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Effects of low frequency and low intensity repetitive paired pulse stimulation of the primary motor cortex

Eman M. Khedr[a], Francesca Gilio[b], John Rothwell[c,*]

[a]*Department of Neurology, The University Hospital, Assiut, Egypt*
[b]*INM Neuromed IRCCS, Pozilli, University of Rome La Sapienza, Rome, Italy*
[c]*Sobell Research Department of Motor Neuroscience and Movement Disorders, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK*

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Abstract

Objective: Following a previous report [Bestmann et al. Clin Neurophysiol 2004;115:755–64] that pairs of subthreshold pulses of transcranial magnetic stimulation (TMS) can show temporal summation, we explored whether repeated application of pairs of stimulation could produce long-lasting after effects on the excitability of the human motor cortex.

Methods: Twelve healthy subjects received 25 min repetitive paired pulse magnetic stimulation (paired rTMS) given at a frequency of about 0.6 Hz over the left primary motor cortex (500 paired stimuli in total). The interval between the paired stimuli was 3 ms and the intensity of both stimuli was 80% of active motor threshold. The resting and active motor threshold, MEP recruitment curve, short interval intracortical inhibition (SICI) and facilitation, and the duration of the cortical silent period (SP) were tested for the right first interosseous muscle (FDI) before and two times after the end of 25 min paired rTMS.

Results: Prolonged subthreshold paired rTMS produced a significant decrease in excitability in the corticospinal projection to FDI: resting motor threshold was significantly increased and MEP recruitment was significantly decreased, SICI was significantly increased at 2 and 4 ms and the SP was significantly increased in duration.

Conclusions: Prolonged low frequency paired rTMS at subthreshold intensity can modulate cortical excitability by producing inhibitory effects that outlast the period of stimulation.

Keywords: Cortical excitability; Repetitive transcranial magnetic stimulation; Paired pulse stimulation

1. Introduction

A large number of studies have investigated in detail the after effects of repetitive transcranial magnetic stimulation (rTMS) (e.g. Siebner and Rothwell, 2003). In the human motor cortex rTMS can change the excitability of corticospinal projections for minutes and even hours after the period of stimulation (Berardelli et al., 1998; Chen et al., 1997; Maeda et al., 2000; Pascual-Leone et al., 1994, 1998; Peinemann et al., 2000; Rossi et al., 2000; Siebner et al., 2000; Tergau et al., 1997; Wu et al., 2000). These effects depend on the intensity, frequency and duration of the rTMS (Chen et al., 1997; Maeda et al., 2000; Muellbacher et al., 2000; Pascual-Leone et al., 1994; Touge et al., 2001; Tsuji and Rothwell, 2002).

Many authors have concentrated on the effects of low frequency stimulation since this tends to lead to lasting inhibitory effects and is therefore regarded as safer than high frequency stimulation (Wassermann et al., 1998). In motor cortex, 1 Hz stimulation at intensities of around resting motor threshold (RMT) suppresses MEPs. Lower intensities of stimulation (e.g. 80% active motor threshold (AMT) as used by Gerschlager et al., 2001) have no effect on MEP amplitude. None of the studies using 1 Hz rTMS on the motor cortex of healthy subjects have reported any effects on short interval intracortical inhibition (SICI) or silent period (SP).

The implication from these studies is that the after effects of rTMS on MEP amplitude require stimulus intensities that

* Corresponding author.
E-mail address: j.rothwell@ion.ucl.ac.uk (J. Rothwell).

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are higher than the intensities that are known to produce paired pulse inhibition (i.e. around 70% AMT). This may be because low threshold elements do not participate in neural circuits that show long-term adaptation. An alternative explanation is that selective stimulation of these elements requires such low intensities that too small a proportion of the available inhibitory elements are activated to lead to lasting changes in excitability. Unfortunately, increasing the intensity of rTMS in order to recruit a larger fraction of the population would also recruit additional elements, and these might be responsible for the effects that have been observed in previous experiments. To test whether very low intensity rTMS is capable of producing after effects on MEP excitability we employed a new method described by Bestmann et al. (2004) to increase activation of low threshold inhibitory circuits. This involves using pairs of low intensity pulses at short interstimulus intervals. The effects of these two pulses appear to summate, and produce much larger inhibition of test MEPs in a paired pulse SICI paradigm that are expected from the sum of each alone.

