Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation

V. Di Lazzaro,1,2 M. Dileone,1 F. Pilato,1 F. Capone,1 G. Musumeci,1 F. Ranieri,1 V. Ricci,3 P. Bria,3 R. Di Iorio,1 C. de Waure,4 P. Pasqualetti,2 and P. Proffice1

1Institute of Neurology, Università Cattolica, Rome; 2Department of Neuroscience, AFaR-Fatebenefratelli Association for Biomedical Research, “San Giovanni Calibita-Fatebenefratelli” Hospital, Isola Tiberina, Rome; 3Institute of Psychiatry, Università Cattolica, Rome; and 4Institute of Hygiene, Catholic University of the Sacred Heart, Rome, Italy

Submitted 10 September 2010; accepted in final form 15 February 2011

Di Lazzaro V, Dileone M, Pilato F, Capone F, Musumeci G, Ranieri F, Ricci V, Bria P, Di Iorio R, de Waure C, Pasqualetti P, Proffice P. Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. J Neurophysiol 105: 2150–2156, 2011. First published February 23, 2011; doi:10.1152/jn.00781.2010.—Repetitive transcranial magnetic stimulation (TMS) techniques can noninvasively activate 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 aftereffects 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, Hoogendam et al. 2010, and Ziemann et al. 2008 for review). Low-frequency rTMS (stimulus rates of 1 Hz or less) produces a lasting decrease in motor cortex excitability (Chen et al. 1997), whereas 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 is termed theta burst stimulation (TBS) (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 (ISI) slightly longer (25 ms; PAS25) or slightly shorter (10 ms; PAS10) than the time needed for the afferent inputs, generated by peripheral nerve stimulation, to reach the cerebral cortex and if a sufficient number of pair of stimuli is delivered, the excitability of the sensory-motor cortex increases or decreases, respectively. TBS employs brief bursts of high-frequency (50 Hz), low-intensity stimuli. Different patterns of delivery of TBS (continuous vs. intermittent) produce opposite effects on the excitability of the stimulated motor cortex (Huang et al. 2005). The intermittent TBS (iTBS) paradigm produces a prolonged LTP-like increase of motor cortex excitability (Huang et al. 2005), whereas the continuous TBS (cTBS) paradigm 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 have 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 also may occur at distant interconnected sites in the brain and, in particular, in the contralateral nonstimulated 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 have investigated this aspect. Moreover, because almost all of the studies employed a single rTMS protocol and because there is a high interindividual variability of the aftereffects of rTMS (Maeda et al. 2000), at least in part related to genetic features (Cheeran et al. 2000), 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.

Address for reprint requests and other correspondence: V. Di Lazzaro, Istituto di Neurologia, Università Cattolica, L.go A. Gemelli 8, 00168 Rome, Italy (e-mail: vdlazzaro@rm.unicatt.it).

TRANSCRANIAL MAGNETIC STIMULATION (TMS) techniques can noninvasively activate 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 aftereffects 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, Hoogendam et al. 2010, and Ziemann et al. 2008 for review). Low-frequency rTMS (stimulus rates of 1 Hz or less) produces a lasting decrease in motor cortex excitability (Chen et al. 1997), whereas 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 is termed theta burst stimulation (TBS) (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 (ISI) slightly longer (25 ms; PAS25) or slightly shorter (10 ms; PAS10) than the time needed for the afferent inputs, generated by peripheral nerve stimulation, to reach the cerebral cortex and if a sufficient number of pair of stimuli is delivered, the excitability of the sensory-motor cortex increases or decreases, respectively. TBS employs brief bursts of high-frequency (50 Hz), low-intensity stimuli. Different patterns of delivery of TBS (continuous vs. intermittent) produce opposite effects on the excitability of the stimulated motor cortex (Huang et al. 2005). The intermittent TBS (iTBS) paradigm produces a prolonged LTP-like increase of motor cortex excitability (Huang et al. 2005), whereas the continuous TBS (cTBS) paradigm 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 have 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 also may occur at distant interconnected sites in the brain and, in particular, in the contralateral nonstimulated 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 have investigated this aspect. Moreover, because almost all of the studies employed a single rTMS protocol and because there is a high interindividual variability of the aftereffects of rTMS (Maeda et al. 2000), at least in part related to genetic features (Cheeran et al. 2000), 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 were the effects of TBS on threshold and amplitude of MEPs compared with those produced by 1- and 5-Hz rTMS (Zafar et al. 2008). The aim of the present 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.

METHODS

Subjects

Ten healthy volunteers [mean age 26.6 ± 4.1 (SD) yr] participated in the experiments; eight of the subjects were nonsmokers. 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.

