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Repetitive transcranial magnetic stimulation (rTMS) at high and low frequency: an efficacious therapy for major drug-resistant depression?

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Abstract

Objective: Repetitive transcranial magnetic stimulation (rTMS) is proposed for the treatment of drug-resistant depression. Studies performed in accordance with evidence-based medicine (EBM) are scarce, particularly in seeking optimal treatment and evaluation parameters. We aimed to test various types of rTMS in a large sample of depressed patients following EBM rules and to investigate treatment-related changes in plasma levels of neurotransmitters involved in depression.

Methods: Seventy-one drug-resistant depressed patients were randomly assigned to low (1 Hz) or high (17 Hz) rate TMS, applied for 5 days over the left dorsolateral prefrontal cortex (L-DLPFC). Patients were separated into two study designs. One group (20 patients) received only active treatment, while the other entered a double-blind, placebo-controlled, crossover design. Pre- and post-treatment blood samples were taken for evaluation of plasma levels of dopamine and serotonin.

Results: After a week of treatment patients had a measurable benefit. However, overall the placebo stimulation did not differ significantly from real stimulation, nor were differences observed between the two rates of rTMS. The only difference emerged when the real stimulation was applied at 17 Hz following placebo treatment. Plasma levels of neurotransmitters between active and placebo rTMS were similar.

Conclusions: Using the treatment schedule of 1 week, although a clinical improvement after active treatment was indeed observed, this was both clinically and biochemically indistinguishable from that seen in the placebo arm.

Significance: This suggests that most of the previous emphasis, for short period of treatment, should be tempered down and that further work is required in order to verify whether optimal stimulation and evaluation parameters for TMS-treatment of depression beyond the placebo effect may be found following EBM rules.

Keywords: Mood disorders; Depression; Transcranial magnetic stimulation treatment; rTMS; Prefrontal cortex

1. Introduction

Drug resistance represents a severe problem for major depression and nearly 35–40% of patients do not respond effectively to pharmacological medication. In this case, non-pharmacological treatments are also often utilized, with the best known being sleep deprivation (Wirz-Justice and Van den Hoofdakker, 1999) and electro-convulsive therapy (ECT). Transcranial magnetic stimulation (TMS)—a technique which allows the induction of brief electric currents within discrete brain areas via pulsed magnetic fields on the corresponding scalp location—has also been regarded as a promising tool for treating medication-resistant depressive patients (George et al., 2003; Gershon et al., 2003; Hausmann et al., 2004; Kauffmann et al., 2004; Lisanby et al., 2002; Loo et al., 2003; Martis et al., 2003; Padberg and Moller, 2003; Schlaepfer et al., 2003). This involves the discharge of a painless transient electro-magnetic field through the skull, thus allowing transynaptic depolarization of cortical neurons. However, studies of large patient

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populations with methods strictly following the evidence-based medicine rules are still scarce, so that the cochrane collaboration group (Martin et al., 2004) has not listed this treatment as fully proven to be efficacious. Unlike ECT, TMS has a good safety record, allows focal brain stimulation, it neither requires anesthesia nor induces short- or long-term cognitive side effects (Little et al., 2000).

The possibility of inducing long-lasting changes in cortical excitability might explain the beneficial results obtained in depressed patients (Siebner and Rothwell, 2003). These long-lasting changes in cortical excitability are dependent upon a number of variables, such as the frequency of stimulation, stimulus intensity, the site and number of applications. While some of these parameters meet relative consensus in the scientific community as critical determinants for the treatment of depression (e.g. site of stimulation on the scalp overlying the left dorsolateral prefrontal cortex [L-DLPFC]), others are still the subject of much debate. One such parameter is the frequency of stimulation used. High (>1 Hz) and low (≤ 1 Hz) TMS have been employed, in the belief that the former has a mainly excitatory, and the latter mainly an inhibitory, net effect (George et al., 2002; Hoffman and Cavus, 2002). Although differing in experimental designs, several studies have demonstrated that both types of stimulation (both low and high frequencies) have similar, positive, effects on the mood of depressed patients (Conca et al., 2002; Fitzgerald et al., 2003; Padberg et al., 1999). This is quite counterintuitive since it would be expected that opposite changes in cortical excitability modulation should produce opposite effects on mood or, in the best of cases, that only one type of stimulation would be effective. In reality it seems that, when applied over the L-DLPFC, only high frequency-TMS is effective in the treatment of depression, while low frequency-TMS is effective only after stimulation of the right dorsolateral prefrontal cortex (R-DLPFC) (George et al., 2002; Lisanby et al., 2002). This is in line with several studies using functional neuroimaging techniques suggesting that prefrontal cortex is implicated in emotion regulation (Ochsner et al., 2004; Phan et al., 2002). Specifically, the prefrontal cortex may be differentially involved in mood regulation (i.e. the right hemisphere is believed to mediate negative mood while left to mediate positive mood), Generally, unbalanced functioning of the two cortices due to hypoactivation of the left cortex is associated with depressive symptoms, corroborating previous TMS results (George et al., 2002; Lisanby et al., 2002; for a review, see Davidson 2004).

