

Lateralized and frequency-dependent effects of prefrontal rTMS on regional cerebral blood flow

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Repetitive transcranial magnetic stimulation (rTMS) is a means to study the function and connectivity of brain areas. The present study addressed the question of hemispheric asymmetry of frontal regions and aimed to further understand the acute effects of high- and low-frequency rTMS on regional cerebral blood flow (rCBF). Sixteen healthy right-handed men were imaged using H₂¹⁵O positron emission tomography (PET) immediately after stimulation. High (10 Hz)- and low (1 Hz)-frequency suprathreshold short-duration rTMS was applied over either the left or right dorsolateral prefrontal cortex (DLPFC). Slow and fast rTMS applied over the *left* DLPFC significantly increased CBF in the stimulated area. Compared to baseline, slow rTMS induced a significant increase in CBF contralateral to the stimulation site, in the right caudate body and in the anterior cingulum. Furthermore, slow rTMS decreased CBF in the orbitofrontal cortex (OFC, ipsilateral to stimulation side). Fast rTMS applied over the *right* DLPFC was associated with increased activity at the stimulation site, in the bilateral orbitofrontal cortex and in the left medial thalamus compared to 1-Hz rTMS. These results show that rCBF changes induced by prefrontal rTMS differ upon hemisphere stimulated and vary with stimulation frequency. These differential neurophysiological effects of short-train rTMS with respect to side and frequency suggest hemisphere-dependent functional circuits of frontal cortico-subcortical areas.

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Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a research tool to study neural connectivity at the system level and

is used therapeutically in a number of neuropsychiatric disorders. It has been extensively studied as a treatment for depression, and the majority of clinical trials that applied rTMS to the prefrontal cortex report results superior to placebo (Burt et al., 2002; Martin et al., 2003). However, the neurophysiological effects of rTMS, particularly as a function of the stimulation parameters (e.g., location, frequency and intensity), remain unclear.

The application of rTMS in depression suggests a rather strong laterality effect. *High*-frequency stimulation (10 Hz to 20 Hz) was found to induce an antidepressive effect when administered over the *left* prefrontal cortex (George et al., 1995; Pascual-Leone et al., 1996a), but not when applied over the *right* prefrontal cortex. Later studies reported effects that depended on stimulation frequency. Thus, *low*-frequency rTMS over the *right* prefrontal cortex has an antidepressant effect (Klein et al., 1999; Feinsod et al., 1998; Schutter et al., 2001), whereas the same parameters over the left side are ineffective. In healthy subjects, however, changes in mood observed after prefrontal rTMS are the opposite of those in depressed patients. In healthy subjects, *high*-frequency rTMS increases feelings of sadness when administered to the *left* prefrontal cortex but increases feelings of happiness if administered to the *right* prefrontal area (George et al., 1996; Pascual-Leone et al., 1996b; but see Jenkins et al., 2002; Mosimann et al., 2000; Padberg et al., 2001).

Thus, both the side of stimulation and the frequency of stimulation seem to be highly relevant to the therapeutic effect achieved and, in healthy subjects, to the direction of the mood changes. The strong association between the affected hemisphere of a frontal lesion and the behavioral manifestation is well known from patient studies on mood disorders and impulse control disorders. As, for example, in the disinhibition syndrome and secondary mania, ample evidence indicates that mainly right-sided lesions are associated with manic-like behavior symptoms (Cummins and Mega, 2003). Interestingly, in patients with mania, high-frequency rTMS over the right prefrontal cortex appears to be associated with antimanic effects, whereas the same stimulation on the left side is ineffective

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(Grisaru et al., 1998; Erfurth et al., 2000; Michael and Erfurth, 2004). Furthermore, we recently reported evidence that prefrontal rTMS affects cognitive processing in a frequency-dependent manner (Knoch et al., 2005).

In contrast to the rapidly growing literature with regard to the laterality- and frequency-dependent swing in mood or therapeutic antidepressant rTMS effects and effects on cognitive performance, there have been few studies examining the potential neurophysiological mechanisms of TMS. To further analyze the role of the stimulation frequency and laterality, we conducted a combined TMS-PET study in healthy subjects focusing on acute rTMS effects. The majority of neuroimaging studies analyzing the acute effects of prefrontal rTMS in healthy subjects conducted so far stimulated only the *left* DLPFC (Barrett et al., 2004; George et al., 1999; Kimbrell et al., 2002; Nahas et al., 2001; Speer et al., 2003). Since the prefrontal cortex is asymmetric in both structure and function, it is feasible to hypothesize that left and right rTMS over the DLPFC would differentially affect CBF changes. In addition, as mounting evidence suggests that rTMS has not only local effects (e.g., Bestmann et al., 2004; Li et al., 2004; Nahas et al., 2001; Paus et al., 2001), we hypothesize that the frequency- and laterality-dependent effects are not limited to the cortical area targeted by rTMS (at stimulated site) but could also occur at remote interconnected areas. *Suprathreshold* stimulations were chosen because larger and more widespread rCBF changes can be expected (Kähkönen et al., 2005; Nahas et al., 2001).

Knowledge of potential frequency-dependent and/or laterality-dependent prefrontal rTMS effects on cortical excitability and neural connectivity of the stimulated area may contribute to our understanding of rTMS mechanisms of the regulation of mood and impulse.

Materials and methods

Subjects

Sixteen right-handed healthy men (mean age 27 years, SD 4 years) participated in the study, having given written informed consent as per approval by the local ethics committee. All subjects were naive to TMS and had no history of psychiatric illness or neurological disorders. Subjects received 300 Swiss Francs for their participation and were randomly assigned to receive either left or right prefrontal rTMS.