We therefore used repeated pairs of low intensity TMS in the present experiments to condition the motor cortex and examine whether this would lead to long-term effects on MEP size, threshold and measures of intracortical inhibition. Even though the maximum frequency that we could use was limited to 0.6 Hz, we observed substantial after effects that had not previously been reported using single pulses of the same intensity.

2. Methods

2.1. Subjects

We studied 12 right-handed healthy volunteers (6 males and 6 females; mean age 35.5 ± 8.43 years; range 18–44 years old). All of them gave their informed oral consent to the study, which was approved by the ethical committee. The subjects had neither a psychiatric medical history nor contraindications to TMS (Wassermann et al., 1998). They were seated comfortably in a reclining chair and instructed to relax throughout the experiment.

2.2. Stimulation technique

2.2.1. Single and paired TMS

Single and paired TMS were performed using two High Power Magstim 200 stimulators connected through a Y cable with a figure-of-eight coil (mean loop diameter of 9 cm). The coil was placed tangentially over the left primary motor cortex in the optimal position for activation of the right FDI muscle. Resting and active motor thresholds (RMT, AMT) were defined as the minimum output of the stimulator that induced reliable MEP (50 and 200 μV in amplitude, respectively, at rest and during contraction) in at least 5 of 10 consecutive trials when the FDI muscle was completely relaxed or during tonic contraction of the contralateral FDI muscle. The level of contraction was maintained constant with reference to an oscilloscope display and additional auditory feedback. Values of AMT lay between 75 and 85% RMT.

The MEP recruitment curve was made by increasing the intensity of stimulation using steps of 10% starting from 110 to 150% of RMT. At each intensity, 10 trials were collected with intertrial intervals of 5 s and averaged.

Paired TMS as described by Kujirai et al. (1993) was delivered using a conditioning stimulus intensity of 80% AMT and a test stimulus capable of producing an MEP in relaxed muscle of ~1 mV peak to peak. Conditioning-test intervals were 2, 4, 6, 8, 10, and 12 ms given in random order and intermixed with responses to the test stimulus alone in one block of 70 trials with an intertrial interval of 4 s.

Single TMS stimuli were also delivered during isometric voluntary contraction (~50% maximal contraction) of the right FDI muscle in order to measure the duration of the silent period. The intensity of stimulation was the same used to evoke the test stimulus in the Kujirai's paradigm. Ten trials were collected with intertrial intervals of 5 s.

2.2.2. Paired rTMS

Repetitive paired pulse stimulation was delivered using two Magstim 200 stimulators and one rapid rate Magstim stimulator connected with a figure-of-eight coil through a purpose built combining box. The intensity of all stimuli was set at 80% AMT and was defined separately for all of the stimulators. The first stimulus was delivered alternately from one of the two Magstim 200 stimulators and the second stimulus was always delivered from the repetitive Magstim stimulator with an interstimulus interval of 3 ms. 500 paired stimuli were delivered with a frequency of 0.6 Hz (25 min in total). Thus the first stimulus of the pair was a monophasic stimulus that induced an anterior–posterior current in the brain, whilst the second was a biphasic stimulus with a PA/AP waveform.

2.3. Recording system

EMG activity was recorded through a pair of Ag–AgCl surface electrodes (1 cm-diameter) placed over the right FDI muscle, using a belly-tendon montage. The signal was amplified, analog filtered (5 Hz–1 kHz) by Digitimer D 360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK) and acquired at a sample rate of 5 kHz. Data were stored on a personal computer for off-line analysis (Signal software; Cambridge Electronic Devices, Cambridge, UK).

2.4. Measurements

The MEP amplitude was measured peak to peak (mV). In the paired TMS paradigm the amplitude of the test MEP was expressed as a percentage of the control MEPs. The duration of the SP was measured in each individual rectified trial

from the end of the MEP elicited by the suprathreshold TMS pulse to the onset of continuous EMG activity after the period of EMG suppression.

2.5. Experimental paradigms

Before paired rTMS (pre), and two times after the end of the stimulation over the left motor area, we evaluated the resting and active motor thresholds, the MEP recruitment curve, the time course of intracortical inhibition and facilitation and the duration of the SP. The first evaluation was performed immediately following paired rTMS (post1) and the second block started 30 min after the end of paired rTMS (post2).

