

## FEATURE ARTICLE

**Suppressing versus Releasing a Habit: Frequency-dependent Effects of Prefrontal Transcranial Magnetic Stimulation**

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**When subjects are required to generate a random sequence of numbers they typically produce too many forward and backward 'counts' (e.g. 5-6, 4-3). This counting bias is interpreted as the consequence of an interference by overlearned tendencies to arrange numbers according to their natural order. Inhibition of such well-learned routines is known to rely on frontal lobe functioning. We examined differential effects of slow (1 Hz) and fast (10 Hz) repetitive transcranial magnetic stimulation (rTMS) over the left or right dorsolateral prefrontal cortex (DLPFC) on random number generation (RNG) performance. Eighteen healthy men performed an RNG task. Those subjects stimulated over the left DLPFC showed a frequency-dependent rTMS effect: counting bias was significantly reduced after the 1 Hz stimulation compared with baseline, but significantly exaggerated after the 10 Hz stimulation compared with 1 Hz stimulation. In contrast, the sequences of the subjects stimulated over the right DLPFC showed the well-known excess of counting in all conditions (i.e. baseline, 1 Hz and 10 Hz). These findings confirm the functional importance of specifically the left DLPFC in sequential response production and show, for the first time, that rTMS affects cognitive processing in a frequency-dependent manner.**

**Keywords:** dorsolateral prefrontal cortex, pulse frequency, random number generation, response suppression, transcranial magnetic stimulation

**Introduction**

The inhibition of impulses or inappropriate responses constitutes a fundamental neuronal principle essential for normal behavior and thinking. A task paradigmatic to assess inhibitory control is random number generation (RNG), in which effective performance requires the suppression of habitual sequential counting. By employing prefrontal repetitive transcranial magnetic stimulation (rTMS), we show here that counting habits can be abolished or exaggerated depending on stimulation frequency.

rTMS is widely employed as a research tool in cognitive neuroscience. Extent and type of a neurophysiologic response can be altered differentially by rTMS frequency. Slow ( $\leq 1$  Hz) rTMS over the motor cortex decreased the excitability and resulted in a long-lasting depression of motor evoked potentials (Chen *et al.*, 1997). Conversely, fast ( $\geq 5$  Hz) rTMS increased cortical excitability (Pascual-Leone *et al.*, 1994). Given these frequency-dependent effects of rTMS on motor cortex, it seems reasonable to assume analogously opposite effects of slow and fast rTMS on cognitive functioning (Robertson *et al.*, 2003). This assumption has not, however, been empirically tested. We set out to investigate the effects of slow and fast rTMS on cognition by employing an RNG paradigm.

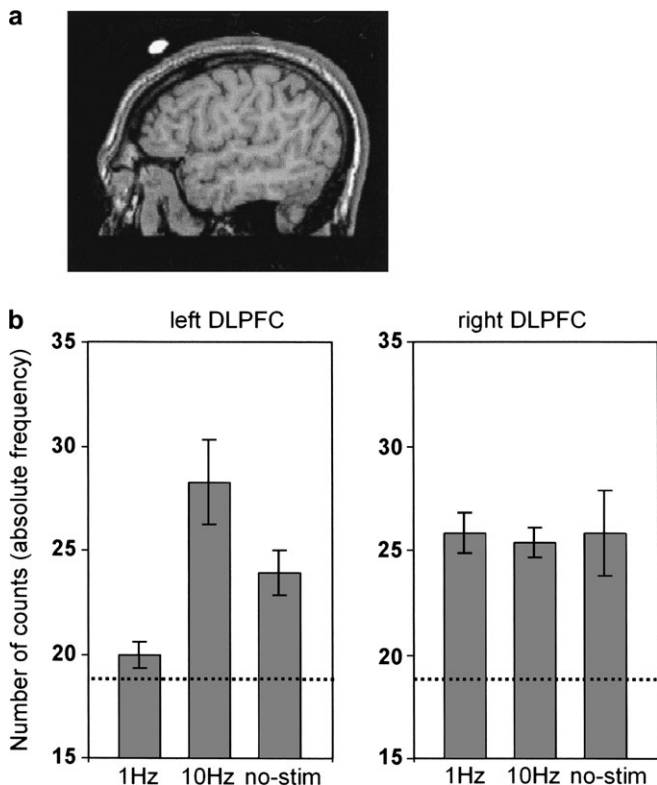
RNG requires subjects to generate numbers in a random fashion for a number of trials. Previous studies have provided

