

Theta-burst transcranial magnetic stimulation over the supplementary motor area decreases variability of temporal estimates

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Abstract

OBJECTIVES: Supplementary motor area (SMA) was suggested to have a dominant role in the temporal control of behavior by many neuroimaging studies. The aim of this study was to support this hypothesis by influencing time estimates with theta burst transcranial magnetic stimulation (TMS) over the SMA.

METHODS: Nineteen healthy volunteers with a mean age 25.9 ± 3 (SD) years performed the time reproduction task (TRT) before and after 190 seconds of intermittent theta-burst TMS over SMA and the precuneus (total 600 pulses). The TRT consisted of an encoding phase (during which visual stimuli with durations of 5, 10 and 16.82 seconds were presented pseudorandomly) and a reproduction phase (during which interval durations were reproduced by pressing a button). Mean subjects' interval estimates as a measure of accuracy and standard deviation as a measure of variability pre-TMS and post-TMS were compared.

RESULTS: Theta-burst TMS over both areas had no effect on the accuracy of duration estimates. An increased variability of interval reproduction was present after stimulation of the precuneus ($p < 0.01$) with the biggest effect on the five second interval. Stimulation of SMA caused a decrease of variability in the ten second interval only ($p < 0.05$).

CONCLUSION: It is likely that increased variability of time estimates is a non-specific result of impaired attention and working memory after theta-burst TMS. Decreased variability after stimulation over the SMA could be explained in terms of enhanced activity of the physiological oscillator with a frequency close to 0.1 Hz.

INTRODUCTION

Keeping track of time is an important cognitive function. Whereas perception of environmental sensory stimuli and planning of motor response requires measurements in the range of hundreds of milliseconds, human subjective perception of time flow is supposedly based on the perception of intervals ranging from 3 to dozens of seconds (Wackermann 2007). Neuroimaging research and lesion studies have found several brain areas which are involved in interval timing (for review see (Coull *et al.* 2011)). The cerebellum and primary sensorimotor cortex (SM1) are more likely activated by tasks using time intervals in the millisecond range. The network comprising of the basal ganglia, insular cortex, posterior parietal cortex (PPC) and dorsolateral prefrontal cortex (DLPFC) are more likely activated by tasks involving suprasecond time intervals (Lewis and Miall 2003; Koch *et al.* 2009). Recent voxel-wise meta-analysis stressed the importance of the supplementary motor area (SMA) and right inferior frontal gyrus for time perception across various perceptual and motor timing tasks (Wiener *et al.* 2010).

Besides functional imaging studies, repetitive transcranial magnetic stimulation (rTMS) has been successfully used to investigate brain areas involved in temporal processing. Two rTMS studies showed an underestimation of time intervals in the seconds range after stimulation of the right DLPFC (Jones *et al.* 2004; Koch *et al.* 2003) and two another studies showed disruption of temporal processing in the milliseconds range after stimulation of cerebellum (Del Olmo *et al.* 2007; Fierro *et al.* 2007) supporting the predicted roles of these areas in the interval timing. Repetitive TMS over the right PPC caused an underestimation of reproduced time in the time bisection task (Oliveri *et al.* 2009). Recently, rTMS over the primary auditory cortex has been shown to impair the estimation of the duration of visual and auditory stimuli in the milliseconds range (Kanai *et al.* 2011).