This difference also raises the possibility that other factors, in addition to cortical excitability modulation, might be responsible for the improvement in clinical outcome observed in depressed patients treated with TMS. One candidate, besides changes in cortical excitability, might be TMS-induced modulation of biohumoral profile. To date, few studies have analyzed possible changes in plasma level of neuroactive substances after TMS in humans (Strafella et al., 2001, 2003; but see Shaul et al. 2003 in human neuroblastoma cells). These studies mainly focused onto post-TMS changes in plasma concentration of hormones, above all thyroid hormone, in normals (Szuba et al., 2001). Normalization of the dexamethasone suppression test (DST) in depressed patients after TMS treatment has also been shown, suggesting possible influences onto the hypothalamic-pituitary-adrenocortical function (Pridmore, 1999). No previous study has evaluated TMS-induced changes in plasma level of the neurotransmitters mostly involved in the pathogenesis of depression, such as serotonin and dopamine, in patients with affective disorders. Only animal models exist, where TMS-induced changes in brain neurochemistry have been described with features strikingly resembling those observed after ECT treatment (Ben-Shachar et al., 1997, 1999; Keck et al., 2001; Ohnishi et al., 2004).

Against this background, the efficacy of rTMS for the currently published therapeutical protocols for the treatment of depression is still an unanswered question. In particular, it is not yet established whether the clinical improvement following the 1 week epoch of daily treatment with TMS (Figiel et al., 1998; George et al., 1995; Padberg et al., 1999; Pascual-Leone et al., 1996) can be fully attributable to biological effects, namely whether TMS-induced therapeutic effects can be disentangled from non-specific placebo effects.

The aim of the present study was to test the efficacy of a 5 days treatment regimen with high and low TMS rates in a relatively large population of depressed patients, by following evidence-based medicine criteria in order to distinguish 'real' therapeutic effects of TMS from placebo effects. As a corollary aim, we also wanted to evaluate TMS-induced changes in plasma levels of neurotransmitters involved in the pathogenesis of depression.

The study was divided into two experimental protocols. First, we delivered TMS for 1 week at both high and low frequencies, with parameters equivalent to those mainly employed in the relevant literature, to two groups of depressed inpatients, in order to verify whether it had any effect on their clinical outcome and biohumoral profile. After that, the same experimental protocol, with the additional introduction of controlled sham stimulation, was carried out in another group of outpatients, in order to differentiate real TMS-induced therapeutic potential from placebo effects.

2. Patients and methods

2.1. First experiment

Twenty patients (5 male and 15 female) of mean age 55 (range 35–81) were included in this part of the study. All of them were inpatients and none had shown clinical

Table 1
Clinical data for both studies

Protocol	Ss with psychotic symptoms	Duration of current episode (months)		HDRS score in		Number of failed antidepressant trials	
		Mean	Range	Mean	Range (SD)	Mean	ECT (Ss)
Inpatients (first experiment)							
17R	4	11.33	2–36	20.50	16–26 (3.75)	>5	0
1R	3	18.14	6–36	22.70	12–37 (7.20)	>5	2

Protocol	Ss with psychotic symptoms	Duration of current episode (months)		HDRS score in		Number of failed antidepressant trials		
		Mean	Range	Mean	Range (SD)	Mean	Range	ECT (Ss)
Outpatients (second experiment)								
17RI	2	9.60	2–24	19.40	13–29 (4.43)	3.33	2–5	0
1RI	2	8.00	2–36	24.90	15–35 (5.97)	3.70	2–7	1
17RII	1	7.66	3–12	23.00	13–32 (6.00)	2.75	2–6	0
1RII	1	11.22	2–36	20.90	13–35 (5.66)	3.30	2–5	1

The table reports duration of current episode, HDRS score at the beginning of the study (mean, minimum and maximum), number of previous failed antidepressant trials, episodes of electro-convulsive therapy and number of patients with psychotic symptoms according to treatment group (SD, standard deviation).

improvement following pharmacological antidepressant medication. They had no history of epilepsy or other neurological disorders.

Criteria for inclusion were as follow: 21 items Hamilton rating scale depression (HRSD) ≥ 12 or clinical improvement on the HRSD $\leq 50\%$ obtained after treatment with at least two classes of antidepressive drugs (see Table 1) (clinical improvement on the HDRS was calculated by subtracting the score after rTMS from the score before rTMS, dividing this figure by the score before rTMS, and finally multiplying by 100 [i.e. $100*(HDRS_1 - HDRS_2)/HDRS_1$], with a time lag of 5 days between T1 and T2). All patients had a period of constant medication for 3 months prior to the inclusion in the study and continued the medication they were taken at the enrolment for the duration of the study, therefore pharmacological dosages were kept constant during the trial. Informed consent was obtained, and the local ethics committee approved the protocol. Patients were randomly assigned to the two groups of TMS-treatment: group 17R for high-frequency (17 Hz) TMS and group 1R for low-frequency (1 Hz) TMS. The mean age of group 17R was 58 years, while for group 1R the mean age was 52; the mean intensity of stimulation was 10% higher than the motor threshold and was 84% for group 17R and 78% for group 1R of the maximum stimulator output;[1] in group 17R, 7 patients suffered from major depression, two from bipolar disorder in the depressed phase and one was schizoaffective, while for group 1R 7 patients suffered from major depression, two from dysthymia and one from schizoaffective disorder. All diagnoses were DSM-IV based and were formulated, by an expert psychiatrist, after meticulous diagnostic interview. Pharmacological treatments are detailed in Table 2.