2.6. Statistical analysis

All results were expressed as mean ± SE. The effects of paired rTMS on the MEP recruitment curve were evaluated using the natural logarithm of the data by two-way repeated-measures ANOVA with *time* before and after paired rTMS and *intensity* of stimulation as within-subject main factors. The effects of paired rTMS on the time course of SICI–ICF were evaluated by two-way ANOVA with *time* and *interstimulus interval* (ISI) as within-subject main factors. To increase the power of the analysis we reduced the number of ISI entered into the analysis by averaging data from adjacent intervals for the inhibition period (ISIs of 2 and 4 ms) and the facilitation period (ISIs of 10 and 12 ms). Post-hoc analysis was performed by paired sample *t* test.

The effects on the RMT and AMT, on SP duration and MEP size during voluntary muscle contraction were evaluated by one-way ANOVA with time before and after paired rTMS as within-subject main factor. The Greenhouse–Geisser correction was used when necessary to correct for non-sphericity. A P value of <0.05 was considered significant for all statistical analyses.

3. Results

With the parameters of stimulation used in this experiment, none of the subjects reported adverse effects during and after paired rTMS.

25 min paired rTMS had a significant effect on RMT (one way ANOVA: pre, post1, post2; $F_{(2,20)} = 10.9$, $P < 0.001$). Post-hoc testing showed that there was a significant increase after paired rTMS (pre vs. post1 $P = 0.001$; pre vs. post2 $P = 0.019$). There was no effect on AMT (Fig. 1A).

Fig. 1B illustrates the effect of 25 min paired rTMS on the MEP recruitment curve. A two-way repeated measures ANOVA was performed after log transformation of the amplitude data to normalize its distribution. It revealed a significant effect of the factor time ($F_{(1.6,16)} = 6.94$, $P = 0.005$) and intensity ($F_{(1.7,17.5)} = 68.6$, $P < 0.0001$)

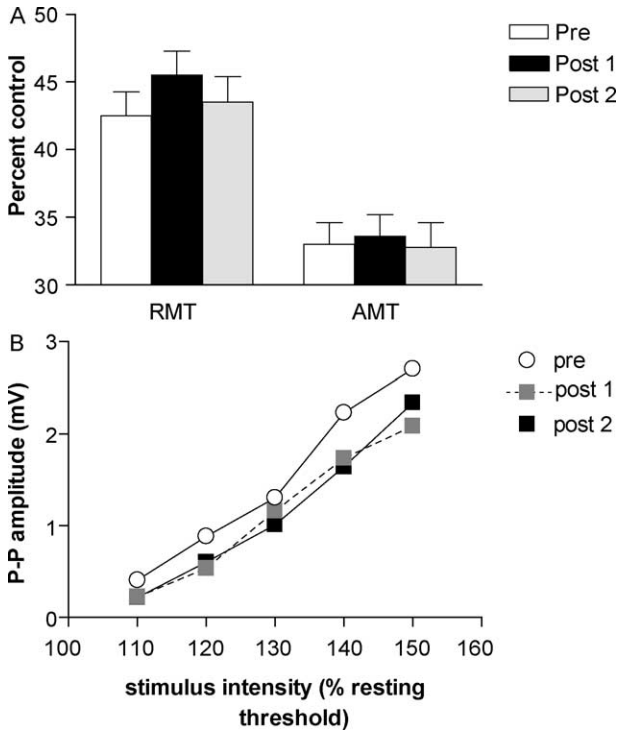

Fig. 1. (A) Effects of 25 min paired pulse rTMS delivered over the left motor cortex on resting and active motor thresholds. Values correspond to the stimulator power expressed as means ± SE%. (B) Effects of 25 min paired pulse rTMS on the input–output curve of the left primary motor cortex. Pre, MEP amplitude before repetitive stimulation; Post1, immediately after repetitive stimulation; Post2, 30 min after repetitive stimulation. Data correspond to the amplitude of the MEP expressed as means ± SE mV.

(Fig. 2). MEPs were smaller at all intensities in both of the post-rTMS testing periods compared with control.

