Modulation of Motor Cortex Neuronal Networks by rTMS:

Comparison of Local and Remote Effects of Six Different Protocols of Stimulation

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) of human motor cortex can produce long-lasting changes in the excitability of excitatory and inhibitory neuronal networks. The effects of rTMS depend critically on stimulus frequency. The aim of present study was to compare the effects of different rTMS protocols. We compared the after-effects of six different rTMS protocols [paired associative stimulation at interstimulus intervals of 25 ms (PAS25), and 10 ms (PAS10); theta-burst stimulation delivered as continuous (cTBS) or intermittent (iTBS) delivery pattern; 1Hz and 5Hz rTMS] on the excitability of stimulated and contralateral motor cortex in 10 healthy subjects. A pronounced increase of cortical excitability, evaluated by measuring the amplitude of motor evoked potentials, was produced by iTBS (+56%) and PAS25 (+45%). Five Hz rTMS did not produce a significant increase of MEPs. A pronounced decrease of cortical excitability was produced by PAS10 (-31%), cTBS (-29%), and 1 Hz rTMS (-20%). Short interval intracortical inhibition was suppressed by PAS10. Cortical silent period duration was increased by 1 Hz stimulation. No significant effect was observed in the contralateral hemisphere. Head-to-head comparison of the different protocols enabled us to identify the most effective paradigms for modulating the excitatory and inhibitory circuits activated by TMS.

Key words: brain stimulation, TMS, rTMS, brain plasticity, LTP, LTD
Introduction

Transcranial magnetic stimulation (TMS) techniques can activate non-invasively the human brain evoking artificial activity in cortical neuronal networks (Hallett 2007). Technical advances have offered the possibility of delivering repetitive TMS (rTMS) and it has been observed that rTMS may induce changes in brain excitability that outlast the stimulation period. The after-effects of rTMS might relate to activity-dependent changes in the effectiveness of synaptic connections between cortical neurons reflecting plasticity mechanisms of the brain (see (Fitzgerald et al. 2006), (Ziemann U et al. 2008) and (Hoogendam et al. 2010) for a review). Low frequency rTMS (stimulus rates of 1 Hz or less) produces a lasting decrease in motor cortex excitability (Chen et al. 1997) while high frequency rTMS (stimulus rates of 5 Hz or higher) (Berardelli et al. 1998; Maeda et al. 2000; Peinemann et al. 2000) promotes a short term increase in cortical excitability. More recently, two novel protocols of rTMS that resemble experimental models of induction of long term potentiation (LTP) and long term depression (LTD) of synaptic activity have been introduced: the first is the so called paired associative stimulation (PAS) (Stefan et al. 2000) and the second one is termed theta-burst stimulation (Huang et al. 2005). PAS is based on the Hebbian concept of spike-timing-dependent plasticity: two inputs, the first arising from electrical peripheral nerve stimulation and the second delivered over the motor cortex using TMS, are
paired to activate brain networks at approximately the same time. If the TMS pulse is applied at an interstimulus interval slightly longer (PAS25) or slightly shorter (PAS10) than the time needed for the afferent inputs, generated by peripheral nerve stimulation, to reach the cerebral cortex and if a sufficient pairs of stimuli is delivered, the excitability of the sensory-motor cortex increases or decreases respectively. The second protocol is named theta burst stimulation (TBS) (Huang et al. 2005), and employs brief bursts of high frequency (50 Hz) low intensity stimuli. Different patterns of delivery of TBS (continuous versus intermittent) produce opposite effects on the excitability of the stimulated motor cortex (Huang et al. 2005). The paradigm named intermittent theta-burst stimulation (iTBS) produces a prolonged LTP-like increase of motor cortex excitability (Huang et al. 2005) while the paradigm named continuous theta-burst stimulation (cTBS) produces a prolonged LTD-like decrease of motor cortex excitability.