Methodological remarks

Shielding requirements

Whether or not PET scanners should be shielded from the magnetic field induced by TMS coils is still under debate. Several groups shield the scanner with a mu-metal cylinder positioned in the gantry (Thompson et al., 1998; Siebner et al., 2000). Others claim that shielding is not necessary (Lee et al., 2003; Speer et al., 2003). To test whether shielding is required in our PET system (GE Advance, in 3D mode), we have performed phantom experiments. For this purpose, a standard GE phantom was filled with an appropriate concentration of ^{18}F -FDG (20 kBq/ml) and scans were compared with and without TMS stimulation (100% output, 10 Hz). Statistical parametric mapping of the images did not reveal any differences between the two groups of scans. For these reasons, we chose to perform the study without shielding.

Optimal timing of scan

We conducted a combined TMS/PET study with a constant H_2^{15}O infusion protocol, which continuously monitors cerebral blood flow (see Weber et al., 2004) to evaluate the optimal time point to scan the CBF changes after TMS stimulation for 1 min using 1-Hz and 10-Hz rTMS ($N = 6$). The maximal signal increase over the stimulated area was reached 90 s after the onset of TMS. Taking into account the lag of the signal change due to a CBF increase (a characteristic inherent in all continuous infusion protocols, for details see Weber et al., 2004), we determined the optimal scan window in the bolus protocol from 60 to 120 s following TMS start. The baseline CBF level was reached 7 min after the onset of TMS. The continuous infusion protocol was not chosen for the actual experiments because of its considerably more complicated application and inferior signal-to-noise ratio.

Sham stimulation

The choice of the appropriate “baseline condition” in TMS and PET experiments is not trivial. Several aspects have to be taken into consideration: a.) acoustic stimulation, i.e., click sound of TMS stimulus, b.) facial nerve and scalp stimulation, c.) proprioception of muscle contraction. The air-cooled TMS coil produces a loud broadband noise that covers the click sound of the stimulations. Moreover, the volunteers wore earplugs, and clicks were reported not to be audible. Aspects b.) and c.) cannot be controlled using a second sham coil as used by other groups. For these reasons, the baseline scans were carried out with the coil in position and with fan noise but with no current applied.

Location of the target region and positioning of the TMS coil

One day before the rTMS/PET experiment, the site of prefrontal stimulation was determined from the location of the motor cortex for each subject. Transcranial magnetic stimulation was administered using a Magstim (Rapid Magnetic Stimulator, Magstim, Winchester, MA) stimulator and a figure-eight air-cooled coil (70-mm diameter double circle). The coil was systematically displaced over the primary motor cortex until the largest consistent movement in the contralateral index finger was detected. This position was marked on the scalp (position A). The stimulation intensity was gradually decreased until muscle twitches were no longer observed. Thereafter, the individual motor threshold was defined as the intensity setting on the Magstim (in 1% increments) that produced a visible muscle twitch in the contralateral index finger in at least five out ten consecutive stimulations. The prefrontal stimulation site was 5 cm anterior in a parasagittal line (position B). This site was chosen in order to target the middle frontal gyrus in the DLPFC. An anatomical T1-weighted MRI scan was then obtained with vitamin E capsules at positions A and B. Based on the anatomical MRI scan, the position for the DLPFC stimulation was adjusted to be over the middle frontal gyrus in approximately the same position for all subjects in order to render a group statistics feasible. In the actual rTMS/PET experiment, the coil was positioned in the same coil orientation used for determining the motor threshold over the motor cortex, i.e., with the junction of the coil at 45° to the sagittal plane and tangential to the curvature of the head, with an articulated arm fixated at the front-end of the scanner table.

Stimulation and PET procedures

A total of 12 H₂¹⁵O PET scans were performed in each subject (baseline, 1-Hz and 10-Hz rTMS performed 4 times, repeated 3 times). All participants first underwent the baseline condition. The order of the two rTMS conditions was pseudo-randomized across participants. The interval between the scans was 10 min. Subjects lay with eyes closed and wore foam earplugs. Head movements were minimized by a toby collar and fixation straps. For the 1-Hz condition, a continuous 60-s stimulation was applied, whereas in the 10-Hz condition, pairs of 5-s stimulation and 5-s rest were repeated 6 times. The stimulation intensity was set at 110% of the individual motor threshold. Stimulation began 10 s before the intravenous bolus injection. Sixty-second PET acquisitions started immediately after each cessation of TMS. Two transmission scans were performed with the coil positioned over the DLPFC target region: a short one used as a localizer (approximately 3 min) before the first scan and the second (10 min) subsequent to the sixth scan used for attenuation correction. PET scans were acquired on a whole-body scanner (Advance GE Medical Systems, Waukesha, WI) in 3D mode with a 15-cm axial field of view. For each scan, 400 MBq H₂¹⁵O were administered as a slow bolus with a remotely controlled injection device. Attenuation-corrected data were

reconstructed into 35 image planes. The accumulated radioactivity counts over 60 s were taken as measure for cerebral blood flow.

Analysis

Statistical parametric mapping was performed as follows. First, head movement between the scans was corrected using the least-squares method implemented in the statistical parametric mapping software SPM99 (Friston et al., 1995). Then, all images of each subject were summed and transformed into stereotaxic space [Montreal Neurological Institute coordinates (MNI) as provided by SPM99]. The normalization included linear transformations and deformations based on non-linear basis function. The resulting transformation matrix was subsequently used to transform each individual scan. A proportional scaling was applied to remove global effects. To ameliorate residual interindividual anatomical and functional differences after spatial normalization, the scans were smoothed with a Gaussian filter of 15 mm full width at half maximum (FWHM). The difference between conditions was then evaluated voxel by voxel.