Fig. 2A illustrates the effect of paired rTMS on the time course of SICI/ICF. The data are plotted as the peak–peak amplitude of the MEP responses. A two-way ANOVA showed a significant effect of the factor time on the mean MEP amplitude ($F_{(1.8,18.5)} = 4.1, p = 0.03$) and ISI ($F_{(1.6,16.3)} = 18.2$, $P < 0.0001$) without significant interaction time–ISI. Post-hoc analysis showed that immediately after the end of paired rTMS the mean value of the MEP amplitude significantly decreased in comparison with the pre-rTMS condition (pre-rTMS vs post1: $F_{(1,11)} = 6.8$, $P = 0.02$; pre-rTMS vs post2: $F_{(1,10)} = 1.8$, $P = 0.2$). Fig. 2B plots the same data as percentage inhibition for the inhibitory (ISI = 2, 4 ms) and facilitatory (ISI = 10, 12 ms) periods. A two-way ANOVA showed a significant effect of factor time ($F_{(2,20)} = 4.9$, $P = 0.018$) and ISI ($F_{(1,10)} = 81.5$, $P < 0.0001$). Post-hoc analysis showed a significant increase in SICI at post1 in comparison with pre-paired rTMS (pre vs. post1 $P < 0.0001$; pre vs. post2 $P = 0.06$). There were no significant paired differences for ICF.

Time considerations did not allow us to re-evaluate the strength of SICI and ICF after adjusting the stimulus intensity to correct for the change in amplitude of

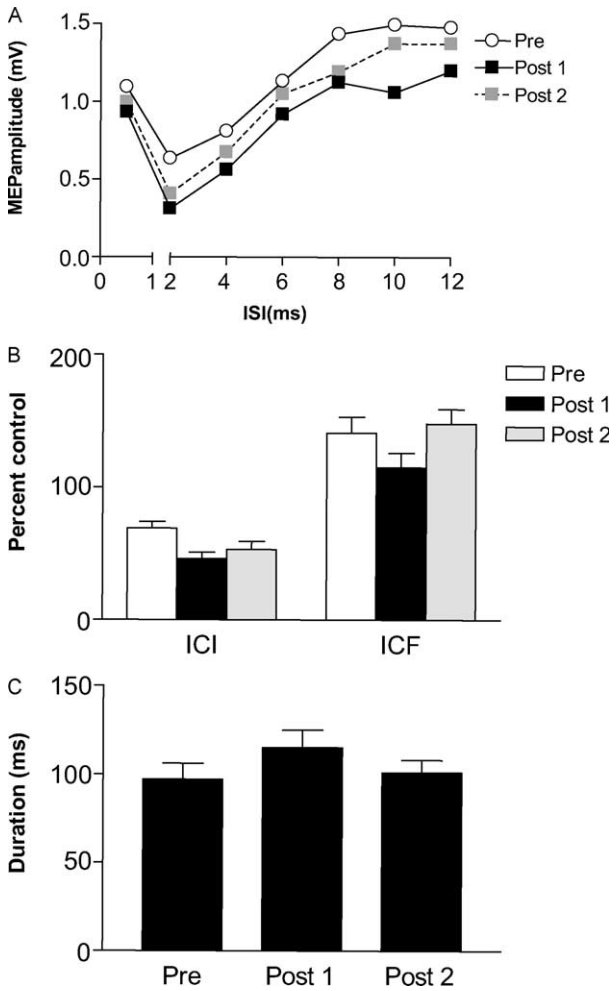

Fig. 2. (A) Effects of repetitive paired pulse stimulation on the SICI/ICF of the left primary motor cortex. Pre, MEP amplitude before repetitive stimulation; Post1, immediately after repetitive stimulation; Post2, 30 min after repetitive stimulation. Data correspond to the amplitude of the test MEP expressed as means ± SE mV (upper panel) and as means ± SE percentage of the control MEP (lower panel). (B) Effects of 25 min paired pulse-rTMS on the inhibitory (SICI corresponds to the average of 2 and 4 ms) and facilitatory (ICF corresponds to the average of 10 and 12 ms) intervals. Data correspond to the amplitude of the test MEP expressed as means ± SE percentage of the control MEP. (C) Effects of paired pulse-rTMS on the duration of the cortical SP. Data correspond to the duration of the contralateral SP expressed as means ± SE ms.

the control MEP. However, given that this effect was small, we think it likely that that we were operating in the linear range of control MEP amplitudes, where small differences in size have little effect on calculations of percent SICI/ICF (Ridding et al., 1995; Sanger et al., 2001).