Repetitive TMS can influence brain function depending on the stimulation protocol. Previous studies mostly employed protocols with the intention to cause “virtual lesion” of the stimulated cortex. A supposedly inhibiting protocol, rTMS train of frequency 1 Hz lasting 10 minutes preceding the task, was used in two studies (Oliveri *et al.* 2009; Koch *et al.* 2003). Jones *et al.* used a protocol of four stimuli at 20 Hz over the right DLPFC and SMA during an estimation or reproduction phase of the time reproduction task (Jones *et al.* 2004). Theta-burst TMS (TBS) is another protocol, which comprises of three pulses at 50Hz repeated at intervals of 200ms. It has been shown to elicit a long-lasting (up to an hour) and powerful stimulating or inhibiting effect on the motor cortex depending on the pattern of stimulation (Huang *et al.* 2005). Intermittent TBS, a train of stimuli, where this pattern is repeated for 2 seconds and is followed by 8 seconds of rest, has

been shown to increase excitability of the motor cortex (Huang *et al.* 2005). Continuous TBS lasting 40 seconds has been recently used to document roles of primary visual and auditory cortices in time perception (Kanai *et al.* 2011).

Our previous functional magnetic resonance imaging (fMRI) study using a time reproduction task with durations ranging from 5 to 16.82 seconds showed several brain areas with gradual activation or deactivation during the encoding or reproduction of time intervals. Among these areas, SMA showed a gradual activation and the precuneus showed a gradual deactivation in the reproduction phase of the task. We interpreted the gradual activation of SMA as a possible “time accumulator” (Jech *et al.* 2005). Surprisingly, previous studies examining the effect of rTMS over SMA on time perception have been negative (Jones *et al.* 2004; Koch *et al.* 2004). The aim of this study was to support the role of SMA in time perception. Contrary to previous studies, we did not intend to make a “virtual lesion”; instead we hypothesized that the supposedly stimulating intermittent TBS over SMA might lead to more precise time estimates.

We compared the effect of stimulation over SMA to the effect of stimulation over the precuneus. Unlike SMA, the precuneus has not been convincingly associated with time perception in humans, but several studies suggested its role in interval timing (Harrington *et al.* 2004; Wittmann *et al.* 2010). The connection between its gradual deactivation found in our previous study and time estimation thus remains unclear. The precuneus is part of the so called “default network” which decreases its activity during tasks requiring externally oriented attention (Buckner *et al.* 2008). The precuneus has been shown to have a role in autobiographical memory retrieval (Cavanna & Trimble 2006). A recent study showed facilitation of performance in a delayed match-to-sample task during 5Hz rTMS over the precuneus (Luber *et al.* 2007). Thus, we did not have a specific hypothesis regarding the effect of TMS over the precuneus.

METHODS

Participants

19 right-handed healthy volunteers (8 males; mean age 25.9 ± 3 (SD) years) participated in the study. A signed, informed consent was obtained from all participants and the study was approved by the local ethics committee.

Task

The time reproduction task consisted of two phases: encoding and reproduction. During the encoding phase, participants had to retain the duration of a presented visual stimulus – a gray square with a centered red cross. The encoding interval was followed by a 3 seconds interstimulus interval, during which an indifferent

stimulus (gray cross) was displayed. The reproduction phase started by the appearance of a gray square with a centered green cross and participants were required to reproduce the retained duration by pressing a button with their right index finger. The button pressing was followed by a 3 second interstimulus interval. Three interval durations (5, 10 and 16.82 seconds) were repeated six-times in a pseudorandom order in every experimental block. The 16.82 second interval was chosen, because it is part of a geometric sequence $x_{i+1} = x_i \cdot 2^{1/4}$ (Jech *et al.* 2005). It took approximately twelve minutes to complete the task. Participants were instructed to fixate centrally on the cross throughout the task and to avoid mental counting. The subjects practiced this task before the experiment.

Transcranial magnetic stimulation procedure and target sites

Theta-burst TMS was performed using a figure-eight 70-mm air-cooled double coil attached to a Magstim Rapid stimulator (Magstim, Whitland, UK). Intermittent TBS at 100% the intensity of the resting right tibial anterior muscle motor threshold was used. Intermittent TBS procedure is described in (Huang *et al.* 2005) and comprises of 3 stimuli at 50Hz repeated ten-times at intervals of 200ms, followed by a pause of eight seconds. This sequence was repeated twenty-times, so that 600 pulses were delivered during the train. The motor threshold was based on the intensity of the leg motor area stimulation, which elicited motor responses in the right tibial anterior muscle in four out of eight single TMS pulses. Motor response was measured by electromyography and defined as a muscle contraction of 150 μ V amplitude. The target position of the coil was reached using the frameless stereotaxy navigation system Brainsight (Rogue Research, Montreal, Canada).