In a first level of analysis, we calculated contrasts between the four conditions for each subject (“1 Hz–baseline”, “10 Hz–baseline”, “1 Hz–10 Hz”, “10 Hz–1 Hz”, “baseline–1 Hz”, “baseline–10 Hz”). To

Table 1
Coordinates and statistical values of six *t*-contrasts of the SPM analyses

Left-sided stimulation						Right-sided stimulation							
Contrasts and regions	BA	Side	Coordinates			<i>T</i>	Contrasts and regions	BA	Side	Coordinates			<i>T</i>
			<i>x</i>	<i>y</i>	<i>z</i>					<i>x</i>	<i>y</i>	<i>z</i>	
10 Hz–base						10 Hz–base							
Middle frontal gyrus	10	l	–42	50	20	6.37 ^a	Middle frontal gyrus	10	r	42	46	16	6.69 ^a
Middle frontal gyrus	10	l	–44	48	18	6.57 ^a	Uncus		l	–18	8	–26	6.10 ^a
Inferior frontal gyrus	45	r	62	24	14	9.63 ^a	Caudate body		r	10	8	10	5.83 ^a
1 Hz–base						1 Hz–base							
Middle frontal gyrus	8	r	28	42	46	5.13 ^b	Postcentral gyrus	43	l	–64	–8	20	5.85 ^a
Middle frontal gyrus	10	l	–38	50	18	5.93 ^a	Precentral gyrus	6	r	56	–4	34	9.81 ^a
Middle frontal gyrus	6/8	r	54	6	46	5.45 ^a	Cingulate gyrus		r	14	6	34	7.36 ^a
Precentral gyrus	6/43	r	56	–4	10	7.08 ^a							
Caudate body		r	20	12	14	10.48 ^a							
Anterior cingulate	32	l	–6	34	26	5.47 ^a							
10 Hz–1 Hz						10 Hz–1 Hz							
Cingulate gyrus	24		–2	–4	30	7.00 ^a	Thalamus (MDN)		l	–4	–14	2	8.66 ^a
							Inferior frontal gyrus (OFC)	47	r	32	26	–24	6.45 ^a
							Inferior frontal gyrus (OFC)	47	l	–38	24	–20	6.25 ^a
							Medial frontal gyrus	11/25	l	–8	22	–20	5.87 ^a
							Parahippocampal gyrus	34	r	16	–10	–20	6.00 ^a
1 Hz–10 Hz						1 Hz–10 Hz							
Middle frontal gyrus	9	r	38	36	40	6.38 ^a	Precentral gyrus	6	r	58	–6	32	12.07 ^a
Caudate body		r	18	0	16	8.00 ^a							
Superior frontal gyrus	6	r	20	12	60	7.13 ^a							
Base–10 Hz						Base–10 Hz							
Parahippocampal gyrus	35	l	–26	–12	–32	5.48 ^a	Superior frontal gyrus	10	r	30	68	–6	5.10 ^b
Medial frontal gyrus	6	r	14	6	54	8.24 ^a	Middle frontal gyrus	8	r	32	16	48	8.39 ^a
							Medial frontal gyrus	6	l	–8	–14	52	6.85 ^a
Base–1 Hz						Base–1 Hz							
Parahippocampal gyrus	36	l	–38	–22	–20	5.23 ^b	Parahippocampal gyrus		l	–16	–32	–16	10.48 ^a
Parahippocampal gyrus	19	r	22	–54	–12	5.20 ^b	Posterior cingulate	30		4	–60	8	5.35 ^b
Inferior frontal gyrus (OFC)	47	l	–54	38	0	6.37 ^a							

l = left; r = right; BA = Brodmann area.

^a *P* < 0.001.

^b *P* < 0.001.

evaluate the results for each group (left-sided or right-sided stimulation), we performed a random effects analysis over the contrast images of the first-level analysis using a one-sample t test. To test for differences between groups, we performed a two-sample t test on the contrast images of the first-level analysis. In all studies, we accepted results with $P < 0.001$ uncorrected for multiple comparisons. We did not consider whole brain corrections for multiple comparisons because of a priori regions of interest as defined by the position of the TMS coil and by activations reported in other studies (e.g., Li et al., 2004; Speer et al., 2003).

The results of the between-group analysis left group contrast “1 Hz–10 Hz” minus right group contrasts “10 Hz–1 Hz” were used to determine regions of interest which were activated differentially in both groups as well as in both stimulation conditions. The contrast images of the group stimulated on the right were mirrored along the vertical midline left to right beforehand to facilitate the analysis. The regions of interest were drawn around the activated cluster incorporating all pixels with $T > 2.62$ ($P < 0.01$). If the cluster comprised more than one anatomical region, the authors only outlined the pixel in the respective region of interest. This was the case for the posterior OFC. Homotopic contralateral regions were defined by mirroring the regions of interest. Regions of interest analyses were performed with PMOD (medical image quantitation and modeling software, Mikolajczyk et al., 1998) and SAS 8.02 (SAS Institute Inc. 2000).

Results

None of the subjects reported any adverse side effects concerning pain on the scalp or headaches after the experiment. There were no significant differences between groups with respect to age ($t = 0.66$, $df = 14$, $P = 0.52$) and motor threshold ($t = 0.68$, $df = 14$, $P = 0.51$).

SPM analyses

The results are displayed in Table 1.

Stimulation over the left hemisphere

Relative to baseline, both 10-Hz rTMS and 1-Hz rTMS induced an increased CBF in the stimulated area (left middle frontal gyrus; Brodmann area [BA] 10) (Fig. 1). Aspects of the contralateral middle frontal gyrus showed increased CBF when 1-Hz rTMS was compared to baseline (BA 8) and when 1-Hz rTMS was compared to 10-Hz rTMS (BA 9). 1-Hz rTMS induced a signal increase in the right caudate body compared to baseline as well as to 10-Hz rTMS. Regions close to the corpus callosum showed an increased signal in the 1-Hz rTMS condition when compared to baseline and in a more anterior part in the 10-Hz rTMS condition when compared to the 1-Hz rTMS. Furthermore, the comparison between 10-Hz and 1-Hz rTMS revealed a differential activation of the cingulate gyrus (BA 24) in the sense of a larger CBF increase from baseline in the 10-Hz rTMS condition. The anterior cingulate (BA 32) revealed a higher CBF in the 1-Hz condition when compared to the baseline.