The majority of previous studies investigated the effects of rTMS on cortical excitatory circuits by measuring the amplitude of motor evoked potentials (MEPs) elicited by single pulse TMS, before and after rTMS; only a few studies evaluated the effects of rTMS on cortical inhibitory circuits (Daskalakis et al. 2006; Fitzgerald et al. 2006). The modulatory effects of rTMS are not limited to the cortical area targeted by rTMS but may also occur at distant interconnected sites in the brain and in particular in the contralateral, non
stimulated, motor cortex (Di Lazzaro et al. 2008; Gilio et al. 2003; Schambra et al. 2003; Suppa et al. 2008; Wassermann et al. 1998). Only a limited number of studies investigated this aspect. Moreover, because almost all of the studies employed a single rTMS protocol and there is a high inter individual variability of the after effects of rTMS (Maeda et al. 2000), at least in part related to genetic features (Cheeran et al. 2009), it is difficult to compare the results reported for different paradigms and it is still unclear which are the most effective protocols in modulating specific cerebral cortex circuits. In only one study, the effects of TBS on threshold and amplitude of MEPs were compared with those produced by 1 Hz and 5 Hz rTMS (Zafar et al. 2008).

The aim of this study was to compare in a group of healthy subjects the effects of different protocols of rTMS on the excitability of the excitatory and inhibitory circuits of the stimulated and contralateral motor cortex.

**Subjects and methods**

**Subjects**

Ten healthy volunteers (mean age 26.6±4.1 (SD) years) participated in the experiments. All gave their written informed consent. The study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the Medical Faculty of the Catholic University of Rome.

Eight of the subjects were non smokers.
TMS measures

TMS was performed with a high power Magstim 200 (Magstim Co., Whitland, Dyfed UK). A figure-of-eight coil, with external loop diameters of 9 cm, was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous (FDI) muscle. The optimum scalp position to elicit MEPs in contralateral FDI was determined on each session and was marked on the scalp with a felt-tip pen to assure a stable coil placement throughout the session. The experimenter performing TMS was the same across all six sessions. The induced current in the brain flowed in a posterior-to-anterior direction. MEPs were recorded via two 9 mm diameter Ag-AgCl surface electrodes with the active electrode over the motor point of the left FDI and the reference on the metacarpophalangeal joint of the index finger. The EMG was amplified and filtered (bandwidth 3 Hz-3 kHz) by D360 amplifiers (Digitimer, Welwyn Garden City, Herts UK). Data were collected on a computer with a sampling rate of 10 kHz per channel and stored for later analysis using a CED 1401 A/D converter (Cambridge Electronic Design, Cambridge UK).

Resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a liminal MEP (> 50 µV in at least 5 of 10 trials) at rest. Active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a small MEP (> 200 µV in 5 of 10 trials) during isometric contraction of the FDI at about 20% of maximum voluntary strength. A constant level of
voluntary contraction was maintained with reference to an oscilloscope display of the EMG signal in front of the subject. Auditory feedback of the EMG activity was also provided. RMT and AMT are given in percentage of maximum stimulator output (% MSO).

MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Ten sweeps of the data were collected, and the mean peak-to-peak amplitude of the MEPs was calculated. Contralateral SP were elicited whilst subjects held a tonic voluntary contraction of approximately 50% of MVC of the FDI contralateral to the stimulated hemisphere. Five stimuli at 200% AMT were given. The duration of cSP was measured from the end of MEP to the reappearance of sustained EMG activity. Ipsilateral SP were elicited whilst subjects held a tonic voluntary contraction of approximately 50% of MVC of the FDI ipsilateral to the stimulated hemisphere. Five stimuli at 200% AMT were given. The ipsilateral cortical silent period was measured according to the objective graphical method described by Garvey et al. (Garvey et al. 2001). This method allows an automated and objective estimation of onset and offset points, based on statistical analysis of variation of the baseline EMG activity (Garvey et al. 2001). EMG signal was sampled at 5 kHz. 100 ms of rectified averaged pre-stimulus EMG signal (that is 500 data points) were analysed to calculate the mean EMG level and the mean consecutive difference of the data points. Ipsilateral SP onset was the first point to fall below the lower
variation limit if 50% or more of the data points in the following 5 ms window were also below the lower variation limit. Ipsilateral SP offset was the first point to fall above the lower variation limit if 50% or more of the data points in the following 5 ms window were also above the lower variation limit. In order to automate the procedure, we used a self-made function for the Matlab software (The MathWorks, Inc. ©1984-2005). Ipsilateral SPs were measured before and after rTMS using the same stimulus intensity.