TMS Measures

TMS was performed with a high-power Magstim 200 stimulator (Magstim, Whitland, 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 ensure 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 electromyogram (EMG) was amplified and filtered (bandwidth 3 Hz–3 kHz) by D360 amplifiers (Digitimer, Welwyn Garden City, 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 analog-to-digital 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 ~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 as percentages 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 silent periods (cSP) were elicited while subjects held a tonic voluntary contraction of ~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 ~50% of maximum voluntary contraction (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. (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. We analyzed 100 ms of rectified averaged prestimulus EMG signal (that is, 500 data points) 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. To automate the procedure, we used a self-made function for the MATLAB software (The MathWorks). Ipsilateral SP 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 (Magstim) with the use of a single stimulator for single-pulse and short-latency afferent inhibition studies and two stimulators for paired-pulse paradigms.

Short-interval intracortical inhibition. Short-interval intracortical inhibition (SICI) was studied using the technique of Kujirai et al. (1993). Two magnetic stimuli were given through the same stimulating coil over the motor cortex at an 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% MSO below AMT. The intensity of the test stimulus was adjusted to elicit an unconditioned test MEP in the relaxed FDI of ~1 mV in peak-to-peak amplitude. Subjects were provided with audiovisual feedback of the EMG at high gain (50 μV/D) to assist in maintaining complete relaxation, since 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 intracortical facilitation (ICF) by analyzing the facilitatory interaction that occurs between pairs of magnetic stimuli given over the motor cortex at 15-ms ISI. Five single-pulse stimuli and five paired stimuli at 15-ms ISI were delivered. Subjects were given audiovisual feedback at high gain to assist in maintaining complete relaxation. The amplitude of the conditioned MEPs was expressed as a percentage of the amplitude of the test MEPs.

Short-latency afferent inhibition. Short-latency afferent inhibition (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 ~1 mV in peak-to-peak amplitude.

The conditioning stimulus to the median nerve preceded the TMS test pulse by 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 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 pseudorandomized order. Subjects were given audiovisual 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- and 5-Hz rTMS and for TBS, we used a MagPro stimulator (Medtronic, Copenhagen, Denmark) connected to a figure-of-eight coil (MCF B65; Medtronic). 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 ~280 µs and maximum magnetic field strength of 1.5 T. 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 ~2 min, were delivered.

**One-hertz rTMS.** One-hertz rTMS was performed at 110% RMT. Nine hundred stimuli were delivered in a single train.

**Intermittent TBS.** 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 10 s for a total of 600 pulses (Huang et al. 2005).

**Continuous TBS.** An intensity of 80% AMT was used. We used the cTBS protocol in which 3 pulses of stimulation are given at 50 Hz, 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) 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, followed by TMS at intensities sufficient to produce an unconditioned response amplitude of ~1 mV in the resting FDI. For the PAS25 protocol, 90 pairs were delivered at 0.05 Hz over 30 min at an 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 the PAS10 protocol, 90 pairs were delivered at 0.05 Hz over 30 min at an 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 nondominant hemisphere and all TMS measures were evaluated bilaterally at baseline (T0, before rTMS) and at two time points after rTMS (T1 and T2). Measurements at T1 were performed immediately after rTMS; measurements at T2 were performed 30 min after the end of rTMS. The order of measurement of different parameters and the order of the hemisphere (stimulated or contralateral) was pseudorandomized and counterbalanced across subjects and rTMS protocols but remained constant in pre- and/or postevaluation for each session. All subjects were tested using all rTMS protocols in a randomized crossover design. The intersession interval for a given subject was at least 1 wk 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 relative to the post-rTMS AMT. The time of testing was around 12:00 PM and was the same across all sessions.

**Statistics**

All data are means ± 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, nonstimulated), and time (T0, T1, T2) as within-subject factors. The Mauchly test was used to evaluate the sphericity assumption, and the Greenhouse-Geisser correction was employed when necessary to correct for nonsphericity. The Kruskal-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 nonstimulated hemispheres. The post hoc analyses between times were performed by comparing T0 vs. T1 and T0 vs. T2 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.

To evaluate which was the most effective facilitatory protocol 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, PAS25) and the three inhibitory protocols (1 Hz, cTBS, PAS10) by means of the Sidak procedure. The Sidak procedure is slightly more powerful than the Bonferroni method. Such a procedure is available in many popular statistical software applications (such as SPSS). The alpha adjustment is made by $\alpha_{	ext{adj}} = 1 - (1 - \alpha)^{1/k}$ instead of the Bonferroni approach, $\alpha_{	ext{adj}} = \alpha/k$, where $k$ is the number of comparisons between means (Edwards and Berry 1987). We used SPSS software version 12.0 to perform analyses.