To test for potential deactivations, we performed two contrasts (“baseline–1 Hz”; “baseline–10 Hz”). These contrasts revealed a region in the middle frontal gyrus (BA 6) showing an increased CBF in the baseline compared to the 10-Hz rTMS condition and a region in the left inferior frontal gyrus (BA 47) in the baseline compared to the 1-Hz rTMS condition.

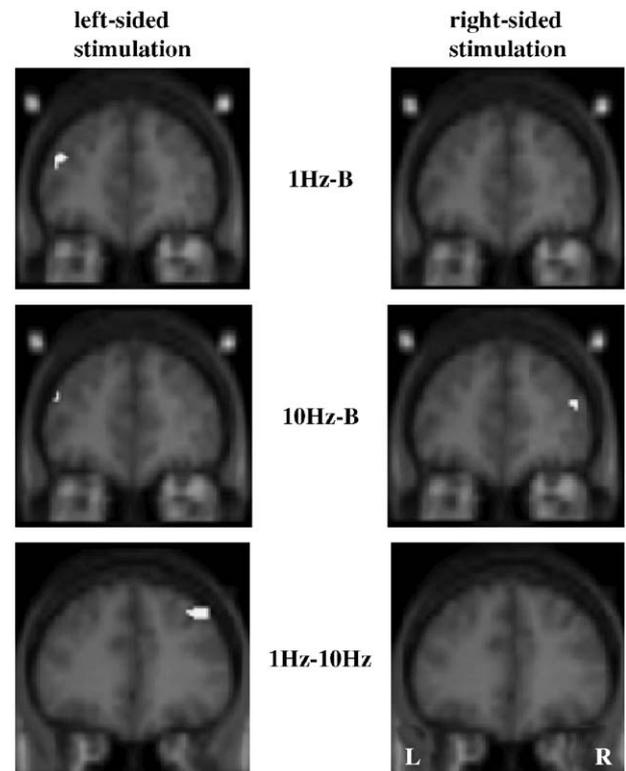


Fig. 1. Statistical parametric maps over the three conditions (1-Hz rTMS, 10-Hz rTMS and baseline [B]) overlaid on an averaged T1-weighted MRI of one subject of each group (left- and right-sided stimulation; the capsules mark the position of the TMS coil, which was directed over the left or right middle frontal gyrus). The top panel of this figure shows that supra-threshold 1-Hz rTMS over the left DLPFC led to significant increases in CBF in the stimulated area, while stimulation over the right DLPFC did not show any change (compared with baseline). The middle panel depicts that 10-Hz rTMS over left and right DLPFC produced significant increases in the stimulated area (compared with baseline) ($y = 48$). The third panel indicates an increase in CBF contralateral to the left-sided stimulation in the 1-Hz rTMS condition compared with 10-Hz rTMS ($y = 36$).

Stimulation over the right hemisphere

The results of this group revealed a somewhat different picture (Fig. 1). Relative to baseline, 10-Hz rTMS was associated with increased CBF in the middle frontal gyrus close to the stimulated area. This effect was absent when the 1-Hz condition was compared to baseline. 1-Hz rTMS led to small signal increases in the left postcentral gyrus (BA 43), the right precentral gyrus (BA 6) and the cingulate gyrus close to the midbody of the corpus callosum. Relative to baseline, 10-Hz rTMS resulted in increased CBF in subcortical regions such as the uncus (contralateral to stimulation) and the caudate body (ipsilateral to stimulation). The direct contrast between 10-Hz rTMS and 1-Hz rTMS showed a signal increase in the left and right OFC (left/right BA 47 and left BA 11/25) as well as in the left medial thalamus and the parahippocampal gyrus (BA 34). The direct comparison between 1-Hz rTMS and 10-Hz rTMS revealed only a signal increase in the precentral gyrus (BA 6).

The contrast baseline minus 10-Hz rTMS, as a test for potential deactivations, revealed right middle and superior frontal gyrus regions (BA 10 and 8) and a left-sided region in the middle frontal gyrus (BA 6). The contrast “baseline–1 Hz” resulted in a

decreased CBF in the left parahippocampal gyrus and the posterior cingulate gyrus in the 1-Hz condition when compared to baseline.

Regions of interest

For further analyses, we chose two regions of interest (ROI) from the between-group contrast (Fig. 2): the OFC ipsilateral to the stimulation side (MNI coordinates and T values of the pixel-wise statistics: $-14; 16; -14; t = 3.05$) and the medial thalamus ipsilateral to the stimulation side ($-8; -14; -6; t = 4.64$). We tested for significant effects with respect of side of stimulation (factor group: left and right) and type of stimulation (factor condition: baseline, 1-Hz rTMS and 10-Hz rTMS).

The ipsilateral thalamus revealed no condition effect [$F(2,13) = 2.86, P = 0.09$] and no group effect [$F(1,14) = 0.18, P = 0.68$] but a significant interaction effect [$F(2,13) = 6.22, P \leq 0.01$]. Two-sided a posteriori contrasts revealed significant group effects for the contrast 1-Hz rTMS versus baseline [$F(1,14) = 7.94, P \leq 0.01$] and for the contrast 10-Hz versus 1-Hz rTMS [$F(1,14) = 9.29, P \leq 0.01$].

The contralateral thalamus ROI revealed no condition effects [$F(2,13) = 0.52, P = 0.60$], no group effect [$F(1,14) = 1.03, P = 0.33$] and no interaction effect [$F(2,13) = 1.74, P = 0.21$]. Nevertheless, the contralateral thalamic ROI revealed the same pattern as the ipsilateral thalamus.