All measurements were performed with two magnetic stimulators connected to a figure of eight coil through a Y-shaped cable (all Magstim Co, UK) using a single stimulator for single pulse and SAI studies and two stimulators for paired-pulse paradigms.

Short Interval Intracortical inhibition (SICI)

SICI was studied using the technique of Kujirai et al. (Kujirai et al. 1993). Two magnetic stimuli were given through the same stimulating coil over the motor cortex at an interstimulus interval (ISI) of 2 ms and the effect of the first (conditioning) stimulus on the second (test) stimulus was investigated. Five single pulse stimuli and five paired stimuli at 2 ms ISI were delivered. The conditioning stimulus was set at an intensity of 5% of MSO below AMT. The intensity of the test stimulus was adjusted to elicit an unconditioned test MEP in
the relaxed FDI of approximately 1 mV in peak-to-peak amplitude. Subjects were provided with audio-visual feedback of the EMG at high gain (50 µV/D) to assist in maintaining complete relaxation, since already slight activation of the target muscle may result in significant SICI reduction (Ridding et al. 1995).

The amplitude of the conditioned MEP was expressed as a percentage of the amplitude of the unconditioned test MEP.

*Intracortical facilitation*

We also evaluated ICF by analysing the facilitatory interaction that occurs between pairs of magnetic stimuli given over the motor cortex at 15 ms interstimulus intervals. Five single pulse stimuli and five paired stimuli at 15 ms ISI were delivered. Subject was given audio-visual feedback at high gain to assist in maintaining complete relaxation. The amplitude of the conditioned MEPs was expressed as percentage of the amplitude of the test MEPs.

*Short Latency Afferent Inhibition (SAI)*

SAI was studied using the technique that we have described previously (Tokimura et al. 2000). Conditioning electrical pulses (constant current square wave pulses; duration, 200 µs) were applied through a bipolar electrode to the median nerve at the wrist (cathode proximal). The intensity of the conditioning stimulus was set to evoke a just visible twitch of the thenar muscles. The intensity of the TMS test pulse over the motor cortex was adjusted to evoke an
unconditioned MEP in the relaxed FDI of approximately 1 mV in peak-to-peak amplitude.

The conditioning stimulus to the median nerve preceded the TMS test pulse by interstimulus intervals (ISIs) that were related to the individual latency of the N20 component of the median nerve somatosensory evoked potential. To record somatosensory evoked potentials, the active electrode was attached 3 cm posterior to C4 or to C3 (according to the 10-20 International EEG system) and the reference was 3 cm posterior to C3 or C4, respectively. Five hundred responses were averaged to identify the latency of the N20 peak. ISIs corresponding to the N20 latency plus 2, 3, and 4 ms were investigated (Tokimura et al. 2000) with five repeats per ISI in a pseudo-randomized order. Subjects were given audio-visual feedback of the EMG signal at high gain (50 µV/D) to assist in maintaining complete relaxation of the FDI. The mean amplitudes of the conditioned MEPS at the various ISIs were expressed as a percentage of the mean amplitude of the unconditioned test MEP. These data were averaged across all ISIs to obtain a grand mean single value of SAI.

**Repetitive TMS**

Repetitive TMS was delivered over the right motor cortex “hot spot” for MEPS in the contralateral FDI muscle. The coil was held over the motor cortex with the handle pointing posteriorly and approximately perpendicular to the central sulcus.
For 1 Hz and 5 Hz rTMS and for TBS, we used a MagPro stimulator (Medtronic A/S Denmark) connected to a figure-of-eight coil (MCF B65; Medtronic A/S Denmark). The initial direction of the current induced in the brain was anterior to posterior. The magnetic stimulus had a biphasic waveform with a pulse width of about 280 µs and maximum magnetic field strength of 1.5 tesla. The stimulation intensity was defined in relation to AMT or RMT evaluated using the MagPro stimulator.

