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ARTICLE

Antidepressant effects of different schedules of repetitive transcranial magnetic stimulation vs. clomipramine in patients with major depression: relationship to changes in cortical excitability

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Abstract

The antidepressant effects of repetitive transcranial magnetic stimulation (rTMS) that have been demonstrated in recent studies could be related to its ability to modulate cortical excitability. Yet, the relationship between stimulus location and frequency and treatment outcome has not been established. The aim of the present study was to compare efficacy of rTMS in various configurations and clomipramine treatment in patients with major depression (MD) and to evaluate the relationship between clinical outcome and changes in cortical excitability. Fifty-nine MD patients were randomized to receive (1) left ($n=12$) or right ($n=12$) 3 Hz rTMS with placebo medication; (2) left ($n=10$) or right ($n=9$) 10 Hz rTMS with placebo medication; (3) active medication (clomipramine) with sham rTMS ($n=16$). Both 3 Hz and 10 Hz rTMS were administered to the prefrontal cortex by a circular coil at an intensity of 110% and 100% of the resting motor threshold (rMT) respectively. Measurements of cortical excitability were performed prior to and 24 h after completion of 2 wk of daily rTMS or pharmacological treatments. These included the rMT, silent period threshold (SPT), inter-threshold difference (ITD), MEP/M-wave amplitude ratio and silent period duration (SPD). Severity of depression was blindly assessed by the Hamilton Depression Rating Scale (HDRS). The best improvement scores were seen in patients who received left 3 Hz rTMS. The 10 Hz rTMS treatment was less tolerated resulting in a significantly higher dropout rate. A significant increase of the MEP/M wave amplitude ratio accompanied by a shortening of the SPD was evidenced in patients who showed marked clinical improvement (reduction in HDRS by 50% or more) following left rTMS regardless of stimulation frequency. Our results suggest that 3 Hz left rTMS has a higher therapeutic efficacy and tolerability in patients with MD. The enhancement of cortical excitability may be related to the antidepressant action of rTMS.

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Key words: Cortical excitability, depression, transcranial magnetic stimulation.

Introduction

Since its introduction (Barker et al., 1985), single-pulse transcranial magnetic stimulation (sTMS) has been widely used as a non-invasive technique to evaluate brain function in healthy subjects and patients in various clinical conditions. Therapeutic use of repetitive TMS (rTMS) in neuropsychiatric disorders in general and in major depression (MD) in particular has been studied in recent years. Thus, left high-frequency (>1 Hz) (George et al., 1995) and right low-frequency ($\leqslant 1$ Hz) (Klein et al., 1999) rTMS to the prefrontal cortex have been reported to be effective in the treatment of MD suggesting that rTMS might become an additional treatment modality and in some cases even an alternative to electroconvulsive therapy (Grunhaus et al., 2003; Pridmore, 2000). However, the relationship between rTMS parameters (i.e. stimulus location,

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intensity and frequency) and treatment outcome is still not clear and needs to be investigated. Previous work has shown comparable clinical response in MD patients to 5 Hz and 20 Hz left prefrontal rTMS with better treatment tolerability in the low-frequency group (George et al., 2000). Furthermore, the decrease of stimulation frequency permits the increase of stimulus intensity without compromising safety. This may be important given that intensity appears to be a critical factor in determining rTMS efficacy (Gershon et al., 2003). Thus, further decrease of left-sided stimulation frequency within the acceptable high-frequency range (>1 Hz) might be a logical approach for the optimization of treatment parameters.

The most prominent feature of sTMS is an ability to produce both excitatory and inhibitory effects on the human cerebral cortex. When applied over the motor cortex during voluntary target muscle contraction, sTMS elicits a motor-evoked potential (MEP) followed by a temporary suppression of the EMG activity, known as the cortical silent period (SP). Similar excitatory and inhibitory responses can be obtained following sTMS of somatosensory and visual cortex (Amassian et al., 1998; Seyal et al., 1992, 1993). Likewise, rTMS may exert opposite effects on cortical excitability depending on stimulation frequency and intensity (Maeda et al., 2000b,c). High-frequency rTMS can rapidly but temporarily facilitate cortical responses to sTMS (Pascual-Leone et al., 1998) while low-frequency rTMS has been shown to transiently inhibit cortical excitability (Boroojerdi et al., 2000; Chen et al., 1997). Triggs and colleagues reported that a 10-d course of left prefrontal high-frequency rTMS was associated with a decrease in MEP threshold (Triggs et al., 1999). It has been also demonstrated that electroconvulsive therapy (Amiaz et al., 2001; Coffey et al., 1995) and pharmacological treatment (Pisani et al., 2002) can affect seizure threshold. This suggests that mechanisms of the antidepressant action of rTMS could be related to its modulatory effects on cortical excitability. However, to our knowledge no data are available in the literature on long-lasting changes in excitatory and inhibitory intra-cortical mechanisms following rTMS or pharmacological treatments in depressed patients.