There were two TMS sites tested, the precuneus and the SMA. Positions were defined in a standard Montreal Neurological Institute (MNI) stereotactic space and the coordinates were adapted from our previous fMRI study ($x=0, y=-62, z=48$) for the precuneus and ($x=0, y=2, z=48$) for the SMA (Jech *et al.* 2005). These positions were transformed through reverse normalization back into the native space of each subject. The SPM5 software (The Wellcome Department of Imaging,

London, UK) was employed for forward and reverse normalization procedures.

Each subject performed the task four-times, before (pre-TMS session) and after stimulation (post-TMS session) over the precuneus and before and after stimulation over the SMA. TBS started approximately ten minutes after the completion of the pre-TMS session and the post-TMS session started right after the TBS procedure. The study protocol was divided into two consecutive days due to safety reasons and in order to reduce fatigue. The order of site stimulation for the precuneus and SMA was counterbalanced across participants. Subjects were asked to rate the pain induced by the TMS procedure as none (0), mild (1), moderate (2) or severe (3). Pain induced by TMS was not significantly different when comparing the stimulation sites (SMA 1.45 (mean) \pm 0.6 (SD), precuneus 1.10 \pm 0.8, paired t-test, $p=0.11$).

Analysis

Four two-way ANOVA with repeated measures and factors of condition (pre-TMS, post-TMS) and duration (5, 10, 16.82 s) were used for the analysis. It was carried out separately for both sites (SMA, precuneus) on two indices: mean subjects' interval estimates as a measure of accuracy and standard deviation as a measure of variability. Post hoc tests were corrected for multiple comparisons by Bonferroni correction.

RESULTS

There was no significant difference in accuracy of reproduced durations after TMS over the SMA ($F(1,18) = 2.02, p=0.17$) and over the precuneus ($F(1,18) = 0.13, p= 0.73$) (Figure 1, Table 1). ANOVA carried out on the index of variability (SD) yielded a significant effect of condition after TMS over the precuneus ($F(1,18) = 12.26, p<0.01$) and a significant interaction condition \times duration after TMS over the SMA ($F(2,36) = 3.36, p<0.05$). Four *post-hoc* paired t-test were performed and showed, that the SD of a 5 second interval was increased in the precuneus condition (paired t-test, $p<0.001$) and the SD of the 10 second interval was decreased in the SMA condition (paired t-test, $p<0.05$). SD was increased in all other post-TMS conditions, however not significantly.

Tab. 1. Mean reproductions (seconds) and mean SD for every condition and interval duration.

	SMA				precuneus			
	mean		SD		mean		SD	
	pre TMS	post TMS	pre TMS	post TMS	pre TMS	post TMS	pre TMS	post TMS
5 sec	5.09 \pm 0.8	5.23 \pm 0.7	1.18 \pm 0.3	1.38 \pm 0.4	5.30 \pm 0.8	5.34 \pm 0.9	1.05 \pm 0.4	1.57 \pm 0.5
10 sec	9.20 \pm 1.6	9.44 \pm 1.3	2.1 \pm 0.8	1.56 \pm 0.6	9.41 \pm 1.3	9.45 \pm 1.5	1.80 \pm 0.7	2.35 \pm 0.8
16.82 sec	13.38 \pm 2.3	13.70 \pm 1.9	2.69 \pm 1.1	2.95 \pm 1.3	13.69 \pm 1.7	13.38 \pm 2.3	2.62 \pm 1.1	2.82 \pm 1.1