**RESULTS**

Results are summarized in Tables 1, 2, 3 and 4.

**Baseline Analysis**

The Kruskal-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, nonstimulated), 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 T0 and T1 for PAS10 ($P = 0.041$) and a significant decrease between the same two time points for PAS25 ($P = 0.047$) for the stimulated hemisphere.

**MEP amplitude**

The rmANOVA with rTMS protocol, hemisphere, and time as within-subject factors showed a significant association with time ($F_{2,18} = 7.31, P = 0.01$) and hemisphere ($F_{1,9} = 9.94, P = 0.01$) and a significant interaction between protocol and hemisphere ($F_{5,45} = 2.99, P = 0.05$) and between time, protocol, and hemisphere ($F_{10,90} = 4.49, P < 0.01$). Post hoc analysis showed a significant increase in MEP amplitude between T0 and T1 for iTBS ($P = 0.015$) and PAS25 ($P = 0.021$) and between T0 and T2 for PAS25 ($P = 0.027$) for the stimulated hemisphere (Fig. 1). Moreover, post hoc analysis showed a significant decrease in MEP amplitude between T0 and T2.
Table 1. Facilitatory protocols: study of the stimulated hemisphere

<table>
<thead>
<tr>
<th>Measure</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMT</td>
<td>45.7 ± 12.2</td>
<td>45.3 ± 12.1</td>
<td>47.1 ± 11.9</td>
<td>46.4 ± 12</td>
<td>45.7 ± 11.9</td>
<td>46.4 ± 12</td>
<td>46 ± 12</td>
<td>44.8 ± 12.5</td>
<td>45.2 ± 12.5</td>
</tr>
<tr>
<td>AMT</td>
<td>35.9 ± 8.4</td>
<td>35.3 ± 8.6</td>
<td>36.1 ± 7.5</td>
<td>36.4 ± 8.1</td>
<td>35.7 ± 8.9</td>
<td>35.8 ± 7.7</td>
<td>34.9 ± 8.9</td>
<td>34.7 ± 9.3</td>
<td>34.4 ± 8.9</td>
</tr>
<tr>
<td>MEP</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.4</td>
<td>0.64 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.67 ± 0.35</td>
<td>0.97 ± 0.39</td>
<td>0.92 ± 0.27</td>
</tr>
<tr>
<td>cSP</td>
<td>153 ± 43</td>
<td>146 ± 27</td>
<td>143 ± 49</td>
<td>149 ± 49</td>
<td>154 ± 36</td>
<td>154 ± 50</td>
<td>144 ± 22</td>
<td>149 ± 15</td>
<td>150 ± 15</td>
</tr>
<tr>
<td>iSP</td>
<td>33.9 ± 24.2</td>
<td>31.5 ± 38.5</td>
<td>41.1 ± 45.5</td>
<td>37.1 ± 13.6</td>
<td>30.9 ± 14.9</td>
<td>33.6 ± 20.1</td>
<td>33.9 ± 13.7</td>
<td>27 ± 22.1</td>
<td>35.1 ± 18.3</td>
</tr>
<tr>
<td>SICI</td>
<td>39 ± 14.2</td>
<td>50.4 ± 28.3</td>
<td>34.7 ± 21.3</td>
<td>37.1 ± 19</td>
<td>36 ± 20</td>
<td>32.8 ± 14.8</td>
<td>38.9 ± 20.2</td>
<td>39.7 ± 22.1</td>
<td>44.4 ± 28.5</td>
</tr>
<tr>
<td>ICF</td>
<td>128 ± 33</td>
<td>157.1 ± 95.3</td>
<td>134.1 ± 44.4</td>
<td>143.3 ± 37.2</td>
<td>146.4 ± 49.9</td>
<td>120.9 ± 37.1</td>
<td>129.4 ± 52.3</td>
<td>114.4 ± 56.9</td>
<td>113.5 ± 31.4</td>
</tr>
<tr>
<td>SAI</td>
<td>44.4 ± 14.9</td>
<td>43.26.8</td>
<td>61.1 ± 23.3</td>
<td>41.4 ± 17.4</td>
<td>46.4 ± 17.7</td>
<td>40.2 ± 12.8</td>
<td>41.7 ± 11.1</td>
<td>44.2 ± 9.8</td>
<td>48.5 ± 15.5</td>
</tr>
</tbody>
</table>

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 vs. T0 (P < 0.05) are reported in bold.

DISCUSSION

The results of our present study confirm that it is possible to produce lasting effects on cortical excitability in both the stimulated and contralateral motor cortex by using different protocols, and hemispheres and no significant interaction between the within-subject factors.