Fig. 2C illustrates the duration of the SP before and after paired rTMS. One-way ANOVA showed a significant effect of the factor time ($F_{(2,20)} = 4.7$, $P = 0.02$). Post-hoc analysis demonstrated a significant increase in the SP duration at post1 in comparison with the basal SP (pre vs. post1 $P = 0.006$; pre vs. post2 $P = 0.68$; post1 vs. post2 $P = 0.08$).

4. Discussion

The main finding of this study is that 25 min of repetitive paired pulse stimulation at low frequency and subthreshold intensity delivered over the left primary motor cortex produces a significant reduction of cortical excitability at the point of stimulation. Thus, MEP amplitudes are reduced and resting thresholds increased whilst two measures of cortical inhibition, SICI and silent period are enhanced.

Previous studies of low frequency rTMS with single pulses of the same intensity as we used here did not have any effect on MEP amplitude (Gerschlager et al., 2001; 1 Hz, 80% AMT), and no effects on SICI or SP have been described in healthy subjects after 1 Hz rTMS even with higher intensities of stimulation (Fitzgerald et al., 2002; 1 Hz up to 115% RMT). Thus the present results indicate that pairs of pulses have an unexpectedly powerful influence on motor cortex. As we outlined in the Introduction, the reason for this may well be that pairs of low intensity pulses can summate and activate cortical inhibitory elements more effectively than single pulse stimulation.

We did not test directly whether any of the effects on MEP amplitude were due to changes in spinal rather than cortical excitability. However, given the low intensity of the conditioning pulses, we do not think there was any direct activation of corticospinal projections to spinal cord during the rTMS. In addition, we observed changes in SICI, which is thought to be a test of excitability in motor cortical circuits independent of spinal cord. Thus, it seems likely that the majority, if not all, of the effects we observed were due to changes in motor cortex rather than subcortical structures.

Repetitive pairs of TMS pulses were also used in a study by Sommer et al. (2001, 2002). Although they also used a 3 ms (and a 10 ms) interval, their pulses were of unequal size: the first was 90% AMT whilst the second was capable of eliciting an MEP of about 1 mV in relaxed muscle. In the first study (Sommer et al., 2001), they gave only 80 pairs of pulses at frequencies from 1 to 5 Hz, and compared the effects with single stimuli given at the same frequency. At 1 Hz, neither single nor paired pulses at 3 ms had any effect on MEP amplitude during or after the conditioning train. At 5 Hz, pairs of stimuli at 3 ms produced a slight increase in MEP during but not after the conditioning. The authors concluded that pairs of stimuli were not superior to single pulse rTMS in producing lasting inhibition or facilitation. In a second study (Sommer et al., 2002), they applied a larger number (900) of unequal pulses at 1 Hz and observed a transient decrease in MEP amplitude if the interval between the pairs was 3 ms. Longer trains of single pulse conditioning stimuli have also been reported to have a longer lasting and more robust effect on motor cortex by Touge et al. (2001) when they used rTMS at 1 Hz.

The present data were similar to the second study of Sommer et al. (2002), and showed a clear advantage of paired over single pulse rTMS. We could also show effects

on ICI/ICF and silent period. The conclusion is that the paired pulse approach can be successful even when both stimuli of the pair are below active motor threshold. Indeed, the unequal combination of sub-threshold and supra-threshold stimuli used by Sommer et al. (2001, 2002) may activate a combination of inhibitory and excitatory elements in the cortex and spinal cord that could potentially lead to opposing effects at the end of a train of rTMS and complicate the interpretation of the results. By limiting our stimuli to below AMT, we may have favoured effects at a cortical level.

Finally, what is the explanation of the effects we have observed? Why do pairs of subthreshold stimuli lead to an increase in SICI and SP, and a decrease in excitability of the MEP? We would like to suggest that the primary effect of the paired rTMS is to increase the effectiveness of inhibition and that this secondarily depresses MEPs. Although we cannot rule out the possibility that the pairs of stimuli had direct effects on excitatory systems, the data would be consistent with the fact that low intensity pairs of TMS pulses produce particularly strong activation of cortical inhibitory circuits. One possible explanation of the after effects would be to view the repeated pairs of pulses as equivalent to a single highly effective inhibitory stimulus. If this were the case, then we would have to assume that repeated stimulation at the low frequency of 0.6 Hz produced facilitation of this system. Another possibility is that the pairs of pulses could be regarded as a short burst of high frequency stimulation (i.e. a two pulse burst at 333 Hz). This might tend to produce facilitation that is reinforced by repeated presentation at 0.6 Hz. If this were the case, then larger effects might be predicted if the number of pulses in each burst was larger, or if the bursts were presented at a higher frequency. Unfortunately this cannot be tested with presently available stimulators, and may have to await further developments in stimulator design.