Each block of TMS treatment consisted of 5 consecutive sessions of stimulation separated by 24 h. Treatment started on Monday and each subject underwent stimulation at the same time each day. Before starting the block of stimulation, clinical evaluation was performed by means of HRSD, and BPRS. The same tests were repeated after completion of the block of stimulation. An expert psychiatrist blind to the treatment performed the ratings. Furthermore, a blood sample was taken from each subject for biohumoral evaluation on the first and the last day of treatment. The frequency of stimulation and drug dosages were unchanged throughout the study.

2.2. Second experiment

The first experiment allowed us to obtain information useful for defining the sample size required to verify the hypothesis behind the second experiment. The standard deviation of HDRS changes (before–after treatment) was 5.0. We assumed that the sham stimulation could decrease the mean HDRS by 2.5 points and considered that a clinically relevant decrease after real stimulation should be at least 5.0 (a real effect size as large as two times the sham effect). Setting the criterion for significance (α) at 0.05 two-tailed, the recruitment of 40 patients would provide a power ($1-\beta$) of 0.87. On this basis, we increased the sample size at 51 patients, considering a high rate of possible drop-outs (around 30%). It is worth noting that this sample size determination was planned to assess the difference between real and sham stimulation in a paired sample. For the evaluation of potential differences between the two stimulation frequencies (between-subjects factor: 1 vs. 17 Hz), this study could be underpowered. Finally, 51 subjects were enrolled in this part of the study. All of them

[1] The high intensity of TMS stimulation in these patients may be explained by a reduction of excitability of the cortex due to several aspects like the age of the patients (Rossini et al., 1992) and mainly the pharmacological treatment (Palmieri et al., 1999; Ziemann et al., 1998).

Table 2
Concomitant pharmacological medications are detailed for all patients

Pharmacological treatment	1R	17R
Inpatients (first experiment)		
AD+BZD	3	4
AD+BZD	4	2
AD+BZD+Aneuro	1	1
AD+BZD+Tneuro	2	2
AD+BZD+Tneuro+MS		1

Pharmacological treatment	1RII	17RII	1RI	17RI
Outpatients (second experiment)				
AD		4	2	3
AD+Aneuro	1		1	
AD+Tneuro				1
AD+BZD	4	4	3	3
AD+BZD+Aneuro				1
AD+BZD+AP+Aneuro				1
AD+BZD+MS				1
AD+BZD+MS+Tneuro	1			
AD+BZD+Tneuro	1		3	
AD+BZD+Tneuro+Aneuro		1		
AD+MS	2	1		
AD+MS+Tneuro	1			
AD+MS+AP			1	

AD, antidepressant; BZD, benzodiazepines; MS, mood stabilizer; Tneuro, typical neuroleptic; Aneuro, atypical neuroleptic; AP, anti parkinson. The pharmacological treatment taken by patients were distributed as follow: First experiment: 17 Hz group 6 patients received serotonine selective reuptake inhibitors (SSRI), 3 received tricyclics, one received serotonin norepinephrine reuptake inhibitor (SNRI), two received selective norepinephrine reuptake inhibitor (NARI) and one received serotonin2-antagonists/serotonin reuptake inhibitors (SARI). In group 1 Hz, 9 patients received SSRI and 5 patients received tricyclics. Second experiment: group 17 Hz, 12 patients were taking SSRI, two received tricyclic, 3 received SNRI, one received NARI, 3 received SARI; while in group 1 Hz 10 were taking SSRI, 3 received tricyclics, 6 received SNRI and one received NARI.

were outpatients who were recruited following the same clinical criteria adopted for the previous experiment. Pharmacological treatment was kept constant during the trial (namely 10 weeks); patients who changed their drug therapy or dosage during the study were excluded from analyses.