The medial thalamus of the group with left-sided stimulation showed a large signal increase after the 1-Hz rTMS stimulation. Whereas, after right-sided stimulation, the medial thalamus did not respond after the 1-Hz condition.

The regions of interest analyses upon the orbitofrontal ROI revealed the same tendency, but the effects were not significant in the repeated-measurement analysis [ipsilateral OFC: no condition effect: $F(2,13) = 0.61, P = 0.56$; no group effect: $F(1,14) = 0.08, P = 0.79$; no interaction effect: $F(2,13) = 3.29, P = 0.07$; contralateral OFC: no condition effect: $F(2,13) = 0.07, P = 0.93$; no group effect: $F(1,14) = 0.68, P = 0.43$; no interaction effect: $F(2,13) = 1.51, P = 0.26$]. However, when comparing only the two stimulation conditions (excluding the baseline condition from analyses), significant interaction effects between factor group and condition were revealed in the ipsilateral OFC [$F(1,14) = 6.75, P = 0.02$]. After left-sided stimulation similar to the medial thalamus ROI, the OFC also revealed a higher CBF in the 1-Hz rTMS condition, and, after right-sided stimulation, the opposite pattern is observable.

Discussion

To our knowledge, the present experiment includes the first comparison of left and right DLPFC stimulation in combination with different stimulation frequencies within a single study protocol. The results demonstrate that right prefrontal rTMS induces a different pattern of rCBF changes than left prefrontal rTMS (laterality effect). Moreover, high-frequency and low-frequency rTMS lead to diverse frontal and remote area CBF changes (frequency effect). CBF changes were not restricted to the stimulated area but involved a range of areas of the fronto-limbic circuits. Crucially, low-frequency rTMS applied over the left DLPFC affects the same parts of the fronto-limbic circuits as does

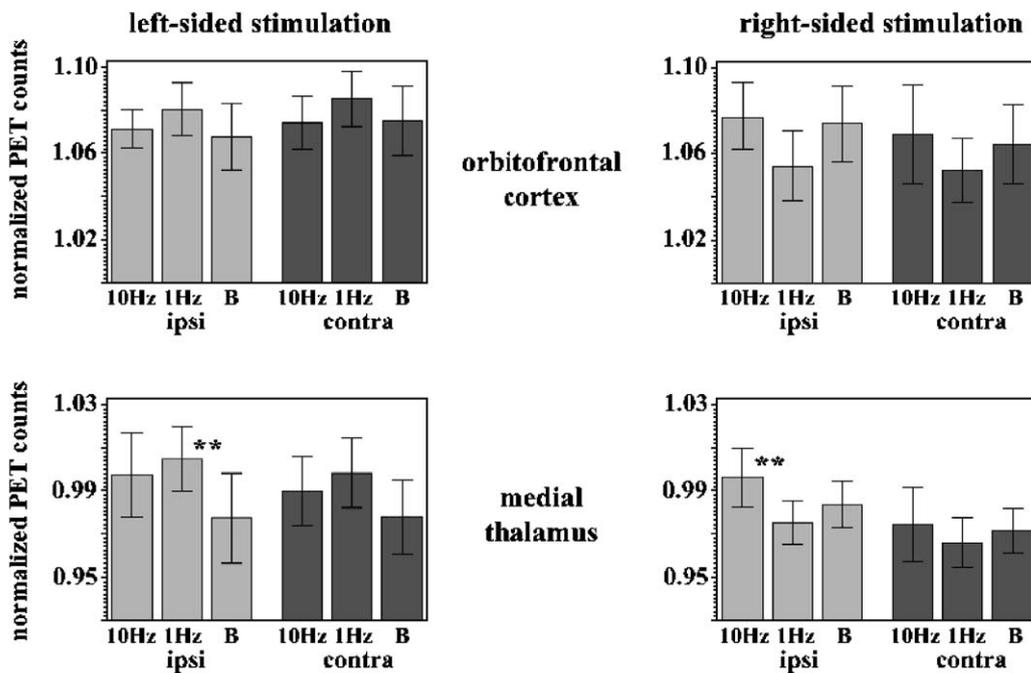


Fig. 2. Results of regions of interest (ROI) analyses over the three conditions (1-Hz rTMS, 10-Hz rTMS and baseline [B]). The upper panel depicts results of the OFC ROI and the lower panel for the ROI in the medial thalamus. Bar charts (mean \pm standard deviation) are drawn for the stimulation over the left DLPFC on the left side of the figure and for the stimulation over the right DLPFC on the right side of the figure. Results of the ROI ipsilateral to the side of stimulation are drawn in light gray, and those of the contralateral to the side of stimulation are drawn in dark gray. Arrows indicate the location of the ROIs on a representative axial slice ($z = -16; x = 4$).

high-frequency rTMS applied over the right DLPFC, suggesting a laterality–frequency interaction.

rTMS over the left DLPFC

One prominent notion, derived from motor cortex stimulation, is that fast rTMS induces neuronal excitation and slow rTMS neuronal inhibition of the target region (Pascual-Leone et al., 1994; Chen et al., 1997). Our results reveal that high- and low-frequency rTMS over the left DLPFC do *not* exert effects with respect to changes in CBF that are *opposite* in direction. Rather, they lead to comparable changes in the stimulated area (i.e., increase in rCBF). The rCBF *increase* in the stimulated area after low-frequency rTMS may be explained by the application of *suprathreshold* stimuli (i.e., 10% above the resting motor threshold). In fact, recent research has provided evidence for excitatory effects of slow rTMS when high stimulation intensities or intensities at motor threshold are applied (Li et al., 2004; but see Speer et al., 2003 for an inverse relationship between rCBF and the intensity of low-frequency rTMS). Furthermore, the same direction of CBF in response to putative excitatory (high-frequency rTMS) or inhibitory (low frequency rTMS) stimuli is not necessarily a contradiction. The effects of inhibition and deactivation on CBF and cerebral metabolism will continue to be an ongoing research challenge.