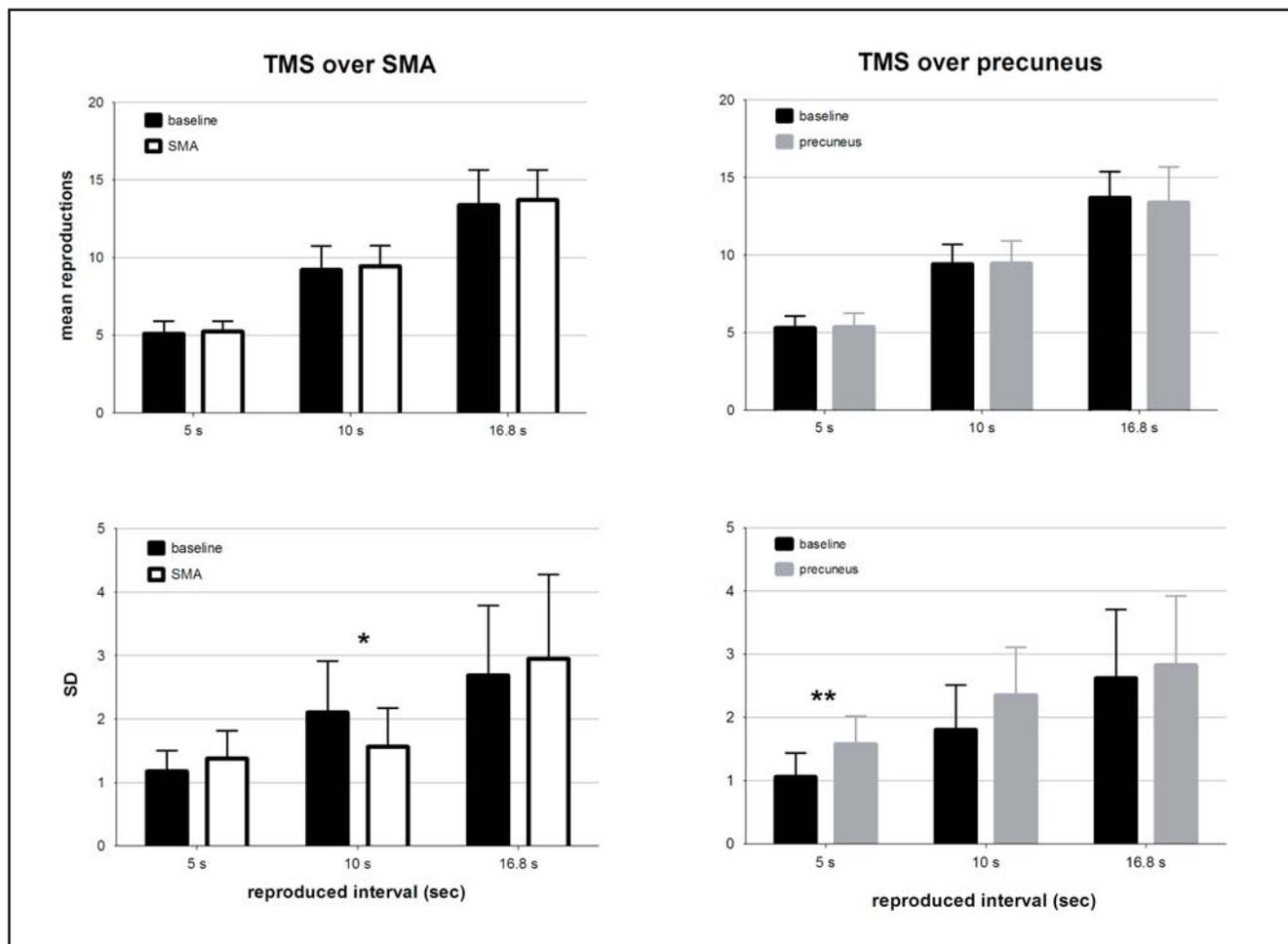


Fig. 1. Mean reproductions (accuracy) and mean standard deviations (variability) of interval duration estimates in all conditions. Values for pre and post TMS conditions are depicted separately for the SMA (left) and the precuneus (right). Significant differences in *post-hoc* paired t-tests are shown above the bars.