The aim of the present study was twofold. Our first objective was to assess in patients with MD the clinical efficacy of left vs. right rTMS administered at two frequencies, 3 Hz and 10 Hz (both in the high-frequency range) in comparison with clomipramine treatment. Our main hypothesis was that after 2 wk of treatment left rTMS would have higher clinical efficacy than both right rTMS and clomipramine, and that both 3 Hz and 10 Hz left rTMS would be equally effective. Our second objective was to evaluate changes in cortical excitability in rTMS and clomipramine-treated patients with relationship to clinical outcome. Based on the above-mentioned literature and theoretical considerations, we hypothesized that (i) rTMS and clomipramine treatment would be associated with alteration of the motor threshold, (ii) changes in cortical excitatory and inhibitory responses (i.e. MEP and SP) following treatment would differ in responders and non-responders.

Subjects and methods

Subjects

Participants in this study were 59 in-patients who met DSM-IV criteria for MD (44 females and 15 males; mean age 60.46 ± 15.02 yr, range 22–80 yr). Diagnosis was made by two senior psychiatrists based on an extended clinical interview and chart review. Patients were all recruited from the Department of Psychiatry at the Rambam Medical Center where they were hospitalized for treatment of an acute episode of MD. All provided written informed consent to participate in the study, which was approved by the institutional review board. Exclusion criteria were: (1) suicidal risk; (2) documented history of head injury or seizure disorder; (3) documented evidence of a disorder which could affect peripheral and central conduction such as multiple sclerosis, motor neuron disease, carpal tunnel syndrome, cervical spondylosis, diabetic neuropathy, etc. and (4) any other contraindication to TMS as specified in the safety guidelines for rTMS (Wassermann, 1998) such as cardiac pacemakers or metallic deep brain electrodes.

After signing informed consent patients were randomly assigned to one of the five treatment conditions: (1) 3 Hz left prefrontal rTMS treatment with placebo medication ($n=12$); (2) 3 Hz right prefrontal rTMS treatment with placebo medication ($n=12$); (3) 10 Hz left prefrontal rTMS treatment with placebo medication ($n=10$); (4) 10 Hz right prefrontal rTMS treatment with placebo medication ($n=9$); (5) sham rTMS with active medication (clomipramine 150 mg/d, $n=16$).

Table 1 summarizes demographic and clinical characteristics of the five groups. There were no significant between-group differences on any of the demographic and clinical variables.

Previous antidepressant medications were tapered and discontinued at least 1 wk prior to beginning rTMS treatment. None of the patients was receiving

Table 1. Socio-demographic and clinical characteristics of the five subgroups (mean ± S.D.)

	Right prefrontal rTMS (with placebo medication)		Left prefrontal rTMS (with placebo medication)			
	3 Hz	10 Hz	3 Hz	10 Hz	Clomipramine (with sham rTMS)	$F_{(4, 54)}/\chi^2_{(4)}$
Age (yr)	61.6 ± 8.7	61.6 ± 22.0	57.4 ± 12.9	59.3 ± 19.8	61.6 ± 13.8	0.17, $p > 0.05$
Age at onset (yr)	47.5 ± 14.4	47.8 ± 17.8	47.5 ± 13.5	43.0 ± 19.3	41.6 ± 16.8	0.42, $p > 0.05$
Length of illness (yr)	14.3 ± 12.8	16.6 ± 10.9	10.1 ± 9.8	16.3 ± 18.7	20.7 ± 20.3	0.88, $p > 0.05$
Number of episodes	3.0 ± 1.8	2.6 ± 1.3	2.8 ± 1.8	4.5 ± 3.5	3.3 ± 3.1	0.82, $p > 0.05$
HDRS at baseline	26.8 ± 6.7	26.5 ± 3.0	24.4 ± 5.4	27.3 ± 5.3	26.0 ± 5.0	0.57, $p > 0.05$
Gender (M/F)	3/9	3/6	2/10	5/5	2/14	6.6, $p > 0.05$
Family status (M/S)	6/6	2/7	5/7	4/6	3/13	5.12, $p > 0.05$

anticonvulsant mood stabilizers or structured psychotherapy except for standard milieu therapeutic activities.

Clinical ratings

Clinical ratings were assessed at baseline (before treatment), after five treatment sessions (1 wk), and 24 h after the last rTMS treatment. The Hamilton Rating Scale for Depression (HDRS) was used to assess depressive symptoms. The raters (O.S., O.R., I.K.) were senior psychiatrists who were blind to the nature of treatment which was delivered outside the in-patient unit. In addition, the raters were instructed to avoid asking questions which could disclose the nature of the treatment.

Assessment of cortical excitability by sTMS

Measures of cortical excitability were performed at baseline (prior to beginning treatment) and approximately 24 h after the last rTMS treatment. Patients were seated in a comfortable chair with the elbow semi-flexed. They were instructed to keep their hands as relaxed as possible. A lycra tightly fitting cap was placed on the head to mark sites for single-pulse and repetitive stimulation and ensure accurate repositioning of the coil throughout the study.