As we hypothesized, the rCBF changes were not restricted to the area directly stimulated by rTMS. This confirms the capability of rTMS to act on functionally and anatomically connected areas. Furthermore, the present results demonstrate that 10-Hz rTMS and 1-Hz rTMS led to activation of *different* parts of such a network. For example, despite the lower number of TMS pulses in the 1-Hz condition (60 pulses) compared to the 10-Hz condition (300 pulses), we paradoxically found higher increases contralaterally after 1-Hz rTMS stimulation. However, the site of the contralateral rCBF increase after left 1-Hz rTMS was not in the homologous area but in an adjacent more posterior and medial location. Evidence that different rTMS parameters lead to different patterns of change was reported earlier. Strafella and Paus (2001) have shown that stimulation at the same motor cortex site with paired TMS pulses resulted in different local and remote sites of rCBF change that depended on the interval between the stimuli. However, regarding the parameter intensity in a recent EEG study, Kähkönen et al. (2005) found that potential distributions did not change with stimulus intensities, suggesting that the same cortical structures are activated independently of stimulus intensities.

Apart from the contralateral prefrontal area, frequency-dependent rTMS effects were also observed in a network of brain regions implicated in impulse and mood control, many of which are connected with the stimulated cortex area. One-hertz rTMS decreased CBF compared to baseline in the left OFC and increased CBF in the caudate nucleus and in the anterior cingulum, whereas 10-Hz rTMS increased CBF in a more posterior part of the cingulum. Generally, low-frequency stimulation applied over the left DLPFC affected a larger number of remote areas than did high-frequency stimulation.

rTMS over the right DLPFC

Our results demonstrate that high-frequency rTMS induces a signal increase at the stimulation site. In contrast, low-frequency

rTMS over the right DLPFC did not lead to significant rCBF changes at the site of stimulation. It remains unclear whether this phenomenon is due to a higher threshold of the right DLPFC. In a recent study, Ferrarelli et al. (2004) explored differences in the activation patterns produced by stimulation of different cortical regions. TMS of both right and left DLPFC failed to produce rCBF changes at the site of stimulation. Since they employed a *simultaneous* TMS/PET protocol, one possible explanation of this finding may be that maximal signal increase is in fact reached only *after* stimulation (see Methodological remarks and for rTMS of the motor cortex see Takano et al., 2004).

Interestingly, when left-sided stimulation was applied, low-frequency rTMS affected more areas in the fronto-limbic network than high-frequency stimulation, whereas in applying right-sided stimulation the high-frequency rTMS condition was associated with CBF changes in a larger number of remote areas compared to low-frequency rTMS. Ten-hertz rTMS over the right DLPFC induced CBF changes in the bilateral OFC, the medial thalamus, the uncus and the caudate body.

Laterality effect

One can only speculate about some possible explanations for the laterality effect we found. Hemispheric asymmetry of the frontal lobes in both anatomy and function is well known to exist (LeMay, 1976; for a review, see Toga and Thompson, 2003). Anatomical asymmetry in healthy subjects has been revealed not only in cortical gray matter but also in white matter that interconnects cortical brain regions (Park et al., 2004). Thus, hemispheric differences in fronto-cortical-subcortical anatomical connections may partly explain the observed laterality effect.

Furthermore, Lichter and Cummings (2001) speculated that a differential biochemical response to injury in the two hemispheres could contribute to the known hemispheric dissociation in the manifestation of mood and their disorders. This leads to the question of whether hemisphere-specific biochemical responses to rTMS may exist. Future work with combined TMS and pharmacological fMRI or PET ligands could further investigate the laterality effect we found and potentially provide a method for determining whether the descending pathways from the frontal cortex modulate the release of dopamine in subcortical areas in a frequency-dependent manner.

Another important finding is that the regions of interest analyses upon the medial thalamus (a key structure in brain anatomic circuits potentially involved in the pathophysiology of mood disorders) revealed a clear dissociation with respect to the side of stimulation and stimulation frequency. Left-sided stimulation with low-frequency rTMS led to a larger ipsilateral signal increase than high-frequency stimulation or baseline. When the right DLPFC was stimulated, only high-frequency rTMS induced a ipsilateral signal increase compared to 1-Hz rTMS. Thus, due to this double dissociation, it seems as if the activation of the thalamus after 1-Hz rTMS is diminished when the right DLPFC is stimulated. The regions of interest analyses upon the orbitofrontal region revealed the same dissociation but less concisely. Here, it is again the 1-Hz rTMS condition, which increases the blood flow when the left DLPFC is stimulated and seems to suppress the response when the right side is stimulated. Direct clinical implications of our results are difficult to draw since the present study only assessed the acute effects of prefrontal rTMS, whereas

antidepressant effects generally emerge after a long-term application. However, the observation that the effects of high- and low-frequency rTMS are hemisphere-specific may open new possibilities for treatment with stimulation parameters tailored to specific pathology and pre-treatment condition. Using an analogy with electroconvulsive therapy, for which bilateral stimulation is more efficient than unilateral stimulation, Loo et al. (2003) examined the effects of simultaneous bilateral prefrontal high-frequency rTMS to depressed resistant subjects and failed to find a significant antidepressant benefit. According to our hemisphere-specific results, bilateral rTMS administered to both hemispheres at high frequencies would not be expected to have antidepressant effects. Bearing in mind that low-frequency rTMS applied over the left DLPFC affects the same parts of fronto-limbic circuits as does high-frequency rTMS applied over the right DLPFC, namely the medial thalamus and to a lesser extent also OFC (both involved in the pathophysiology of mood and impulse disorders), a greater effect might be obtained by simultaneously stimulating the left and right DLPFC with opposite frequencies (already suggested by Lisanby, 2003).