evidence that humans are poor at random generation, and that, compared with computer-generated random series, produce characteristic biases, i.e. too few repetitions (e.g. 5-5) and too much counting in steps of one (e.g. 5-6, 3-2) (Brugger, 1997). This latter bias reflects interference by over-learned and highly automatized rules, i.e. forward and backward counting. Thus, for successful task performance individuals must overcome over-learned routines, whose control is typically assigned to the prefrontal cortex. Specifically, Jahanshahi and collaborators suggested that the left dorsolateral prefrontal cortex (DLPFC) exerts a 'controller' function over an associative network, suppressing most habitual responses, i.e. those adjacent in natural order and represented, in neighboring nodes of the network (for number generation, see Jahanshahi *et al.*, 1998; for letter generation, see Jahanshahi and Dirnberger, 1999). Support for the critical role of the DLPFC for the monitoring of habitual responses was provided by both neuroimaging (Daniels *et al.*, 2003; Jahanshahi *et al.*, 2000) and electrophysiological studies (Joppich *et al.*, 2004). Of special importance in the present context is the observation that high frequency (20 Hz) rTMS over the left, but not right, DLPFC increased counting bias in an RNG task (Jahanshahi *et al.*, 1998). This was interpreted as a breakdown in the controlling function of the DLPFC, whose already limited capacity in suppressing habitual responses would be further compromised.

In the present study we applied slow and fast rTMS over the left and right DLPFC immediately before subjects performed an RNG task ('off-line paradigm'). We predicted (i) a TMS effect on counting bias and not on other prominent sequential response stereotypies (e.g. repetition avoidance); (ii) a lateralization of stimulation effects to the left hemisphere; and, crucially, (iii) opposite effects of fast and slow rTMS on the magnitude of counting bias — specifically, an increase with 10 Hz and a decrease with 1 Hz stimulation trains.

**Materials and Methods**

Eighteen healthy right-handed men (mean age = 26.6 years, SD = 4.4 years) were randomly assigned to receive either left or right prefrontal rTMS after giving written informed consent. All subjects were naive to TMS and had no history of psychiatric illness or neurological disorders. Stimulation was administered using a Magstim (Rapid Magnetic Stimulator, Magstim, Winchester, MA) and figure-of-eight coil (70 mm diameter double circle, air-cooled). The position of the DLPFC was defined as 5 cm anterior (in a parasagittal line) to the motor cortex. A T1-weighted MRI was acquired to ensure the proper positioning of the TMS coil (Fig. 1a). The stimulation intensity was set at 110% of the individual resting motor threshold (MT). For each subject, the MT was established using the criterion of the lowest intensity of stimulation over the hand area that would result in a visible twitch in the contralateral index finger at least five out of ten consecutive stimulations. Both groups (left-sided and right-sided stimulation) received three conditions of



**Figure 1.** Site of stimulation and size of response bias in random number generation. (a). Brain MRI from a subject showing the site of stimulation. The capsule marks the position of the TMS coil, which was directed over the gyrus frontalis medialis. (b) Mean ( $\pm$  1SE) number of counts for sequences generated by the subjects stimulated over the left DLPFC (left panel) and for sequences generated by the subjects stimulated over the right DLPFC (right panel) after the 1 Hz, 10 Hz and no-stimulation conditions. Dotted line corresponds to the mean of 50 computer generated sequences ( $SE = 0.56$ ). Note that (i) no stimulation effects emerged over the right DLPFC and (ii) 10 Hz stimulation over the left DLPFC exaggerated the counting bias, whereas 1 Hz stimulation extinguished it.

stimulation: no-stimulation (control), 1 Hz rTMS and 10 Hz rTMS. For the 1 Hz rTMS stimulation, a continuous 60 s stimulation was applied (total 60 stimuli), whereas in the 10 Hz rTMS stimulation, pairs of 5 s stimulation and 5 s rest were repeated six times (total 300 stimuli). In the no-stimulation control condition the noise of the vacuum unit of the air-cooled coil was on for 60 s. Immediately after the cessation of the sound produced by the vacuum unit, a metronome signal indicated the start of random generation. In the rTMS conditions (1 Hz rTMS and 10 Hz rTMS), the sequence of events was identical, except that a train of TMS was given 60 s before the onset of the metronome. All participants performed first the control no-stimulation trial. The order the two rTMS conditions was pseudo-randomized across participants. The interval between the runs was 10 min.

The task in all three conditions was to generate the numbers 1-6 in a sequence as random as possible (66 trials at a metronomic rate of 1.2 Hz). The concept of randomness was explained by using the analogy of mentally rolling a dice (the 'Mental Dice Task' of Brugger *et al.*, 1996). For each sequence we calculated the number of repetitions and the number of counts in steps of one as in Brugger *et al.* (1996; e.g. in 2-3-2-5-6-1 are three 'counts', i.e. 2-3, 3-2, 5-6). Performance was compared with 50 computer-generated sequences obtained with the pseudo-random generation algorithm provided in Towse and Neil (1998).