The patients were randomly assigned to the two main groups for TMS treatment (i.e. 1 and 17 Hz) according to a double-blind, placebo-controlled, crossover design. Since a simple randomization was chosen, the two groups could be different in terms of size. Within each group, patients were assigned to two subgroups: those who received real stimulation first, and those who first underwent ineffective sham stimulation (placebo). Therefore, there were 4 groups: one group, 1RI (1 Hz real as first), received one block of real 1 Hz-TMS followed by a second block of sham 1 Hz-TMS (placebo); another group, 1RII (1 Hz real as second), received the same stimulation at 1 Hz but in a reverse order (sham first, followed by real TMS second); groups 17RI (17 Hz real as first) and 17RII (17 Hz real as second) included patients who underwent the same protocol as 1RI and 1RII (real/sham; sham/real), respectively, but at 17 Hz stimulation rate. The two blocks of stimulation (real/sham or sham/real) were separated by an interval of 8 weeks (see Fig. 1). The time points considered were: T0, baseline, just before the beginning of treatment; T1, end of the first treatment (i.e. the 5th day); T2, beginning of the second treatment, 8 weeks after T1; T3, last evaluation at the end of the whole study, 5 days following T2.

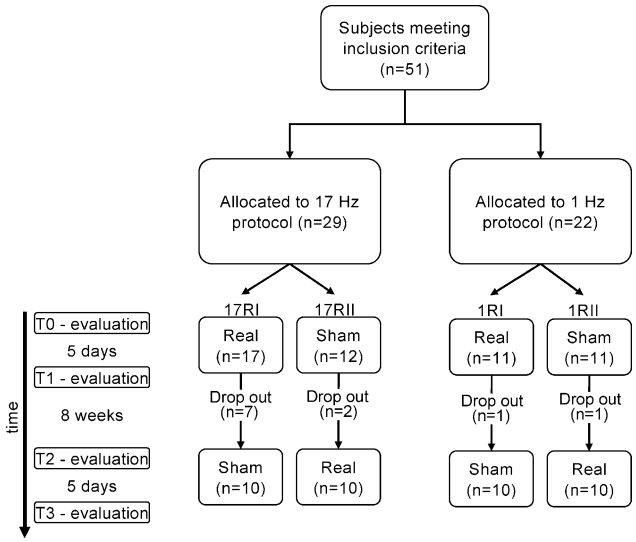

Fig. 1. Diagram showing the flow of participants through each stage of the trial. The time points considered were: T0, baseline, just before the beginning of treatment; T1, end of the first treatment (i.e. the 5th day); T2, beginning of the second treatment, 8 weeks after T1; T3, last evaluation at the end of the whole study, 6 days following T2.

Of the 51 patients, 11 left the study after the first treatment (time interval T0–T1) (Fig. 1). They were distributed as follows: group 17RI, so after real 17 Hz stimulation, $n=7$, (mean improvement HDRS 24%); group 1RI, so after real 1 Hz stimulation, $n=1$ (mean improvement HDRS 0%); group 17RII, so after Sham 17 Hz stimulation, $n=2$ (mean improvement HDRS 3%); group 1RII, so after Sham 1 Hz stimulation, $n=1$ (mean improvement HDRS 7%). The detailed reasons which led them to leave the study were: two patients from group 17RI thought that their mood had improved significantly, so they decided not to undergo the second phase (a significant improvement of tests score was indeed observed in both, 35 and 50%, respectively); one from group 17RII cited family reasons (his scores showed no difference after the treatment); all others wished to change their therapy because they were not satisfied with the rTMS treatment. The high intensity stimulation used in the 17RI condition might explain such a high drop-out rate.

The remaining 40 subjects, 10 for each group, were distributed as follows: the mean age was 54 years for group 17RI, 48 years for group 1RI, 53 years for group 17RII and 59 years for group 1RII. The percentage of stimulation of the maximum stimulator output was 75% for group 17RI, 78% for group 1RI, 79% for group 17RII and 80% for group 1RII. In group 17RI, one patient suffered from dysthymia and one was schizoaffective; in group 1RI one patient had dysthymia, one a bipolar disorder and two were schizoaffective; in group 17RII, two patients suffered from bipolar disorder and one had dysthymia; in group 1RII one was a bipolar patient and one schizoaffective. All the other patients suffered from major depressive disorders (see also Table 1). Pharmacological treatments are summarized in Table 2.

2.3. Magnetic stimulation

TMS was delivered by a Magstim-Rapid Magnetic Stimulator with a figure 8-shaped coil. Before starting TMS treatment, motor threshold (MT) was determined for each subject following international standards (Rossini et al., 1994): TMS was then delivered at 10% > MT on the frontal scalp area overlying the L-DLPFC, localized according to previous reports 5 cm in front of the best spot for inducing MEPs from the APB muscle (Pascual-Leone et al., 1996).

In the sham condition, a 25 mm thick plywood shield, build to appear as an integral part of the apparatus, was interposed between the coil itself and the scalp, separating the two. Moreover, the ventral surface of the coil from which the magnetic field was delivered was upside down and stimulus intensity was substantially decreased at 60% below MT. This 'placebo condition' was tested in a different control-group of patients on the motor cortex, showing that it was completely ineffective in inducing cortical excitability.