For assessment of cortical excitability, MEPs and SPs following sTMS were recorded from the abductor pollicis brevis (APB) muscle using an Amplaid EMG machine (Amplaid, Milan, Italy). TMS was applied by a Magstim Rapid magnetic stimulator (Magstim, Whitland, UK) with a 9-cm mean diameter circular coil. The coil was positioned tangentially to the skull with the handle pointing backwards and moved over the presumed hand area of the motor cortex in 1-cm steps to determine the optimal position for eliciting

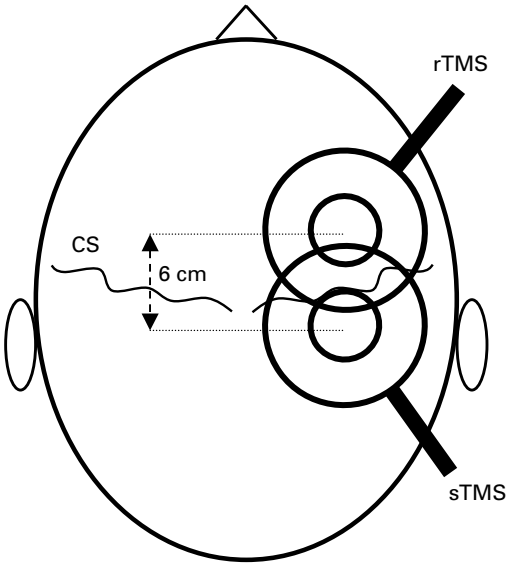

Figure 1. Schematic illustration of coil positions for single-pulse TMS (sTMS) and repetitive TMS (rTMS). For sTMS, the anterior winding of the stimulation coil was placed over the hand area of the motor cortex. In rTMS, the anterior segment of the stimulation coil covered the prefrontal cortex while its posterior tip was located near the presumed line of the central sulcus (CS).

MEPs of maximal amplitude (lowest threshold) in the contralateral APB. Greatest responses were achieved with the centre of the coil 2–3 cm posterior and 5–6 cm lateral to the vertex, when the anterior segment of the coil windings covered the hand motor area (Figure 1). This coil position was marked on the scalp cap and maintained fixed by manual handling throughout the sTMS session. The initial current direction in the coil was counterclockwise for stimulation of the right hemisphere and clockwise for the left hemisphere. Such a coil orientation resulted in the most effective

activation of the motor cortex in response to a biphasic pulse generated by a Magstim Rapid stimulator (Kammer et al., 2001). MEP and SP parameters were evaluated in a similar way as described in a previous paper (Chistyakov et al., 2001). Shortly, the resting motor threshold (rMT) was defined as the lowest stimulus intensity capable of eliciting in the relaxed APB at least 5 MEPs with amplitude of at least 50 μV in a series of 10 consecutive trials of sTMS. The SP threshold (SPT) was defined as the minimum stimulus intensity that elicited the SP lasting at least 40 ms in three consecutive trials while the subjects provided a voluntary muscle contraction of approx. 30% of maximal EMG. The MEP amplitude was measured peak-to-peak at TMS intensity of 120% of the rMT during muscle relaxation. The MEP/M-wave amplitude ratio was then calculated by dividing the MEP amplitude by the maximal M-wave amplitude obtained after supramaximal peripheral electrical stimulation of the median nerve at the wrist. The MEP/M-wave amplitude ratio was the result of averaging eight consecutive sTMS trials and was expressed as the percentage ratio (MEP amplitude $\times$ 100/maximal M-wave amplitude). The SP was elicited while the subjects maintained a steady voluntary isometric contraction of the contralateral APB at approx. 30% of its maximal level. Magnetic stimuli were delivered over the same scalp position (as for producing MEPs) at intensities of 125, 150 and 175% of the SPT. The silent period duration (SPD) was measured from the end of MEP to the point when continuous EMG activity returned to its average level in the 100 ms before the stimulus. For assessment of the balance between excitatory and inhibitory central mechanisms, the inter-threshold difference (ITD) was calculated as the difference between the rMT and SPT.

Audio-visual EMG feedback was given in order to maintain the correct level of voluntary isometric contraction or ensure complete relaxation of the APB muscle. Trials in which EMG activity occurred were discarded from analysis. In all sTMS procedures, the interval between single trials was at least 8 s.

rTMS treatment

Repetitive magnetic stimuli were delivered to the same side and with the same stimulator devices (circular coil connected to a Magstim Rapid magnetic stimulator) as used for assessment of cortical excitability in the sTMS procedures. The coil was held tangentially to the skull for active rTMS and perpendicularly to the scalp surface for sham rTMS with the handle pointing forward. The coil was positioned 6 cm anterior to the site optimal for producing the motor response in the contralateral APB muscle. In such a location the anterior segment of the coil covered the area of the dorsolateral prefrontal cortex (George et al., 1995) while its posterior segment was in close vicinity to the motor cortex (Figure 1). Given that the highest magnetic field strength occurs near the inner turn of the coil, it is reasonable to assume that the distance between the site of maximal tissue current induced by the posterior winding and the hand motor area was approximately 1–2 cm. Therefore, the use of the circular coil for rTMS treatments could lead to simultaneous excitation of the prefrontal and motor cortices. However, in none of the patients who received 10 Hz rTMS and in only one patient who received 3 Hz rTMS was EMG activity in the contralateral wrist muscles detected during repetitive stimulation. In this patient, the coil was moved along the parasagittal line until the motor responses disappeared. Nevertheless, the effect of subthreshold stimulation of the motor cortex could not be excluded in both 3 Hz and 10 Hz rTMS treatment conditions.