*Five Hertz rTMS*

The intensity of rTMS was set at 90% RMT, three rTMS trains of 300 pulses, each separated by approximately two minutes, were delivered.

*One Hertz rTMS*

One Hertz rTMS was performed at 110% RMT, 900 stimuli were delivered in a single train.

*Intermittent theta-burst (iTBS)*

An intensity of 80% AMT was used. We used the iTBS protocol in which 10 bursts of high frequency stimulation (3 pulses at 50 Hz) are applied at 5 Hz every 10s for a total of 600 pulses (Huang et al. 2005).

*Continuous theta-burst (cTBS)*

An intensity of 80% AMT was used. We used the cTBS protocol in which 3
pulses of stimulation are given at 50Hz, repeated every 200 ms for a total of 600 pulses (Huang et al. 2005).

Paired associative stimulation

We used a high power Magstim 200 (Magstim Co., Whitland, Dyfed UK) connected to a figure-of-eight coil, with external loop diameters of 9 cm held over the right motor cortex at the optimum scalp position to elicit MEPs in the contralateral FDI. The induced monophasic current in the brain flowed in a posterior-to-anterior direction.

The intervention consisted of single electrical stimuli delivered to the left ulnar nerve at the wrist at 300 % of the perceptual threshold and followed by TMS at intensities sufficient to produce an unconditioned response amplitude of approximately 1 mV in the resting FDI. For PAS_25 protocol ninety pairs were delivered at 0.05 Hz over 30 min at an interstimulus interval (ISI) of 25 ms because this interval had been shown in previous experiments to be effective in increasing cortical excitability (Stefan et al. 2000). For PAS_10 protocol ninety pairs were delivered at 0.05 Hz over 30 min at an interstimulus interval (ISI) of 10 ms because this interval had been shown in previous experiments to be effective in decreasing cortical excitability (Wolters et al. 2003).
Experimental design

In all subjects rTMS was performed on the non-dominant hemisphere and all TMS measures were evaluated bilaterally at baseline (T0, before rTMS) and at two time points after rTMS (T1-T2). Measurements at T1 were performed immediately after rTMS, measurements at T2 were performed 30 minutes after the end of rTMS. The order of measurement of different parameters and the order of the hemisphere (stimulated or contralateral) was pseudo-randomized and counterbalanced across subjects and rTMS protocols, but remained constant in pre/post evaluation for each session. All subjects were tested using all rTMS protocols in a randomized crossover design. The inter-session interval for a given subject was at least one week to exclude interactions between sessions.

For MEP amplitude measurement the same stimulus intensity was used before and after rTMS, for the paired pulse TMS protocols (SICI and ICF) the intensity of the test stimulus after rTMS was adjusted whenever necessary to ensure that the test MEP matched the amplitude to the baseline test MEP before rTMS and the conditioning stimulus intensity was adjusted relatively to the post rTMS AMT.

For SP evaluation the stimulus intensity after rTMS was adjusted relatively to the post rTMS AMT.
The time of testing was around 12 pm and was the same across all sessions.

Statistics

All data are expressed as mean ± 1 standard deviation of the mean (SD). The effects of different rTMS protocols were tested separately for each TMS parameter (RMT, AMT, MEP amplitude, CSP, iSP, SAI, SICI, ICF), using a repeated measures analysis of variance (rmANOVA) with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (2 levels: stimulated and non-stimulated) and time (T0, T1, T2) as within-subject factors. The Mauchly's test was used to evaluate the sphericity assumption and the Greenhouse-Geisser correction was employed when necessary to correct for non-sphericity. The Kruskall-Wallis test was performed to test differences between the values of each TMS parameter at baseline; the test was carried out separately for stimulated and non-stimulated hemisphere. The post-hoc analysis between times were performed comparing time 0 vs time 1 and time 0 vs time 2 for each TMS parameter, protocol and hemisphere. The level of significance was set at $p = 0.05$ and Sidak correction was applied for post-hoc comparisons.