For the low-rate condition, trains of stimuli at 1 Hz-frequency and 10 s-duration separated by an inter stimulus interval (ISI) of 20 s were employed. For the high-rate condition, trains of stimuli of 17 Hz-frequency and 3 s-duration separated by a 120 s ISI were utilized. These parameters are in line with safety recommendations for rTMS (Wassermann, 1998; Wassermann and Lisanby, 2001). Each session lasted 20 min and the total number of pulses was similar between the two stimulation paradigms (in total 2000 for the 1 Hz-TMS and 2040 for the 17 Hz-TMS).

2.4. High performance liquid chromatographic (HPLC) technique

Plasma levels of dopamine (DA), its main metabolite homovanillic acid (HVA), serotonin (5-HT) and its main metabolite 5-hydroxyindolacetic acid (5HIAA) were determined according to the method described by Cheng et al. (1992). HPLC apparatus consisted of a GT-103 degasser (GASTORR), a PU-980 pump (JASCO), a reverse phase column (TRACER), an electrochemical analytical cell model 5011 and a detector coulochemII (ESA). The applied potentials were set at +300 and −250 mV. The flow rate was set at 1 ml/min. All the analyses were carried out in the same run of about 40 min. Proteins were precipitated by adding 20 µl of concentrated perchloric acid to 500 µl of plasma. The sample was mixed, centrifuged, filtered and, finally, injected in HPLC with electrochemical detection. Concentrations of all analyses were calculated by interpolation of their respective standard curves.

2.5. Data analysis

The effect of rTMS treatment on the clinical outcomes (HDRS, BPRS) was assessed by means of repeated-measures analyses of variance (ANOVAs); additional post hoc analyses were performed when necessary. The main factors were: *time* (within-subjects: T0, T1, T2, and T3) *rTMS frequency* (between-subjects: 17 Hz, 1 Hz) and *sequence* (RI: real → sham, RII: sham → real). In such analysis the effect of *type of rTMS* (within-subjects: real, sham) should be revealed by the interaction *time* × *sequence* and, in case of differences due to levels of *rTMS frequency*, by the interaction *time* × *sequence* × *rTMS Frequency*. This analysis was performed also adding the baseline measure (T0) as covariate, since—even for slight and not significant baseline differences between groups—the initial status could influence the follow-up changes. For a better comprehension of treatments effects, we computed the before–after treatment changes and represented them along with appropriate 95% confidence intervals.

In addition, exploratory analyses were also performed on the age of patients (≤ 50; >50), diagnosis and baseline scores. Further analyses were carried out by using the same factors in order to detect possible changes in the plasma levels of neurotransmitters (DA; 5-HIAA; HVA; 5HT).

3. Results

For both experiments, no significant differences emerged in the exploratory analyses for group assignment at baseline, age and diagnosis (i.e. only major depression); therefore, these factors were not considered further.

3.1. First experiment

The analysis performed on HRSD scores revealed a main effect of *time* [$P = 0.001$], while no differences were observed for the others factors nor was there any interaction between the factors. The same results were obtained for the BPRS scores [*time*, $P < 0.001$]. Mean of percentage changes on the HDRS according to group can be found in Table 3.

Table 3
Mean of percentage changes on the HDRS scores, divided according to experiment and type of TMS and frequency

		Real-rTMS (SD)	Sham-rTMS (SD)
% improvement at HDRS			
Outpatients	17RI	35 (23)	7 (23)
	1RI	20 (20)	13 (23)
	17RII	26 (14)	34 (32)
	1RII	23 (25)	18 (15)
Inpatients	17R	20 (24)	–
	1R	22 (14)	–

Overall, improvement after real stimulation at 17 Hz was 32%; 23% at 1 Hz and 17% after sham stimulation (SD, standard deviation).

Analyses of plasma levels of neurotransmitters did not reveal any significant difference between conditions.

3.2. Second experiment

The main analysis (outcome measure: HDRS) indicated that the only significant effect was *time* [$P<0.001$], and no other main or interactive terms reached the statistical threshold (0.05). As evident in Fig. 2, significant changes occurred between T0 and T1 [$P<0.001$] and between T2 and T3 [$P<0.001$], whilst no changes were observed between T1 and T2 [wash-out period, $P=0.519$]. The lack of significant interactions indicated that this trend was