## Results

A repeated-measures analysis of variance with stimulation condition (no-stimulation, 1 Hz rTMS, 10 Hz rTMS) as the within subjects factor revealed that subjects stimulated over the

left DLPFC (Fig. 1b, left panel) showed a frequency-dependent effect for counting in steps of one [ $F(2,16) = 12.31$ ,  $P < 0.001$ ]. Post-hoc analyses (Scheffé-tests) showed that the counting bias was significantly smaller in the sequences generated after the 1 Hz rTMS stimulation compared with the sequences generated after the 10 Hz rTMS stimulation ( $P < 0.001$ ) and compared with the control no-stimulation ( $P < 0.01$ ). Crucially, after 1 Hz rTMS stimulation the pervasive counting bias was not observable, i.e. the number of counts did not differ from that in the computer generated sequences ( $t = 0.72$ ,  $P = 0.48$ , two-tailed). The sequences generated after the 10 Hz rTMS stimulation and after the control no-stimulation showed the well-known excessive counting (compared with pseudo-random sequences, both  $t$ -values  $> 3.41$ , both  $P$ -values  $< 0.001$ , two-tailed). Compared with the sequences generated after the control no-stimulation, those generated after the 10 Hz rTMS stimulation showed a tendency towards a higher number of counts ( $P = 0.06$ ).

In contrast, in the sequences of those subjects stimulated over the right DLPFC (Fig. 1b, right panel) there was no frequency-dependent effect for counting in steps of one [ $F(2,16) = 0.23$ ,  $P = 0.98$ ]. All sequences showed the well-known excess of counting (compared with pseudorandom sequences all  $t$ -values  $> 3.51$ , all  $P$ -values  $< 0.001$ , two-tailed).

As expected, there was no significant difference in the number of counts between the two no-stimulation control conditions [ $F(1,16) = 0.58$ ,  $P = 0.46$ ]. Also, neither the number of repetitions nor that of any other responses pairing of non-adjacent numbers was affected by side of stimulation nor by frequency of stimulation [all  $F(2,16) \leq 0.06$ , all  $P$ -values  $\geq 0.94$ ].

## Discussion

We found a significant influence of rTMS on the subjects' counting bias, i.e. the tendency to arrange consecutive numbers according to their natural order, despite the instruction to randomize. Other response pairings (e.g. repetitions) were not affected by TMS. This result replicates previous findings and supports the role of the DLPFC in controlling specifically over-learned habits of response production (Jahanshahi *et al.*, 1998). Our observation that left, but not right, rTMS modified RNG performance is in line with the findings of both neuroimaging (Jahanshahi *et al.*, 2000) and electrophysiological studies (Joppich *et al.*, 2004), which indicate that areas of left DLPFC become activated during this RNG task. However, the principal goal of the present experiment was to address frequency-dependent effects of TMS on cognitive function. Slow rTMS to the left DLPFC resulted in a suppression of habitual counting, whereas fast rTMS at the same site rather enhanced this bias. While an exaggerated tendency to arrange responses in a natural order has previously been described as a consequence of fast rTMS (Jahanshahi *et al.*, 1998; Jahanshahi and Dirnberger, 1999), the suppression of this bias by *slow* rTMS constitutes a novel finding.

TMS studies of cognition using an 'off-line' paradigm usually stimulate with low frequencies for a duration of 5-15 min (for a review, see Robertson *et al.*, 2003). This type of stimulation is expected to transiently disrupt the cortical function by inducing a depression of excitability that outlasts the duration of the rTMS train itself (Chen *et al.*, 1997; Maeda *et al.*, 2000). However, since the present experiment aimed to compare the effects of slow rTMS with those of high rTMS and since longer trains at high stimulation frequencies are increasingly risky with

respect to seizure induction (Pascual-Leone *et al.*, 1994), we stimulated for a duration of only 1 min. This procedural step renders difficult a direct comparison with previously employed low-frequency off-line paradigms.

The physiological mechanisms of the observed frequency-dependent behavior changes remain unclear. One prominent notion, derived from motor cortex stimulation, is that fast rTMS induces neuronal excitation and slow rTMS neuronal inhibition of the target region. If equally applicable to stimulation of the DLPFC, this hypothesis would predict fast rTMS to reduce counting bias (by activating this structure's known function of habitual response suppression) and slow rTMS to enhance this bias (by disrupting its inhibitory function). Both predictions are opposite to what was found in the present experiment (and, with respect to fast rTMS, in the study by Jahanshahi *et al.*, 1998). This may indicate that findings regarding frequency-dependent TMS effects on motor cortex functions may not readily be extrapolated to predict frequency-dependent TMS effects over the DLPFC. In fact, recent research has provided evidence for excitatory effects of slow rTMS if high stimulation intensities are applied. Nahas *et al.* (2001), studying acute rTMS effects by fMRI, found DLPFC activations after 1 Hz rTMS at 100% MT and 120% MT. As we also focused on acute stimulation effects (although immediately after and not during stimulation), it appears highly conceivable that, in our experiment, supra-threshold 1 Hz rTMS abolished any counting bias by boosting the inhibitory function of the DLPFC.

As a final note, we mention that the effects of rTMS are not necessarily limited to the stimulated area, but are also observed at remote sites (e.g. Nahas *et al.*, 2001; Paus *et al.*, 2001; Strafella *et al.*, 2001; Li *et al.*, 2004). It remains to be established whether potential remote rTMS effects influence the number associative network, supposedly localized in the superior temporal cortex (Jahanshahi *et al.*, 1998) in a frequency-dependent manner.

## Notes

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