In order to evaluate which was the most effective facilitatory and the most effective inhibitory protocol on cortical excitability as evaluated by measuring MEP amplitude, we performed a further post-hoc analysis comparing the effects produced by the three excitatory (5 Hz, iTBS and PAS+) and by the three inhibitory (1 Hz, cTBS and PAS-) protocols by means of Sidak procedure. The
Sidak’s procedure is slightly more powerful than Bonferroni method. Such a procedure is available in many popular statistical software (such as SPSS). The alpha adjustment is made by $\alpha_s = 1 - (1 - \alpha)^{1/k}$ instead of the Bonferroni approach $\alpha_B = \alpha/k$, where $k$ is the number of comparisons between means. (Edwards and Berry 1987)

SPSS software version 12.0 was used for performing analyses.

**Results**

Results are summarised in tables 1 and 2.

**Baseline analysis**

The Kruskall-Wallis test showed no differences between baseline parameters.

**Motor threshold (RMT and AMT)**

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed a significant interaction between time, protocol and hemisphere ($F_{10,90} = 2.99$, $P=0.03$) for RMT. No significant results or interactions were observed for AMT. Post-hoc analysis showed a significant increase in RMT between time 0 and time 1 for PAS10 ($p=0.041$) and a significant decrease between the same two time points for PAS25 ($p=0.047$) for the stimulated hemisphere.
The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and non stimulated) and time as within-subject factors showed a significant association with the time (F2,18=7.31, P=0.01), and the hemisphere (F1,9=9.94, P=0.01) and a significant interaction between protocol and hemisphere (F5,45=2.99, P=0.05) and between time, protocol and hemisphere (F10,90=4.49, P<0.01). The post-hoc analysis showed a significant increase in MEP amplitude between time 0 and time 1 for iTBS (p=0.015) and PAS25 (p=0.021), and between time 0 and time 2 for PAS25 (p = 0.027) for the stimulated hemisphere (Figure 1). Moreover, the post-hoc analysis showed a significant decrease in MEP amplitude between time 0 and time 1 for 1Hz (p=0.027), cTBS (p=0.015) and PAS10 (p=0.021) for the stimulated hemisphere (Figure 1).

The comparison of the effects of the 3 different excitatory protocols on the amplitude of MEPs evoked by the stimulated hemisphere, showed that iTBS (p=0.010) and PAS+ (p=0.035) produced a significant larger increase in MEP amplitude than 5Hz rTMS No difference was found between iTBS and PAS+(p=0.946).

The comparison of the effects of the 3 different inhibitory protocols on the amplitude of MEPs evoked by the stimulated hemisphere, showed that the larger
suppression of MEPs was found with PAS- and this approached statistical significance when compared with 1Hz (p=0.070). No evidence of difference was found for the other comparisons (PAS- vs. cTBS and cTBS vs. 1Hz, consistently p>0.40).

Contralateral silent period

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed a significant effect of time (F2,18=5.97, P=0.02) and hemisphere (F1,9=7.31, P=0.02). Post hoc analysis showed a significant increase in CSP duration between time 0 and time 1 only for 1 Hz protocol (p = 0.038) for the stimulated hemisphere.

Ipsilateral silent period

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed no significant effect of time, protocols and hemispheres and no significant interaction between the within subjects factors.
Short Interval Intracortical Inhibition

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed a significant effect of time ($F_{2,18}=8.83$, $p<0.01$). The post-hoc analysis showed a significant decrease between time 0 and time 2 ($p = 0.021$) for PAS10 protocol for the stimulated hemisphere.

Intracortical facilitation

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed no significant effect of time, protocols and hemispheres and no significant interaction between the within subjects factors.