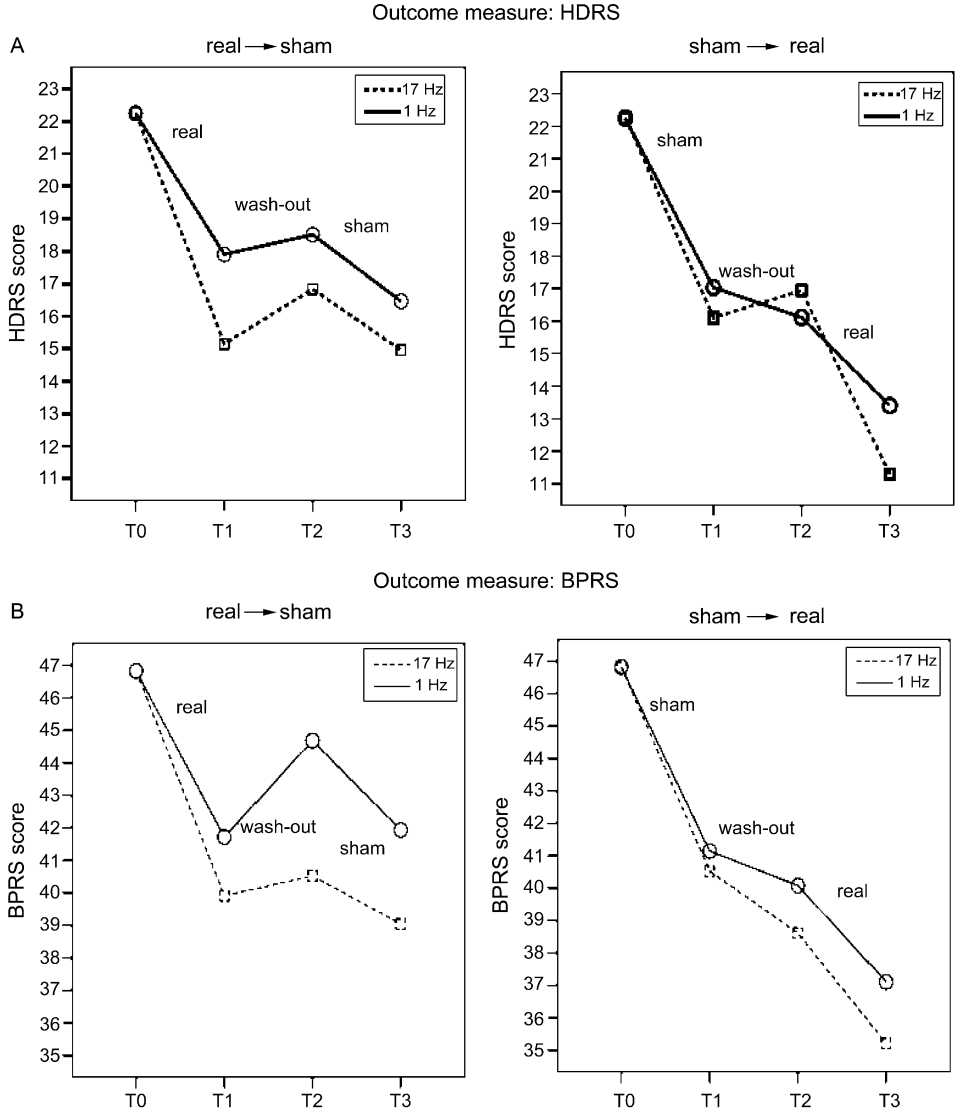

Fig. 2. Marginal mean for the two type of sequence: real first on the left side panel or sham first right side panel and frequency of treatment at the 4 time points. Panel A for HDRS scores and panel B for BPRS scores. In all the conditions T1 is significantly different from T0, while no differences were found between sham or real TMS and high or low frequency. In the last interval T2–T3, the condition 17 Hz real given second emerged from the other treatments; in this condition T3 approached the statistical threshold compare to T2.