To conclude, our results from healthy subjects are well in line with the lateral evidence from clinical studies in patients with unilateral structural or functional disorders associated with the frontal brain regions. TMS combined with brain imaging appears to be a potential method for investigating behavior and its corresponding neuronal activity especially with respect to chemical activity and the likely lateral asymmetry of neurochemical pathways.

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References

- Barrett, J., Della-Maggiore, V., Chouinard, P.A., Paus, T., 2004. Mechanisms of action underlying the effect of repetitive transcranial magnetic stimulation on mood: behavioral and brain imaging studies. *Neuropsychopharmacology* 29, 1172–1189.
- Bestmann, S., Baudewig, J., Siebner, H.R., Rothwell, J.C., Frahm, J., 2004. Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur. J. Neurosci.* 19, 1950–1962.
- Burt, T., Lisanby, S., Sackeim, H., 2002. Neuropsychiatric applications of transcranial magnetic stimulation: a meta analysis. *Int. J. Neuropsychopharmacol.* 5, 73–103.
- Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E.M., Hallett, M., Cohen, L.G., 1997. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology* 48, 1398–1403.
- Cummings, J.L., Mega, M.S., 2003. *Neuropsychiatry and Behavioral Neuroscience*. Oxford Univ. Press.
- Erfurth, A., Michael, N., Mostert, C., Arolt, V., 2000. Euphoric mania and rapid transcranial magnetic stimulation. *Am. J. Psychiatry* 157, 835–836.
- Feinsod, M., Kreinin, B., Christyakov, A., Klein, E., 1998. Preliminary evidence for a beneficial effect of low-frequency repetitive transcranial magnetic stimulation in patients with major depression and schizophrenia. *Depress. Anxiety* 7, 65–68.
- Ferrarelli, F., Haraldsson, H.M., Barnhart, T.E., Roberts, A.D., Oakes, T.R., Massimini, M., Stone, C.K., Kalin, N.H., Tononi, G., 2004. A [17F]-fluoromethane PET/TMS study of effective connectivity. *Brain Res. Bull.* 64, 103–113.
- Friston, K.J., Ashburner, J., Poline, J.B., Frith, C.D., Heather, J.D., Frackowiak, R.S.J., 1995. Spatial registration and normalisation of images. *Hum. Brain. Mapp.* 2, 165–189.
- George, M.S., Wassermann, E.M., Williams, W.A., Callahan, A., Ketter, T.A., Basser, P., Hallett, M., Post, R.M., 1995. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *NeuroReport* 6, 1853–1856.
- George, M.S., Wassermann, E.M., Williams, W.A., Steppel, J., Pascual-Leone, A., Basser, P., Hallett, M., Post, R.M., 1996. Changes in mood and hormone levels after rapid-rate transcranial magnetic stimulation (rTMS) of the prefrontal cortex. *J. Neuropsychiatry Clin. Neurosci.* 8, 172–180.
- George, M.S., Stallings, L.E., Speer, A.M., Nahas, Z., Spicer, K.M., Vincent, D.J., Bohning, D.E., Cheng, K.T., Molloy, M., Teneback, C.C., Risch, S.C., 1999. Prefrontal repetitive transcranial magnetic stimulation (rTMS) changes relative perfusion locally and remotely. *Hum. Psychopharmacol.* 14, 161–170.
- Grisaru, N., Chudakov, B., Yaroslavsky, Y., Belmaker, R.H., 1998. Transcranial magnetic stimulation in mania: a controlled study. *Am. J. Psychiatry* 155, 1608–1610.
- Jenkins, J.A., Shajahan, P.M., Lappin, J.M., Ebmeier, K.P., 2002. Right and left prefrontal transcranial magnetic stimulation at 1 Hz does not affect mood in volunteers. *BMC Psychiatry* 2, 1.
- Kähkönen, S., Komssi, S., Wilenius, J., Ilmoniemi, R.J., 2005. Prefrontal transcranial magnetic stimulation produces intensity-dependent EEG responses in humans. *NeuroImage* 24, 955–960.
- Klein, E., Kreinin, I., Christyakov, A., Koren, D., Mecz, L., Marmur, S., Ben-Shachar, D., Feinsod, M., 1999. Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study. *Arch. Gen. Psychiatry* 56, 315–320.
- Kimbrell, T.A., Dunn, R.T., George, M.S., Danielson, A.L., Willis, M.W., Repella, J.D., Benson, B.E., Herscovitch, P., Post, R.M., Wassermann, E.M., 2002. Left prefrontal-repetitive transcranial magnetic stimulation (rTMS) and regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res.* 115, 101–113.
- Knoch, D., Brugger, P., Regard, M., 2005. Suppressing versus releasing a habit: frequency-dependent effects of prefrontal transcranial magnetic stimulation. *Cereb. Cortex* 15, 885–887.
- Lee, J.S., Narayana, S., Lancaster, J., Jerabek, P., Lee, D.S., Fox, P., 2003. Positron emission tomography during transcranial magnetic stimulation does not require mu-metal shielding. *NeuroImage* 19, 1812–1819.
- LeMay, M., 1976. Morphological cerebral asymmetries of modern man, fossil man, and nonhuman primate. *Ann. Ny Acad. Sci.* 280, 346–366.
- Li, X.B., Nahas, Z., Kozel, F.A., Anderson, B., Bohning, D.E., George, M.S., 2004. Acute left prefrontal transcranial magnetic stimulation in depressed patients is associated with immediately increased activity in prefrontal cortical as well as subcortical regions. *Biol. Psychiatry* 55, 882–890.
- Lichter, D.G., Cummings, J.L., 2001. *Frontal–Subcortical Circuits in Psychiatric and Neurological Disorders*. Guilford Press, New York.
- Lisanby, S.H., 2003. Focal brain stimulation with repetitive transcranial magnetic stimulation (rTMS): implications for the neural circuitry of depression. *Psychol. Med.* 33, 7–13.
- Loo, C.K., Mitchell, P.B., Croker, V.M., Malhi, G.S., Wen, W., Gandevia, S.C., Sachdev, P.S., 2003. Double-blind controlled investigation of bilateral prefrontal transcranial magnetic stimulation for the treatment of resistant major depression. *Psychol. Med.* 33, 33–40.
- Martin, J.L.R., Barbanj, M.J., Schlaepfer, T.E., Thompson, E., Perez, V., Kulisevsky, J., 2003. Repetitive transcranial magnetic stimulation for the treatment of depression. Systematic review and meta-analysis. *Br. J. Psychiatry* 182, 480–491.