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References

Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Curra A, Gilio F, Modugno N, Manfredi M. Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res 1998;122:79–84.

Bestmann S, Siebner HR, Modugno N, Amassian VE, Rothwell JC. Inhibitory interactions between pairs of subthreshold conditioning stimuli in the human motor cortex. Clin Neurophysiol 2004;115:755–64.

Chen R, Gerloff C, Classen J, Wassermann EM, Hallett M, Cohen LG. Safety of different inter-train intervals for repetitive transcranial magnetic stimulation and recommendations for safe ranges of stimulation parameters. Electroencephalogr Clin Neurophysiol 1997; 105:415–21.

Fitzgerald PB, Browna TL, Daskalakisb ZJ, Chenc R, Kulkarnia J. Intensity-dependent effects of 1 Hz rTMS on human corticospinal excitability. Clin Neurophysiol 2002;113:1136–41.

Gerschlager W, Siebner HR, Rothwell JC. Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. Neurology 2001;57(3):449–55.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD. Corticocortical inhibition in human motor cortex. J Physiol (Lond) 1993;509:607–18.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. Clin Neurophysiol 2000;111:800–5.

Muellbacher W, Ziemann U, Boroojerdi B, Hallett M. Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. Clin Neurophysiol 2000;111:1002–7.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994;117:847–58.

Pascual-Leone A, Tormos JM, Keenan J, Tarazona F, Canete C, Catala MD. Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol 1998;1(15): 333–43. (Review).

Peinemann A, Lehner C, Mentschel C, Munchau A, Conrad B, Siebner HR. Subthreshold 5-Hz repetitive transcranial magnetic stimulation of the human primary motor cortex reduces intracortical paired-pulse inhibition. Neurosci Lett 2000;15(296):21–4.

Ridding MC, Taylor JL, Rothwell JC. The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 1995; 487:541–8.

Rossi S, Pasqualetti P, Rossini PM, Feige B, Ulivelli M, Glocker FX, Battistini N, Lucking CH, Kristeva-Feige R. Effects of repetitive transcranial magnetic stimulation on movement-related cortical activity in humans. Cereb Cortex 2000;10:802–8.

Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. J Physiol 2001;530:307–17.

Siebner HR, Rothwell JC. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16.

Siebner HR, Peller M, Willoch F, Minoshima S, Boecker H, Auer C, Drzezga A, Conrad B, Bartenstein P. Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. Neurology 2000;22(54):956–63.

Sommer M, Tergau F, Wischer S, Paulus W. Paired-pulse repetitive transcranial magnetic stimulation of the human motor cortex. Exp Brain Res 2001;139:465–72.

Sommer M, Kamm T, Tergau F, Ulm G, Paulus W. Repetitive paired-pulse transcranial magnetic stimulation affects corticospinal excitability and finger tapping in Parkinson's disease. Clin Neurophysiol 2002;113: 944–50.

Tergau F, Tormos JM, Paulus W, Pascual-Leone A, Ziemann U. Effects of repetitive transcranial magnetic stimulation (rTMS) on cortico-spinal and cortico-cortical excitability. Neurology 1997;48:A107.

Touge T, Gerschlager W, Brown P, Rothwell JC. Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clin Neurophysiol 2001;112:2138–45.

Tsuji T, Rothwell JC. Long lasting effects of rTMS and associated peripheral sensory input on MEPs, SEPs and transcortical reflex excitability in humans. J Physiol 2002;540:367–76.

Wassermann EM, Wedegaertner FR, Ziemann U, George MS, Chen R. Crossed reduction of human motor cortex excitability by 1-Hz transcranial magnetic stimulation. Neurosci Lett 1998;250:141–4.

Wu T, Sommer M, Tergau F, Paulus W. Lasting influence of repetitive transcranial magnetic stimulation on intracortical excitability in human subjects. Neurosci Lett 2000;16(287):37–40.