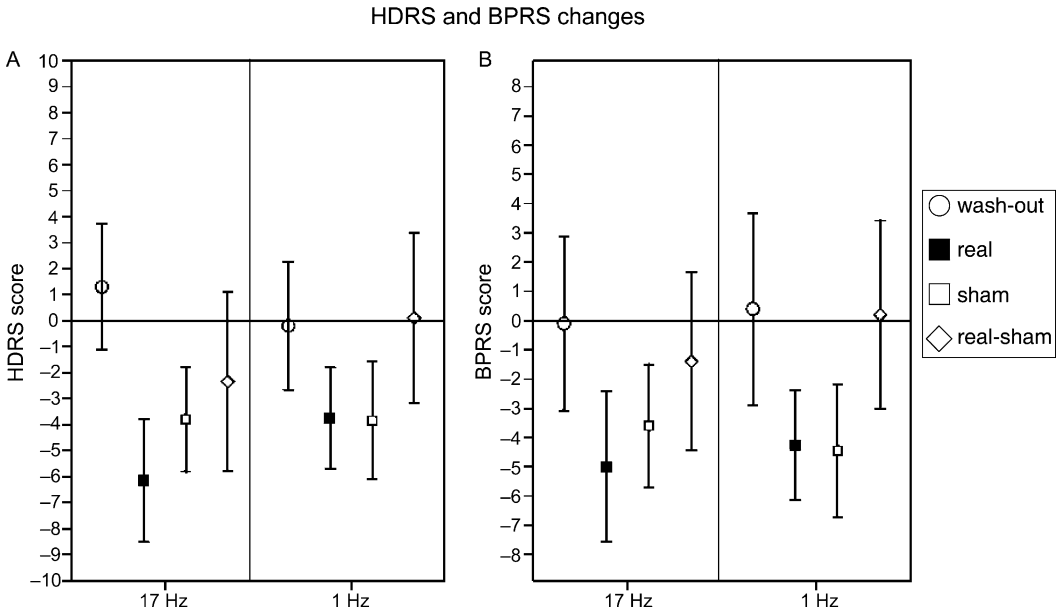

Fig. 3. Effect size with 95% confidence interval for HDRS (panel A) and BPRS (panel B) for real and sham rTMS treatment as well as data during wash-out periods and difference between real minus sham treatment (net effect of real) are reported. As can be noted the reference line at 0 (null effect) was not crossed by both sham and real rTMS delivered at both 1 and 17 Hz, indicating significant HDRS decreases (not found after the wash-out period). However, it is evident that, when the 'net' effect of real rTMS was computed (real–sham), the confidence interval still includes the 0 reference line.

roughly parallel across the levels of the other factors and, therefore, not significantly modulated by real vs. sham rTMS nor by 1 vs. 17 Hz stimulation. Including HDRS baseline evaluation as a covariate did not change these findings. In Fig. 3 (left panel), the effect sizes of each 'treatment' with the 95% confidence intervals are reported. The reference line at 0 (null effect) was not crossed by either sham or real rTMS delivered at both 1 and 17 Hz frequencies, indicating significant HDRS decreases (not found after the wash-out period). However, it is evident that, when the 'pure' effect of real rTMS was computed (real–sham), the confidence interval still includes the 0 reference line.

However, a close inspection of Fig. 2 suggests that, while the HDRS decreases observed in the first period (T0–T1) were very similar, in the third period (T2–T3) the results were slightly different. Especially when the last HDRS measurement (T2) was entered as a covariate, we found that the *time*×*sequence* interaction approached the statistical threshold [$P=0.053$] and this effect seemed to be more specific to the 17 Hz stimulation. However, as mentioned in the data analysis paragraph, our sample was sized to assess just real vs. sham efficacy and the lack of significance of the described effects could be due to insufficient power to detect such fine effects.

Analyses of changes in plasma levels of neurotransmitters did not reveal any significant correlations with HDRS scores.

As for HDRS, an overall, non-specific improvement in the clinical outcome of patients was also found for BPRS scores (Figs. 2 and 3). The main analysis revealed a significant effect of *time* [$P<0.001$] without any significant correlation with other factors. A further analysis performed on 4 subscales included in the BPRS was carried out in order to find possible clinical patterns that might explain the results obtained. These were: excitement, negative symptoms, positive symptoms, and depression (Ventura et al., 2000). A first analysis confirmed the general saturation of the specific items for the 4 subscales, while a further analysis revealed that only two subscales contributed significantly to the result obtained, namely depression and negative symptoms (i.e. only depression and negative symptoms scores were significantly altered by TMS). In the analysis of neurotransmitters changes, we found a correlation between BPRS and HVA [coefficient 0.37; $P=-0.04$] but irrespective of the type of TMS or frequency.

4. Discussion

The results from the first experiment suggest that rTMS may be a potential tool for treating depression, irrespective of the frequency of stimulation used. However, some considerations must be taken into account before any firm conclusion can be drawn from this first study. Based on previous reports, we expected to find differences in the clinical outcome of treated patients between high and low frequency stimulation of the L-DPFC; instead, no significant differences were observed. Secondly, we did not have any real control group undergoing a no-TMS treatment, so that the possibility of a placebo effect being responsible for

the clinical improvement we observed cannot be ruled out. Taking in consideration these two aspects, no definite conclusion could be drawn about the real efficacy of TMS as a therapeutic tool for the treatment of depressed patients. Therefore, we decided to perform the second phase of the study in another group of outpatients subjected to a double-blind, placebo-controlled, crossover design for each frequency of stimulation.

Overall, we have shown that, on the basis of a 5 day stimulation schedule, depressed patients benefit from TMS treatment, but this benefit is not strictly and clearly related to the real effect of TMS itself. In fact, it is not possible to say whether the improvement in the clinical outcome is attributable to real or 'placebo' TMS.

It is known that any remedial effects of treatment can potentially arise from two sources: one related to the specific properties of the treatment, and the other associated with the patient's expectations for the treatment (placebo effect). The magnitude of the placebo effect varies according to its supposed effectiveness and emotional impact on the treated subject (de la Fuente-Fernandez et al., 2002). The placebo phenomenon also seems to have a biological counterpart. In a PET study performed in depressed patients, the response to placebo treatment was associated with changes in brain metabolism partly overlapping the response induced by the active pharmacological treatment (Mayberg et al., 2002). This suggests that some changes in the clinical response are common for both types of TMS (real or placebo) and this may be necessary for remission from severe depression.