Regardless of frequency and type of rTMS (3 Hz, 10 Hz, sham) the treatment protocol in each patient consisted of 10 daily sessions during a 2-wk period. In the 3 Hz rTMS treatment, stimulation intensity was 110% of the rMT and the total number of stimuli per session was 450. Five trains of 30 s duration were applied with 60 s inter-train interval. In the case of 10 Hz rTMS, each session consisted of 500 stimuli delivered at an intensity of 100% of the rMT and given as 10 trains of 5 s duration with 45 s inter-train interval.

Statistical procedures

In order to examine the degree of matching between the five treatment groups on key demographic and clinical characteristics, a set of one-way analyses of variance (ANOVA) was used for continuous variables, and χ^2 tests for categorical variables. The first hypothesis, regarding the superior treatment effect of left rTMS over that of right rTMS or medication, was tested with a set of one-way analyses of covariance (ANCOVA). A separate ANCOVA was performed for each dependent variable (post-treatment scores), with treatment as the between-group factor and pre-treatment scores as the covariate. Preplanned contrasts were used to assess the hypothesized effect of stimulation frequency and side. Between-group comparisons of the frequencies of categorical variables were carried out by the χ^2 test with Yates' correction. Post-hoc tests with a Bonferroni correction for multiple comparisons were used in all other cases in

which the omnibus F was significant but we did not have a-priori hypothesis regarding the pattern of means.

The hypotheses regarding the effects of rTMS and pharmacological treatments on the motor threshold, MEP and SP parameters were tested separately using repeated-measures ANOVA with Treatment group (3 Hz, 10 Hz, clomipramine), Clinical outcome [improvement (reduction in HDRS by more than 50% of baseline values), no response] and Side (left, right) as between-subject factors and Time (baseline, after treatment) as the within-subject term. For significant omnibus F values, the post-hoc paired sample two-tailed t test was performed with a Bonferroni adjustment for multiple comparisons. The results were considered significant if $p < 0.05$.

Results

Clinical findings

Pre-treatment level of depression

First, we examined the degree to which levels of depression at baseline were similar across all five groups in the study. A set of one-way ANOVAs revealed no significant group effect on the HDRS [$F(4, 54) = 0.57$, $p > 0.05$].

Attrition and adverse effects

Of the 59 patients who started the study, 50 completed the entire protocol (10 sessions) and nine withdrew after 3–5 treatment sessions. The reasons for withdrawal were a local scalp discomfort, pain and facial muscle contraction during stimulation ($n = 8$) as well as headache reported by one patient after rTMS procedure. Withdrawal from the study was significantly related to stimulation frequency [$\chi^2_{(2)} = 10.14$, $p = 0.006$); with seven of the nine non-completers dropping out from the two groups that received 10 Hz rTMS treatment (four left, three right), one from the active 3 Hz rTMS treatment (one left), and one from the sham rTMS treatment. To ascertain that dropout from the study was not systematically associated with other key independent variables in the study, we compared completers and non-completers on all socio-demographic and clinical variables. In general, non-completers tended to be younger (54.89 ± 17.22 yr) than their completer fellows (61.46 ± 14.55 yr), with younger age of onset (41.11 ± 16.80 vs. 46.02 ± 15.73 yr) and fewer past episodes (2.43 ± 1.13 vs. 3.30 ± 2.54). However, these differences were not statistically significant. Taken together, these results suggest that low tolerance for stimulation frequency was the main reason for dropout from the study, but that otherwise the completers are a representative sample of those who started the study.

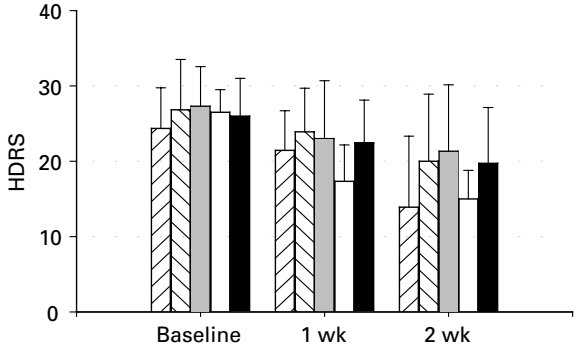

Figure 2. Clinical improvement after rTMS and clomipramine treatments as revealed by the Hamilton Depression Rating Scale (HDRS). ▨, 3 Hz left; ▧, 3 Hz right; ▩, 10 Hz left; □, 10 Hz right; ■, clomipramine.

Treatment efficacy

First, we looked at only those who completed the whole 2-wk protocol. All groups showed similar patterns of slight improvement over time (Figure 2). The ANCOVA, with pre-treatment scores as covariates, failed to detect a significant overall treatment effect on the post-treatment HDRS [$F(4, 44) = 1.24$, $p > 0.05$]. Similarly, a set of preplanned contrast analyses failed to reveal any significant effect of stimulation site or stimulation frequency on any of the post-treatment scores.