Short Latency Afferent Inhibition

The rmANOVA with rTMS protocol (6 levels: 1 Hz, 5 Hz, cTBS, iTBS, PAS25, PAS10), hemisphere (stimulated and not stimulated) and time as within-subject factors showed no significant effect of time, protocols and hemispheres and no significant interaction between the within subjects factors.
Discussion

The results of present study confirm that it is possible to produce lasting effects on cortical excitability both in the stimulated and in contralateral motor cortex using different protocols of rTMS. As previously demonstrated: iTBS and PAS$_{25}$ produced a pronounced increase of MEP amplitude (Huang et al. 2005; Stefan et al. 2000) while one Hz (Chen et al. 1997), cTBS (Huang et al. 2005) and PAS$_{10}$ (Wolters et al. 2003) produced a pronounced suppression of MEP amplitude in the stimulated hemisphere; this was associated with a slight decrease in RMT for PAS$_{25}$ and a slight increase in RMT for PAS$_{10}$; the most prolonged effect on MEP amplitude was found for the PAS$_{25}$ protocol. As previously reported (Russmann et al. 2009), PAS10 produced decrease of SICI of the stimulated hemisphere, however, in our study this effect was slightly delayed. Moreover, as reported by Romeo et al (Romeo et al. 2000) and Daskalakis et al (Daskalakis et al. 2006), 1 Hz rTMS increased contralateral silent period duration after stimulation of the stimulated hemisphere. The head-to-head comparison of the different rTMS protocols in a group of subjects enabled us to overcome the limitation of inter-individual variability and to identify the protocols with the most pronounced effects on the excitability of intracortical excitatory and inhibitory circuits. The most pronounced increase in MEP amplitude was produced by iTBS (+56%) and PAS$_{25}$ (+45%) while the most pronounced MEP suppression was produced by PAS$_{10}$ (-31%) and cTBS.
Several previously reported after-effects of rTMS were not consistently observed in present study: 1) the increase in MEP amplitude after 5 Hz rTMS (Berardelli et al. 1998; Quartarone et al. 2005); 2) the decrease in the amplitude of MEPs evoked by stimulation of the non stimulated hemisphere after iTBS (Di Lazzaro et al. 2008; Suppa et al. 2008); 3) the change in SICI after iTBS (Huang et al. 2007; Suppa et al. 2008), cTBS (Huang et al. 2007; McAllister et al. 2009; Suppa et al. 2008) and five Hz rTMS (Di Lazzaro et al. 2002; Fierro et al. 2007; Koch et al. 2008; Quartarone et al. 2005; Wu et al. 2000). These discrepancies might be explained either by a high variability of all these effects that make them less robust or by a short duration of these after-effects. This latter point appears relevant because we measured a large number of TMS parameters and several minutes were required to complete all the tests. For this reason, short lived changes such as the facilitation of MEPs after five Hz rTMS might be difficult to demonstrate in present experimental setting. Moreover, stimulus parameters might have influenced our results. It has been demonstrated that TBS effects on SICI are highly intensity-dependent and intensities lower than those used in present study may be more effective in modulating SICI (McAllister et al. 2009). Finally, it should be considered that even though some of the changes observed in present study did not reach statistical significance, there was a clear tendency toward a change in the same direction as reported in previous studies.
This is the case for the effects of iTBS on MEPs evoked by stimulation of contralateral hemisphere that resulted significantly suppressed in two previous studies (Di Lazzaro et al. 2008; Suppa et al. 2008) and showed a consistent decrease (-16%) in present study and for the suppression of SICI after five Hz rTMS (Di Lazzaro et al. 2002; Fierro et al. 2007; Koch et al. 2008; Quartarone et al. 2005; Wu et al. 2000), that was observed also in present study though the change did not reach statistical significance. These findings demonstrate that using different protocols of rTMS it is possible to target specific cortical excitatory and inhibitory networks. MEP amplitude mainly reflects excitatory glutamatergic neurotransmission at the level of the motor cortex, short interval intracortical inhibition reflects GABAA intracortical activity, and cortical silent period mainly reflects GABAB intracortical activity (Paulus Walter et al. 2008). Thus, our results confirm that using rTMS it is possible to modulate excitatory glutamatergic neurotransmission in both directions, that it is possible to suppress intracortical inhibitory GABAA activity and to enhance intracortical GABAB activity. Interestingly, the time course of the effects of rTMS might be different for excitatory and inhibitory circuits. We observed a delayed and isolated suppression of intracortical inhibitory activity after PAS10. This confirms the notion that excitatory and inhibitory circuits may be modulated independently (McAllister et al. 2009).

