks, collapsed across ISIs and priming conditions, and plotted separately for rTMS sites in the main experiment (motor cortex, premotor cortex) and the control experiment (somatosensory cortex) and for each hand. Positive values indicate slower responses after rTMS, negative values indicate faster responses after rTMS. Error bars indicate SEM.

interaction were significant (both $F(1,9) < 3.3$, both $P > 0.10$).

3.3. Compatibility effects (see Fig. 3)

Overall, RTs were faster on incompatible than on compatible trials ($F(1,9) = 27.0$, $P = 0.001$), and faster in

ISI-0 blocks than in ISI-150 blocks ($F(1,9) = 6.59$, $P = 0.030$). As expected, ISI interacted with Compatibility ($F(1,9) = 89.2$, $P < 0.001$), with a PCE in ISI-0 blocks (19 ms) and a NCE in ISI-150 blocks (-43 ms; both $t(9) > 7.2$ both $P < 0.001$; similar effects observed in the control experiment will for reasons of brevity not be discussed here). Importantly, there was no interaction of Compatibility and Stimulation ($F(1,9) = 0.4$, $P > 0.5$), and

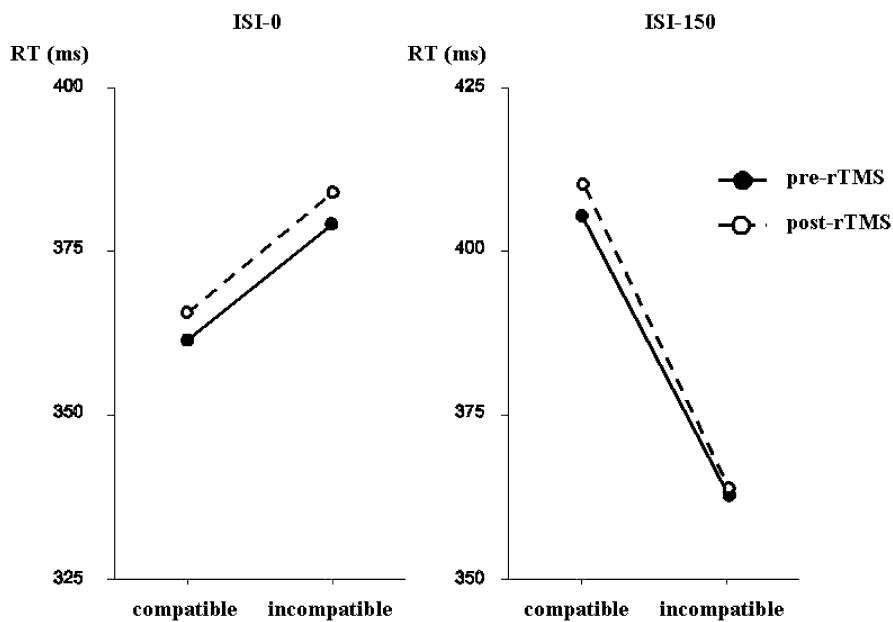


Fig. 3. Priming effects – reaction times (in ms) on compatible and incompatible trials in ISI-0 blocks (left panel) and ISI-150 blocks (right panel), collapsed across rTMS sites and hands, and plotted separately for pre-rTMS blocks (filled circles and solid lines) and post-rTMS blocks (open circles and broken lines).

Table 1

Summary of priming effects in milliseconds (in compatible RT minus incompatible RT) before and after rTMS, and difference between effect sizes before and after rTMS

	ISI-0			ISI-150		
	Pre-rTMS	Post-rTMS	Difference	Pre-rTMS	Post-rTMS	Difference
<i>Motor cortex</i>						
Left hand	2.4 (6.0)	19.2 (3.5)	+16.8**	-43.6 (8.8)	-46.6 (7.7)	+3.0
Right hand	31.1 (3.4)	21.0 (2.6)	-11.1	-52.1 (8.2)	-52.1 (10.2)	0
<i>Pre-motor cortex</i>						
Left hand	21.2 (4.5)	18.1 (6.9)	-3.1	-37.2 (6.2)	-45.9 (4.1)	+8.7*
Right hand	17.2 (3.6)	19.2 (4.3)	+2.0	-51.4 (6.9)	-53.3 (7.6)	+1.9
<i>Somatosensory cortex</i>						
Left hand	12.5 (2.6)	10.9 (4.6)	-1.6	-58.8 (6.5)	-43.0 (8.7)	-15.8
Right hand	16.8 (5.1)	23.2 (2.1)	+6.4	-57.3 (8.5)	-47.6 (11.0)	-9.7

Numbers in brackets indicate one standard error. *Indicates significance at 5% level. **Indicates significance at 1% level.

none of the interactions including both factors reached significance¹.