Next, in order to increase the statistical power of our analyses, we repeated the same set of analyses after imputing post-treatment scores for five patients (four from the 10 Hz frequency, and one from the 3 Hz condition) who completed the first week (five sessions) and then withdrew. The imputation method used for calculation of the missing observations was a regression equation that predicts post-treatment ratings based on age, length of illness, age at onset, and clinical ratings at baseline and after 1 wk of treatment (all participants had data on these variables). The R^2 of these prediction equations was 0.58 for the HDRS. The overall pattern and magnitude of results remained unchanged after repeating the same set of ANCOVAs and contrast analyses on the more extensive set of data. That is, no significant treatment effects were found with any of the clinical measures [for the HDRS, $F(4, 49) = 1.10$, $p > 0.05$].

Finally, we defined marked clinical improvement as a reduction of 50% or more in post-treatment HDRS

Table 2. Number of patients who improved (at least 50% reduction in the Hamilton Depression Rating Scale) after rTMS and clomipramine treatments[a]

	3 Hz protocol	10 Hz protocol
Left active rTMS with placebo medication ($n=17$)	6/11 (54.5%)*	1/6 (16.7%)
Right active rTMS with placebo medication ($n=18$)	2/12 (16.7%)	2/6 (33.3%)
Clomipramine treatment with sham rTMS ($n=15$)	2/15 (13.3%)	

[a] Significant difference as compared to the average rate of improvement in the other four groups.
* $p<0.05$, χ^2 test.

scores compared to baseline scores. Table 2 shows the percentage of patients who reached this criterion in each one of the five groups. Only a small subgroup of patients reached this criterion in most groups. The only exception to this general rule was the left 3 Hz rTMS group in which six patients (54.55%) met this improvement criterion. The higher improvement rate in the left 3 Hz rTMS group was statistically significant when compared to the average rate of improvement in the other four groups (17.94%, Yates' $\chi^2_{(1)}=4.22$, $p=0.039$), and near significant when compared to only the active medications group (Yates' $\chi^2_{(1)}=3.10$, $p=0.068$).

Measures of cortical excitability

Out of 50 patients who completed all 10 treatments 49 patients (mean age 61.1 yr, range 22–80 yr; 36 women, 13 men) were able to cooperate sufficiently for neurophysiological assessment. Results presented below refer to these 49 patients.

Upon completion of the 2-wk treatment period, a significant increase of the rMT was found in patients who received active 10 Hz rTMS with placebo medication [Figure 3, $t(11)=-4.2$, $p=0.001$, paired t test]. The repeated-measures ANOVA showed a significant interaction 'Treatment group × Time' indicating that the effect of 10 Hz rTMS on the rMT was opposite to that of clomipramine [$F(2, 46)=4.12$, $p=0.022$]. The latter caused a reduction of the rMT however this change did not reach statistical significance compared to baseline ($p>0.05$, paired t test). Other measures of cortical excitability were not affected by 3 Hz rTMS, 10 Hz rTMS or pharmacological treatments (Table 3).

In a second comparison, we evaluated changes in MEP and SP parameters in patients who received active rTMS in relation to clinical outcome and stimulation side regardless of stimulation frequency. The effect of rTMS on the rMT was not associated with positive clinical response to the treatment. In contrast, a significant increase of the MEP/M-wave amplitude ratio was observed in patients who had marked

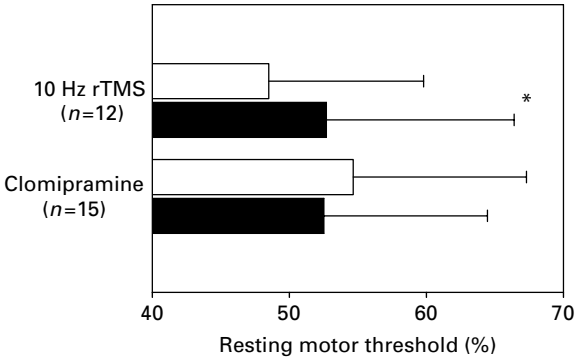

Figure 3. Opposite changes of the resting motor threshold in patients who received active 10 Hz rTMS compared to clomipramine treatment (mean ± S.D., * $p<0.01$, paired t test). □, Baseline; ■, after treatment.

clinical improvement [paired t test: $t(9)=-2.42$, $p=0.038$] but not in those who failed to improve (Figure 4). For this parameter, a significant interaction 'Clinical outcome × Time' [$F(1, 32)=5.09$, $p=0.031$] was found. The increase of the MEP/M-wave amplitude ratio was more prominent and consistent in the left hemisphere in patients who responded to left rTMS [paired t test: $t(5)=-3.57$, $p=0.016$] and was associated with a non-significant trend towards a shorter SPD at an intensity of 125% of the SPT (Figure 5). For the latter, ANOVA revealed a marginally significant interaction between the factors 'Side', 'Clinical outcome' and 'Time' [$F(1, 29)=3.94$, $p=0.057$]. Parameters of right-hemisphere excitability as well as those of left-hemisphere excitability in patients who did not respond to rTMS did not change significantly.