- Michael, N., Erfurth, A., 2004. Treatment of bipolar mania with right prefrontal rapid transcranial magnetic stimulation. *J. Affect. Disord.* 78, 253–257.
- Mikolajczyk, K., Szabatin, M., Rudnicki, P., Grodzki, M., Burger, C., 1998. A JAVA environment for medical image data analysis: initial application for brain PET quantitation. *Med. Inform. (Lond.)* 23, 207–214.
- Mosimann, U.P., Rihs, T.A., Engeler, J., Fisch, H., Schlaepfer, T.E., 2000. Mood effects of repetitive transcranial magnetic stimulation of left prefrontal cortex in healthy volunteers. *Psychiatry Res.* 94, 251–256.
- Nahas, Z., Lomarev, M., Roberts, D.R., Shastri, A., Lorberbaum, J.P., Teneback, C., McConnell, K., Vincent, D.J., Li, X., George, M.S., Bohning, D.E., 2001. Unilateral left prefrontal transcranial magnetic stimulation (TMS) produces intensity-dependent bilateral effects as measured by interleaved BOLD fMRI. *Biol. Psychiatry* 50, 712–720.
- Padberg, F., Juckel, G., Prassl, A., Zwanzger, P., Mavrogiorgou, P., Hegerl, U., Hampel, H., Moller, H.J., 2001. Prefrontal cortex modulation of mood and emotionally induced facial expressions: a transcranial magnetic stimulation study. *J. Neuropsychiatry Clin. Neurosci.* 13, 206–212.
- Park, H.J., Westin, C.-F., Kubicki, M., Maier, S.E., Niznikiewicz, M., Baer, A., Frumin, M., Kikinis, R., Jolesz, F.A., McCarlex, R.W., Shenton, M.E., 2004. White matter hemisphere asymmetries in healthy subjects in schizophrenia: a diffusion tensor MRI study. *NeuroImage* 23, 213–223.
- Pascual-Leone, A., Valls-Sole, J., Wassermann, E.M., Hallett, M., 1994. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain* 117, 847–858.
- Pascual-Leone, A., Rubio, B., Pallardo, F., Catala, M., 1996. Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. *Lancet* 348, 233–237.
- Pascual-Leone, A., Catala, D.M., Pascual-Leone Pascual, A., 1996. Lateralized effect of rapid-rate transcranial magnetic stimulation of the prefrontal cortex on mood. *Neurology* 46, 499–502.
- Paus, T., Castro-Alamancos, M., Petrides, M., 2001. Corticocortical connectivity of the human mid-dorsolateral frontal cortex and its modulation by repetitive transcranial magnetic stimulation. *Eur. J. Neurosci.* 14, 1405–1411.
- Schutter, D.J.L.G., Van Honk, J., D'Alfonso, A.A.L., Postma, A., De Haan, E.H.F., 2001. Effects of slow rTMS at the right dorsolateral prefrontal cortex on EEG asymmetry and mood. *NeuroReport* 12, 345–347.
- Siebner, H.R., Takano, B., Peinemann, A., Schwaiger, M., Conrad, B., Drzezga, A., 2000. Continuous transcranial magnetic stimulation during positron emission tomography: a suitable tool for imaging regional excitability of the human cortex. *NeuroImage* 14, 883–890.
- Speer, A.M., Willis, M.W., Herscovitch, P., Daube-Witherspoon, M., Shelton, J.R., Benson, B.E., Post, R.M., Wassermann, E.M., 2003. Intensity-dependent regional cerebral blood flow during 1-Hz repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers studied with $H_2^{15}O$ positron emission tomography: II. Effects of prefrontal cortex rTMS. *Biol. Psychiatry* 15, 826–832.
- Strafella, A., Paus, T., 2001. Cerebral blood-flow changes induced by paired-pulse transcranial magnetic stimulation of the primary motor cortex. *J. Neurophysiol.* 85, 2624–2629.
- Takano, B., Drzezga, A., Peller, M., Sax, I., Schwaiger, M., Lee, L., Siebner, H.R., 2004. Short-term modulation of regional excitability and blood flow in human motor cortex following rapid-rate transcranial magnetic stimulation. *NeuroImage* 23, 849.
- Thompson, C.J., Paus, T., Clancy, R., 1998. Magnetic shielding requirements for PET detectors during transcranial magnetic stimulation. *IEEE Trans. Nucl. Sci.* 45, 1303–1307.
- Toga, A.W., Thompson, P.M., 2003. Mapping brain asymmetry. *Nature Rev. Neurosci.* 4, 37–48.
- Weber, B., Westera, G., Treyer, V., Burger, C., Khan, N., Buck, A., 2004. Constant-infusion $H_2^{15}O$ PET and acetazolamide challenge in the assessment of cerebral perfusion status. *Nucl. Med.* 45, 1344–1350.