In a TMS treatment setting, the experimental\therapeutic environment seems to exert a strong emotional impact on the subject's expectations. In fact, the presence of a 'technological cutting-edge' device (i.e. TMS) that had direct tangible effects on the patient's brain, such as inducing involuntary hand movements during evaluation of motor threshold, might have been responsible for the observed generalized clinical improvement. However, although we were not able to differentiate between real and sham (placebo) effects of TMS after the first application, we did see a difference for the high-rate TMS after the second treatment. One possible explanation for this might rely on the evolution of the patients' expectations regarding the potential benefits of the very innovative treatment they were receiving. In particular, the positive outcome after the first TMS session might reflect the high expectations of patients, thus mixing-up placebo and real effects, while for the second treatment habituation to the experimental setting attenuated these expectations and real TMS effects—if present—could emerge beyond the placebo effect. Indeed, this differentiation was found only for the high-rate stimulation, which suggests that this particular treatment may have potential.

In addition to the placebo effect, other factors may be responsible for the failure to reach significant results following EBM rules. For example, it may be that some stimulation reached the cortex during the sham trials, as has been suggested by others (Lisanby et al., 2001; Loo et al., 2000). We think this is not the case for the present study, given that during sham stimulation we moved the coil away from the patients' scalp by means of a thick plywood shield and reduced the yielded intensity of the stimulator output to such an extent that no muscular responses were elicited when some subjects were tested with this 'sham' setting applied to the hot spot for inducing MEPs from the hand. It is also possible that the age of our patients could have reduced the clinical response. It has been found that treatment for depression (Manes et al., 2001; Nelson et al., 1995) is less effective in aged patients compared with their younger counterparts. To further test this possibility, we divided our patients into two age ranges, but we found no differences related to age, suggesting that this parameter did not influence the results.

The duration of TMS treatment is probably a key point. A recent meta-analysis by the cochrane collaboration (Martin et al., 2004) concluded that high-rate stimulation of L-DLPFC significantly improves depression only after 2 weeks of treatment. Our experimental protocol was carried out over only 5 days of real TMS, which is a really brief period compared to other antidepressant treatments, in order to test a treatment schedule which has been proposed as efficacious (Martin et al., 2004). Our results suggest that this treatment schedule is probably not long enough to obtain a clinical improvement that could be clearly differentiated beyond a placebo effect. It should be noted from Table 3 that if we consider the 2 weeks of treatment together (real plus sham) a clear improvement is evident which, in some cases, is quite substantial (i.e. in the 17RII about 50%); this is actually in line with results from most of the open studies published so far. If we consider longer treatment periods, recent meta-analyses have found that TMS seems to be effective in treating depression (Gershon et al., 2003), though Fitzgerald et al. (2003) suggest that at least 4 weeks of treatment are necessary to achieve clinically meaningful benefits.

Another important issue is the absence of a significant difference between the two rates of stimulation. The literature, in this respect, is relatively scant. Padberg et al. (1999) did not find any significant differences between low and high frequency stimulation of L-DLPC. The possibility that low frequency rates may be as effective as the high ones (Fitzgerald et al., 2003) may have positive implications in term of better safety profiles and reducing the risk of kindling related to higher rates of stimulation (Wassermann, 1998).

Some additional considerations relate to the BPRS scores. Although BPRS is not a clinical tool generally employed in TMS studies on depressed patients, the finding that only two subscales, namely those evaluating depression and negative symptoms, correlate positively with TMS treatment strengthens the possibility that TMS may indeed induce an improvement in depressed patients. The selective

improvement in negative symptoms might in part be related to the positive effects exerted by rTMS on psychomotor performances of patients suffering from dysfunction of the cortico-subcortical dopaminergic system, as suggested by the fact that motor symptoms are frequently combined with depression in disorders such as Parkinson disease (Pascual-Leone et al., 1994). Since the dopaminergic system is critically involved in the pathogenesis of depression (Brown and Gershon, 1993), the possibility that the post-TMS improvement in BPRS scores of depression and negative symptoms may, in part, be attributable to direct effects of TMS onto cortico-striatal dopaminergic system cannot be ruled out. This is especially the case given the significant correlation found between TMS-treatment and plasma levels of HVA. This also suggests that this metabolite may play a role in determining the clinical response of depressed patients to TMS (Ben-Shachar et al., 1997; Keck et al., 2002; Strafella et al., 2001), irrespective of the type of treatment received and in agreement with data obtained with both pharmacological and non-pharmacological treatments; for instance treatment with Monoamine Oxidase-type A caused significant mean reductions in HVA plasma levels (Markianos et al., 1994).

In conclusion, it was demonstrated that using the treatment schedule of 1 week rTMS produces an antidepressant effect that is not clearly distinguishable from a placebo effect. Moreover, differences in type of treatment or clinical response do not clearly correlate with changes in plasma levels of some of the major neurotransmitters implicated in mood control.

These findings suggest the need for adopting a more prudent view when evaluating the still largely unproven effects of TMS on depression, at least for 1 week of treatment. Considering the potential benefit of an antidepressant treatment like TMS, that it is non-invasive and well-tolerated, further studies on large patients populations and for longer treatment periods are needed, in order to find appropriate stimulation parameters and clinical evaluation tools that will elucidate the role of TMS in the treatment of depression.

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