Discussion

In this study, 10 daily sessions of rTMS administered to the prefrontal cortex by a non-focal circular coil produced only a moderate antidepressant effect in

Table 3. Changes in MEP and silent period (SP) parameters after rTMS and clomipramine treatments (mean ± S.D.)[a]

	rTMS 3 Hz with placebo medication ($n=22$)		rTMS 10 Hz with placebo medication ($n=12$)		Clomipramine with sham rTMS ($n=15$)	
Parameters	Baseline	After treatment	Baseline	After treatment	Baseline	After treatment
Resting motor threshold (rMT)	51.4 ± 15.2	53.3 ± 15.1	48.5 ± 11.3	52.7 ± 13.7*	54.7 ± 12.7	52.5 ± 11.9
Silent period threshold (SPT)	38.5 ± 7.8	37.8 ± 8.1	37.5 ± 6.1	38.8 ± 9.3	39.4 ± 6.0	38.5 ± 5.6
Inter-threshold difference (ITD)	12.9 ± 10.3	15.5 ± 9.5	11.0 ± 7.4	13.9 ± 6.7	15.2 ± 10.2	14.0 ± 8.7
MEP/M-wave amplitude ratio (%)	15.1 ± 12.5	18.1 ± 16.4	10.7 ± 14.5	11.5 ± 18.6	12.5 ± 13.1	15.8 ± 12.9
SP duration at intensity of 125% SPT	83.7 ± 34.8	71.4 ± 35.1	67.8 ± 25.5	73.5 ± 26.4	74.5 ± 31.2	71.6 ± 30.4
SP duration at intensity of 150% SPT	123.3 ± 36.4	118.8 ± 36.0	101.6 ± 29.8	109.1 ± 29.8	111.4 ± 35.2	107.4 ± 34.9
SP duration at intensity of 175% SPT	157.8 ± 41.6	156.1 ± 38.7	122.2 ± 31.3	131.7 ± 25.5	138.3 ± 35.6	145.9 ± 42.0

[a] Significant difference compared to baseline values.
* $p<0.05$, repeated measures ANOVA, paired t test.

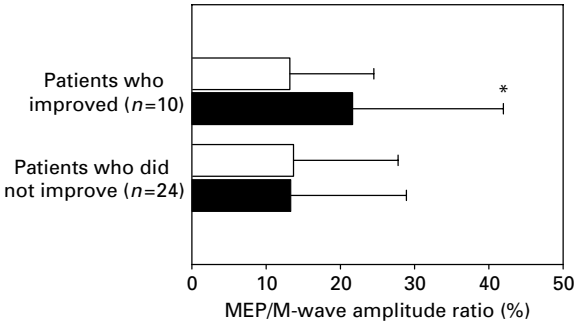

Figure 4. Changes in the MEP/M-wave amplitude ratio (%) following 2 wk of active rTMS compared to baseline with relation to clinical outcome (mean ± S.D., * $p<0.05$, paired t test). □, Baseline; ■, after rTMS treatment.

patients with MD, yet, there seems to be a differential treatment response which depends on treatment parameters. Thus, left 3 Hz rTMS appears to have higher clinical efficacy compared to the other rTMS schedules or 2 wk of clomipramine treatment. The 10 Hz rTMS treatment was less tolerated and, consequently, resulted in a significantly higher dropout rate despite the lower stimulus intensity than that used in 3 Hz rTMS. Numerous studies published over the past few years have reported significant reductions of depressive symptoms following 2 wk of rTMS treatment, employed at high frequencies (5 Hz or more) to the left dorsolateral prefrontal cortex (Avery et al., 1999; Berman et al., 2000; Garcia-Toro et al., 2001; George et al., 1997) or to the right prefrontal cortex using low (1 Hz) frequency (Feinsod et al., 1998; Fitzgerald et al., 2003; Klein et al., 1999). Furthermore, Fitzgerald et al. (2003) showed a lower tolerability of 10 Hz left rTMS in comparison with 1 Hz right rTMS without revealing a significant difference in clinical outcome between these treatment conditions. Likewise, George et al. (2000) did not find significant difference in the degree of clinical improvement in MD patients who received 5 Hz and 20 Hz left prefrontal rTMS. Taken together, these observations suggest that left rTMS at frequencies of 5 Hz or more and right rTMS at a frequency of 1 Hz are equally effective in their antidepressant properties. Our results further show that 3 Hz rTMS is more effective when given to the left hemisphere and thus, maintains the clinical profile of high-frequency stimulation. It is also noteworthy that the adversity of 10 Hz rTMS in our study seemed to be more pronounced than that described in other studies, and this might be related to the diffuse stimulation pattern of the round coil in contrast to the focal figure-of-eight coil usually used for rTMS treatment. Lower frequency rTMS, by virtue of its better tolerability, can be applied at higher stimulus intensity, as in our study (110% rMT for 3 Hz rTMS vs. 100% rMT for 10 Hz rTMS), and the increase of stimulus intensity (amount of the energy delivered) can be done within the recommended safety guidelines