The head-to-head comparison of the effects produced by different
paradigms on specific cortical circuits, could provide valuable information for the development of therapeutical strategies based on neuromodulation in neuropsychiatric disorders characterized by abnormal excitability of cortical circuits that can be targeted and modulated by rTMS.

A main limitation of present study is that we studied only the more commonly used protocols of stimulation but there are many other protocols such as paired pulse rTMS (Thickbroom et al. 2006) and quadripulse rTMS (Hamada et al. 2008) that were not analysed. Moreover, it should be considered that although we attempted to test the effects of the more commonly used protocols, the parameters of these protocols used in different studies are quite variable in that several studies have suggested that higher intensities and longer duration of stimulation will produce stronger effects, also higher frequencies of rTMS such as 10 Hz or 20 Hz seem to have a stronger effect, finally the direction and the phases of the induced current in the brain influences the changes produced in brain excitability. There are also several PAS protocols with variations in the intensity of median nerve stimulation, repetition rates, interstimulus intervals and number of paired stimuli. Therefore, present study does not provide general conclusions regarding the relative effects of the protocols evaluated but only on those commonly used. However, the comparison performed in present study identified the most effective protocols, thus new protocols can be tested against these ones and the results can be easily compared. Another main limitation is
that we only evaluated the effects on young healthy subjects, older healthy subjects or patients with neurological disorders may respond differently to rTMS. A further limitation is represented by our selective study of the motor cortex while other brain areas might represent more effective targets of neuromodulation in conditions such as movement and psychiatric disorders. Finally, it should be considered that present findings are mainly confirmatory in that the effects of individual rTMS protocols have been evaluated in several previous studies.
References


LEGENDS

**Figure 1.** Effects of paired associative stimulation at 25 ms interstimulus interval of the stimulated (PAS\textsubscript{25SH}) and non stimulated (PAS\textsubscript{25nSH}) hemisphere, of paired associative stimulation at 10 ms interstimulus interval of the stimulated (PAS\textsubscript{10SH}) and non stimulated (PAS\textsubscript{10nSH}) hemisphere, of intermittent theta burst stimulation of the stimulated (iTBS-SH) and non stimulated (iTBS-nSH) hemisphere, of continuous theta burst stimulation of the stimulated (cTBS-SH) and non stimulated (cTBS-nSH) hemisphere, of one Hz repetitive stimulation of the stimulated (1Hz-SH) and non stimulated (1Hz-nSH) hemisphere, of five Hz repetitive stimulation of the stimulated (5Hz-SH) and non stimulated (5Hz-nSH) hemisphere, on motor evoked potentials (MEP) amplitude. The means and 95% confidence intervals of the after-before differences in mean amplitudes immediately after rTMS (A) (T1-T0) and 30 minutes after the end of rTMS (B) (T2-T0) are represented. * indicates significant values.
Table 1. Study of the stimulated hemisphere. Motor thresholds (% of maximum stimulator output), MEP amplitude (mV), contralateral and ipsilateral silent period (ms), short interval intracortical inhibition (% of test MEP), intracortical facilitation (% of test MEP), and short latency afferent inhibition (% of test MEP) before (T0) and after (T1-T2) different protocols of rTMS (means ± SD). Significant changes versus T0 (P<0.05) are reported in bold.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Five Hertz</th>
<th>iTBS</th>
<th>PAS25</th>
<th>One Hertz</th>
<th>cTBS</th>
<th>PAS10</th>
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<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
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<td>RMT</td>
<td>45.7±12.2</td>
<td>45.3±12.1</td>
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<td>46.4±12</td>
<td>45.7±11.9</td>
<td>46.4±12</td>
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<td>AMT</td>
<td>35.9±8.4</td>
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<td>36.1±7.5</td>
<td>36.4±8.1</td>
<td>35.7±8.9</td>
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<td>MEP</td>
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<td>0.7±0.4</td>
<td>0.7±0.4</td>
<td>0.6±0.2</td>
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<td>cSP</td>
<td>153±43</td>
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<td>37.1±13.6</td>
<td>30.9±14.9</td>
<td>33.6±20.1</td>
</tr>
<tr>
<td>SICI</td>
<td>39±14.2</td>
<td>50.4±28.3</td>
<td>34.7±21.3</td>
<td>37±19</td>
<td>36±20</td>
<td>32.8±14.8</td>
</tr>
<tr>
<td>ICF</td>
<td>128±33</td>
<td>157±95.3</td>
<td>134±44.4</td>
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<tr>
<td>SAI</td>
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<td>61.1±23.3</td>
<td>41.4±17.4</td>
<td>46.4±17.7</td>
<td>40.2±12.8</td>
</tr>
</tbody>
</table>