Since rTMS effects on RTs were expected – and found – only for the right hand, compatibility effects were re-analysed for right-hand responses separately. Again, none of the interactions including both Compatibility and Stimulation as a factor were significant (all $F < 1.6$, all $P > 0.23$), confirming that rTMS did not systematically affect priming effects (see also Table 1).

4. Discussion

To our knowledge, this is the first study documenting inhibitory effects of long trains of low-frequency, low-intensity motor and premotor rTMS on reaction times in healthy humans. In a speeded choice RT task, responses with the corresponding hand of the stimulated motor/premotor cortex were significantly slower after rTMS than before. Since this effect was not observed for the unstimulated hand, and did not occur after stimulation of the somatosensory cortex, it cannot be explained by general factors like fatigue after the rTMS procedure. In the studies cited above (Chen et al., 1997; Muellbacher et al., 2000), no such effects were observed, although considerably higher stimulation intensities were used (115% resting motor

threshold) than in our study (80% active motor threshold). Several possible explanations for this apparent discrepancy come to mind. First, stimulation at higher intensities may lead to recruitment of other interneurons or exert effects beyond the area of focal stimulation (Gerschlagler et al., 2001; Münchau et al., 2002). Such additional effects might induce an increased variance in the timing of motor output, thereby ‘masking’ any inhibitory effects of motor cortex rTMS on overt responses. Second, since the relationship between stimulation intensity and motor facilitation/inhibition has not been fully investigated yet, the possibility can not be ruled out that with higher stimulation intensities, some facilitatory effects occur together with the intended inhibitory effects. Again, this would result in an increased variance in the timing of motor output. Third, the above-mentioned studies employed tasks that induced low levels of response readiness and a constant response bias. In contrast, we studied visually cued motor behaviour in a task that required high levels of response readiness and unbiased cortical activation levels. Presumably, these conditions made overt performance more susceptible to subtle changes in cortical excitability.

In the light of the present results, the finding that priming effects were not influenced by rTMS is surprising: inhibition of cortical motor neurons – as evidenced by prolonged RTs – might reasonably be expected to result in a decreased responsiveness of these neurons to the relatively ‘weak’ (sub-threshold) activations triggered by masked primes. Consequently, reduced priming effects should have been observed. The fact that this was not the case might indicate that masked priming effects are generated at earlier stages of visuo-motor processing. One candidate structure could be the basal ganglia, which has been implied in a functional magnetic resonance imaging study of masked priming in healthy participants (Aron et al., 2003) and

¹ Three of these approached at least the 10% level (Compatibility × Site × Stimulation × Hand, Compatibility × ISI × Stimulation × Hand, Compatibility × Site × ISI × Stimulation × Hand: all $F > 3.2$, all $P < 0.11$). Remaining 4 interactions: all $F < 1.4$, all other $P > 0.28$. Generally, PCE and NCE were larger for right-hand responses than for left-hand responses ($F(1, 9) = 6.83$, $P = 0.028$), and the overall advantage for incompatible responses was more pronounced in the right hand in the pre-motor rTMS session, but in the left hand in the motor rTMS session: ($F(1, 9) = 8.17$, $P = 0.019$). However, since these effects were not accompanied by corresponding interactions with the factor Stimulation, they can not be regarded as being due to rTMS.

in a behavioural study with patients suffering from Huntington's disease (Aron et al., 2003).

One remaining question is why behavioural effects did not differ significantly between motor and premotor rTMS – especially since with comparable stimulation parameters, motor and premotor rTMS resulted in differential electrophysiological effects (Gerschlagler et al., 2001; Münchau et al., 2002). Possibly, the sample size was not large enough to detect subtle differences with sufficient power. Alternatively, one could hypothesise that the similarity of behavioural effects reflects the intimate topographical and functional cortico-cortical connections between motor and premotor cortex, particularly concerning processing of visuomotor information (Kawashima et al., 1994; Schluter et al., 1998). Changes in motor behaviour might ensue whenever activity in either motor or premotor cortex is altered, implying conjoint or parallel rather than sequential processing in these areas.

To summarise: low intensity 1 Hz rTMS over the motor and premotor cortex can cause

- slowing of responses in visually cued RT tasks in the hand contralateral to stimulation,
- without affecting masked priming effects, supporting the notion that these phenomena are generated outside the motor/premotor area.

Results illustrate that detection of behavioural changes after rTMS not only depend on stimulation parameters, but also on the choice of the performance test. Consequences of rTMS will only become clearer with further studies examining a larger range of motor functions.

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