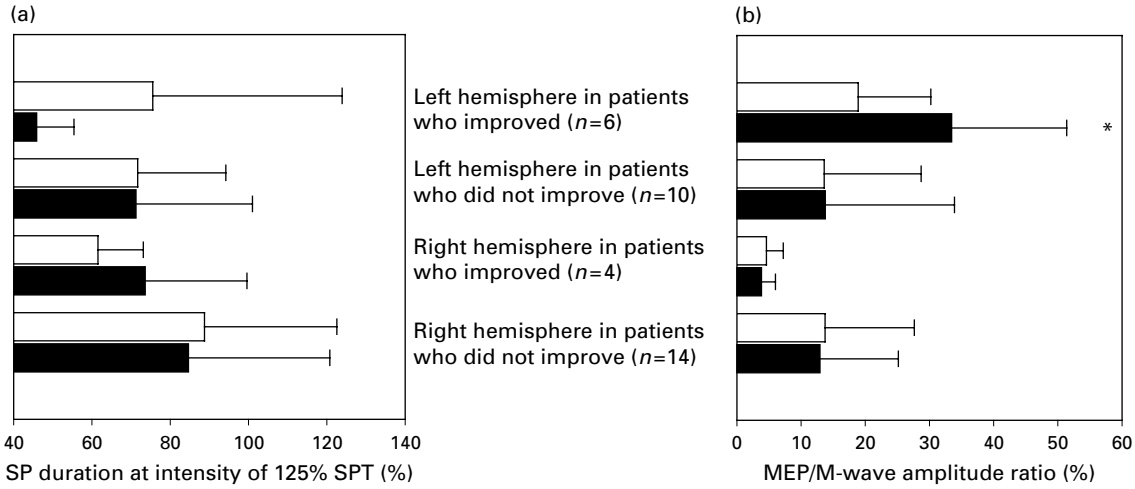

Figure 5. (a) Changes in the silent period duration (SPD) at an intensity of 125% silent period threshold (SPT) and (b) changes in MEP/M-wave amplitude ratio after 10 sessions of rTMS according to the stimulation side (mean ± S.D., * $p < 0.05$, paired t test). □, Baseline; ■, after treatment.

(Wassermann, 1998). This is important since stimulus intensity, rather than stimulation frequency, appears to be more critical in determining the antidepressant efficacy of rTMS (Gershon et al., 2003) especially in elderly patients in whom cortical atrophy could lead to a longer distance between coil and stimulated cortex (Mosimann et al., 2002). The higher stimulus intensity of 3 Hz rTMS may be responsible at least in part for its more prominent antidepressant action in our patients who are relatively old.

Similar to most previously published studies, the duration of our treatment protocol was 2 wk. This is clearly insufficient to obtain a maximal effect of clomipramine and apparently also for rTMS. Recent studies indicate that at least 4 wk of daily rTMS are required to achieve a substantial clinical benefit in MD patients (Fitzgerald et al., 2003). The optimization of treatment parameters and demonstration of significant differential effects of treatment protocols using different stimulation frequencies, intensities and laterality, will need larger samples and longer treatment duration.

Our findings also demonstrate the ability of rTMS to modulate cortical excitability in patients with MD. Several studies have reported that rTMS may induce transient changes in brain functions. Long trains of low-frequency stimulation ($\leqslant$1 Hz) lead to a decrease of MEP amplitude (Touge et al., 2001) lasting for about 30 min whereas high-frequency rTMS ($\geqslant$10 Hz) is associated with a reduction of intra-cortical inhibition and increase of MEP amplitude (Pascual-Leone et al., 1998). Speer and colleagues showed opposite effects of low- and high-frequency rTMS on regional cerebral blood flow observed 72 h after completion of 10 daily treatments in depressed patients (Speer et al., 2000). Likewise, the antidepressant medication has been shown to induce temporary changes in cortical excitability (Manganotti et al., 2001). The increase of the motor threshold and intra-cortical inhibition and suppression of the intra-cortical facilitation were evidenced 4 h following clomipramine administration. On the other hand, clomipramine is known to reduce seizure threshold and to provoke epileptic seizures (Pisani et al., 2002). Our results provide more evidence of long-lasting effects of 2 wk of daily rTMS and clomipramine treatments on cortical excitability. However, in contrast to earlier reports (Triggs et al., 1999), we found a significant increase of the motor threshold in patients who received 10 sessions of 10 Hz rTMS. The possible reason of this contradiction is the use of different coils for rTMS treatments. In most previous studies, focal stimulation of the dorsolateral prefrontal cortex was achieved with the figure-of-eight coil while we used a non-focal circular coil with its anterior segment covering the prefrontal cortex and its posterior tip located near the presumed line of the central sulcus. Such coil position could cause activation of both the prefrontal and motor cortices simultaneously. Therefore, one may assume that the alteration of motor cortex excitability induced by rTMS and assessed by sTMS may at least, in part, reflect analogous changes in excitability of the prefrontal cortex. However, modulatory effects exerted by stimulation of other cortical regions such as the supplementary motor area (Oliveri et al., 2003) and mediated by intra-cortical connections can not be