RMT= resting motor threshold; AMT= active motor threshold; MEP= motor evoked potential; cSP=contralateral silent period; iSP=ipsilateral silent period; SICI=short interval intracortical inhibition; ICF=intracortical facilitation; SAI=short latency afferent inhibition
**Table 2.** Study of the non-stimulated hemisphere. Motor thresholds (% of maximum stimulator output), MEP amplitude (mV), contralateral and ipsilateral silent period (ms), short interval intracortical inhibition (% of test MEP), intracortical facilitation (% of test MEP), and short latency afferent inhibition (% of test MEP) before (T0) and after (T1-T2) different protocols of rTMS (means ± SD). Significant changes versus T0 (P<0.05) are reported in bold.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Five Hertz</th>
<th>iTBS</th>
<th>PAS&lt;sub&gt;25&lt;/sub&gt;</th>
<th>One Hertz</th>
<th>cTBS</th>
<th>PAS&lt;sub&gt;10&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>RMT</td>
<td>44.8±7.6</td>
<td>45.2±7.8</td>
<td>45.5±7.6</td>
<td>47.3±9.7</td>
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<td>47.7±9.4</td>
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<td>AMT</td>
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<td>34.5±5.8</td>
<td>34.8±5.6</td>
<td>35.5±6.3</td>
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<td>MEP</td>
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<td>1.2±0.7</td>
<td>1±0.3</td>
<td>0.79±0.29</td>
<td>0.66±0.23</td>
<td>0.8±0.3</td>
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<td>cSP</td>
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<td>135±29</td>
<td>135±22</td>
<td>131±23</td>
<td>145±51</td>
<td>143±39</td>
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<tr>
<td>iSP</td>
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<td>21.6±22.6</td>
<td>30.3±22.7</td>
<td>27.1±13.4</td>
<td>34±12.1</td>
<td>31.3±14.9</td>
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<tr>
<td>SICI</td>
<td>31.6±8.9</td>
<td>45.7±19.9</td>
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<tr>
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<td>142.8±57.7</td>
<td>139±52.7</td>
<td>150.7±79.7</td>
<td>139.3±64.8</td>
<td>152.6±83.5</td>
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<tr>
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<td>48.1±21.2</td>
<td>41.3±17</td>
<td>40.7±18.5</td>
<td>37.8±13.3</td>
<td>38.5±22</td>
</tr>
</tbody>
</table>

RMT = resting motor threshold; AMT = active motor threshold; MEP = motor evoked potential; cSP = contralateral silent period; iSP = ipsilateral silent period; SICI = short interval intracortical inhibition; ICF = intracortical facilitation; SAI = short latency afferent inhibition