Short-Interval Intracortical Inhibition

The rmANOVA with rTMS protocol, hemisphere, and time as within-subject factors showed a significant effect of time (F2,18 = 8.83, P < 0.01). Post hoc analysis showed a significant decrease between T0 and T2 (P = 0.021) for PAS10 for the stimulated hemisphere.

Intracortical Facilitation

The rmANOVA with rTMS protocol, hemisphere, and time as within-subject factors showed no significant effect of time, protocols, and hemispheres and no significant interaction between the within-subject factors.

Short-Latency Afferent Inhibition

The rmANOVA with rTMS protocol, hemisphere, and time as within-subject factors showed no significant effect of time, protocols, and hemispheres and no significant interaction between the within-subject factors.
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).

Table 3.  
**Facilitatory protocols: study of the nonstimulated hemisphere**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Five Hertz</th>
<th>iTBS</th>
<th>PAS25</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMT</td>
<td>44.8 ± 7.6</td>
<td>47.3 ± 9.7</td>
<td>45.4 ± 9.2</td>
</tr>
<tr>
<td>AMT</td>
<td>34.6 ± 6.2</td>
<td>35.5 ± 6.3</td>
<td>34.9 ± 6.9</td>
</tr>
<tr>
<td>MEP</td>
<td>1 ± 0.6</td>
<td>0.79 ± 0.29</td>
<td>0.87 ± 0.35</td>
</tr>
<tr>
<td>cSP</td>
<td>148 ± 49</td>
<td>131 ± 23</td>
<td>149 ± 23</td>
</tr>
<tr>
<td>iSP</td>
<td>30.6 ± 17.2</td>
<td>27.1 ± 13.4</td>
<td>24.1 ± 21.3</td>
</tr>
<tr>
<td>SICI</td>
<td>31.6 ± 8.9</td>
<td>37.2 ± 17.6</td>
<td>27 ± 19.1</td>
</tr>
<tr>
<td>ICF</td>
<td>125.4 ± 48</td>
<td>150.7 ± 79.7</td>
<td>141.6 ± 55</td>
</tr>
<tr>
<td>SAI</td>
<td>43.5 ± 16.8</td>
<td>40.7 ± 18.5</td>
<td>38.1 ± 12.8</td>
</tr>
</tbody>
</table>

Table 4.  
**Inhibitory protocols: study of the nonstimulated hemisphere**

<table>
<thead>
<tr>
<th>Measure</th>
<th>One Hertz</th>
<th>iTBS</th>
<th>PAS10</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMT</td>
<td>48 ± 12.2</td>
<td>45.5 ± 5.7</td>
<td>45.5 ± 9.2</td>
</tr>
<tr>
<td>AMT</td>
<td>36.1 ± 8.9</td>
<td>34.9 ± 4.7</td>
<td>34.5 ± 6.8</td>
</tr>
<tr>
<td>MEP</td>
<td>0.7 ± 0.3</td>
<td>0.82 ± 0.4</td>
<td>0.76 ± 0.32</td>
</tr>
<tr>
<td>cSP</td>
<td>145 ± 42</td>
<td>141 ± 17</td>
<td>150 ± 44</td>
</tr>
<tr>
<td>iSP</td>
<td>39.7 ± 21.8</td>
<td>33.5 ± 23.6</td>
<td>23.9 ± 22.1</td>
</tr>
<tr>
<td>SICI</td>
<td>33.2 ± 19.4</td>
<td>38.8 ± 17.4</td>
<td>37.4 ± 14.3</td>
</tr>
<tr>
<td>ICF</td>
<td>124 ± 30.9</td>
<td>131.8 ± 25.4</td>
<td>105.4 ± 49.9</td>
</tr>
<tr>
<td>SAI</td>
<td>41 ± 15.7</td>
<td>39.7 ± 14.8</td>
<td>43.2 ± 15.6</td>
</tr>
</tbody>
</table>

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).
sion at the level of the motor cortex, SICI reflects GABA_A intracortical activity, and cSP mainly reflects GABA_B intracortical activity (Paulus et al. 2008). Thus our results confirm that by using rTMS, it is possible to modulate excitatory glutamatergic neurotransmission in both directions, to suppress intracortical inhibitory GABA_A activity, and to enhance intracortical GABA_B 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 PAS_10. This confirms the notion that excitatory and inhibitory circuits may be modulated independently (McAllister et al. 2009). Head-to-head comparison of the effects produced by different paradigms on specific cortical circuits could provide valuable information for the development of therapeutic 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 the 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 quadrupulse rTMS (Hamada et al. 2008), that were not analyzed. 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 that higher frequencies of rTMS such as 10 or 20 Hz seem to have a stronger


REFERENCES

No conflicts of interest, financial or otherwise, are declared by the author(s).

DISCLOSURES