excluded. Besides, the opposite hemisphere could also receive some portion of the magnetic stimulus and, consequently, could influence the motor cortex excitability via transcallosal pathways (Gorsler et al., 2003). Given such a multiple site mode of excitation produced by a large round coil, the increase of the rMT following 10 Hz rTMS is not entirely surprising and may be similar in its mechanism to that underlying the elevation of seizure threshold in electroconvulsive treatment (Amiaz et al., 2001; Coffey et al., 1995). Ebert and Ziemann (1999) reported a suppressive long-term effect of high-frequency rTMS on seizure susceptibility in rats. They found a 55% higher threshold for induction of epileptic after-discharges 2 wk after a single 20 Hz rTMS train of 3 s duration. The decrease in epileptic activity following 1 s rTMS trains at an intensity of 120% motor threshold and frequency of 30 Hz or 50 Hz was also demonstrated in patients with drug-resistant temporal lobe epilepsy (Jennum et al., 1994). These data and results of our study indicate that repeated sessions of non-focal high-frequency rTMS may produce a suppression of neuronal membrane excitability.

Of more interest from a clinical perspective is the relationship between neurophysiological measures and treatment outcome. In our MD patients, changes of the rMT as well as the SPT and the ITD were not related to positive clinical outcome. Moreover, 10 Hz rTMS and clomipramine treatments exerted opposite effects on the rMT whereas there was no significant difference in the therapeutic efficacy of these treatments. This suggests that the motor threshold is not a useful electrophysiological correlate of clinical improvement.

However, the second finding of the present study was a significant increase of the MEP/M-wave amplitude ratio mainly in the left hemisphere in those patients who improved after 2 wk of rTMS. This was associated with a non-significant shortening of the SPD at an intensity of 125% of the SPT. It is currently thought that the MEP/M-wave amplitude ratio is an integrative parameter of cortical excitability which assesses the proportion of the spinal motoneuron pool driven by sTMS. It depends on the number of corticospinal neurons responding to sTMS at a given stimulus intensity and the degree of synchronization of multiple descending volleys [direct (D) and indirect (I) waves] generating an excitatory post-synaptic potential in the spinal motor neurons. The increase of the MEP/M-wave amplitude ratio could be due to shifts in (i) neuronal membrane excitability and/or (ii) excitatory synaptic activity of interneuronal circuits at the cortical level. The former mechanism seems unlikely as the increase of the rMT found in patients of the 10 Hz rTMS group was not associated with reduction of the MEP/M-wave amplitude ratio (Table 3). A more plausible explanation to our findings could be an enhancement of synaptic efficacy or long-term potentiation of trans-synaptic excitation resulting in more effective production of I-waves and increase of the MEP amplitude. The mechanism of I-wave facilitation could also be related to an ability of rTMS to suppress the GABAergic inhibitory influence which controls I-wave interaction (Ziemann et al., 1998) and can be exaggerated in depressed patients (Steele et al., 2000). The shortening of the cortical SPD in patients who responded to rTMS treatment supports this suggestion (Inghilleri et al., 1996; Roick et al., 1993; Werhahn et al., 1999).

Using the paired-pulse TMS paradigm, Maeda and colleagues found a diminished excitability of the left hemisphere compared to the right hemisphere in patients with MD (Maeda et al., 2000a). Such an interhemispheric difference was absent in healthy subjects. The rightward asymmetry was also demonstrated for parameters of regional cerebral blood flow as measured by single photon emission tomography. This asymmetry was reversed after 2 wk of high-frequency rTMS treatment administered to the left dorsolateral prefrontal cortex (Mottaghy et al., 2002). In our other study, we found an increase of the MEP/M-wave amplitude ratio and decrease of the short intra-cortical inhibition in the left hemisphere in MD patients following electroconvulsive treatment (Kaplan et al., 2003). Therefore, the enhancement of excitability of the circuits underlying the MEP and reduction of intracortical inhibition responsible for the SP, as revealed in the present study in patients who improved after left rTMS, could be related to its antidepressant action. However, given the exploratory nature of the findings regarding MEP amplitude and SPD they should be regarded as tentative. Further study using paired-pulse TMS with bilateral MEP recording could enhance our understanding of the role of intra-cortical excitatory and inhibitory mechanisms and their interhemispheric interactions in the pathophysiology of MD. The short-term and long-term evaluations could show whether measures of cortical excitability performed shortly after the beginning of the treatment might be used as predictors of clinical outcome.

In conclusion, the results of this study suggest that rTMS parameters such as stimulation frequency, intensity and location seem to determine treatment response and tolerability. When clinically effective, rTMS causes an overall enhancement of cortical excitability mainly in the left hemisphere, and this could be

related to its antidepressant mechanism of action. Further optimization of these parameters might improve the clinical efficacy of rTMS in patients with MD.

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Statement of Interest

None.

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