DISCUSSION

The accuracy of temporal estimates was not influenced by the stimulation of SMA, which concurs with previous research (Jones *et al.* 2004; Koch *et al.* 2003). Reproduced durations in all experimental conditions complied with the “Vierodt’s law”, i.e. short intervals were overestimated and long intervals underestimated compared to their physical duration (von Vierodt 1868). However, the variability of reproduced durations was altered by TMS over the SMA and precuneus. Increased variability of estimates was observed in all intervals after stimulation of both sites, except for the 10 second interval in the SMA condition. Therefore it might be regarded as an unspecific consequence of stimulation. However, according to *post-hoc* tests, this increase was significant for only the five second interval in the precuneus condition. There is no theoretical basis for why the five seconds interval variability should be influenced. The Perbal *et al* study showed a similar disproportionate increase in variability in the five second interval

reproduction, but this finding was left without explanation (Perbal *et al.* 2001). Increased variability has been observed in many neurodegenerative disorders, focal brain lesions and after traumatic brain injury and this inconsistency of cognitive performance was explained in terms of slower processing speed, impaired attention or decreased capacity of working memory (Gibbon *et al.* 1997; Pouthas & Perbal 2004). Increased variability can thus be regarded as an unspecific effect of acoustic or unpleasant sensory components of the stimulation, which might worsen attention or memory processes.

Decreased variability of time estimates after TMS over the SMA was distinctive for the 10 second interval. This was not the effect of outliers, since there were no outliers among the 10 second interval estimates in the post-TMS SMA condition and very few outliers were filtered in other conditions. It is also unlikely that it was an effect of interference with attention or working memory since these changes would affect all interval durations in a similar manner. The role of pre-SMA and SMA in interval timing is well established. In a recent

voxel-wise meta-analysis, SMA was one of two brain areas uniformly activated by temporal tasks regardless of interval duration and task characteristics (Wiener *et al.* 2010). A microrecording study in monkeys has shown that many neurons in the SMA and pre-SMA respond distinctly to different interval durations in the range of seconds (Mita *et al.* 2009). However, the reason for this effect on the 10 second interval remains unclear. One possible explanation may be the supposed nonlinear nature of timing, i.e. different sensitivity for different durations. It has been shown that rats have the highest sensitivity for timing of intervals in the range 8–12 seconds (Crystal 2001). This fact suggests the presence of a physiological oscillator with a frequency close to 0.1 Hz. Such a low frequency oscillation in the SMA region has been described in fMRI studies, where activity in the SMA was anticorrelated to the resting state default mode network fluctuations at frequency range 0.012–0.1 Hz (Fransson 2005). From this standpoint, it is unclear why only fluctuations at frequency 0.1 Hz and not other frequencies should be influenced and the hypothesis that TBS increased the power of low frequency oscillations in SMA remains highly speculative. Another possible explanation comes from the TBS pattern (Huang *et al.* 2005). The intermittent TBS has an alternating pattern of two seconds of supposedly activating stimulation and eight seconds of rest, giving a 10 second period of stimulation. This could have biased our results since regular temporal pattern of environmental stimuli can enhance accuracy of temporal processing (Coull *et al.* 2011). Nevertheless, it is unlikely that a purely sensory component of stimulation influenced the estimates of the 10 second interval in the SMA condition since stimulation of the precuneus shared the same sensory features. Speculatively, the periodic excitatory stimulation of the SMA could have “programmed” this area for a 10 second interval or even enhanced its intrinsic 0.1 Hz oscillations. This hypothesis should be examined by other studies using theta-burst protocol with different periods.

This study has several other limitations. Despite the fact that several rTMS studies managed to influence activity of the pre-SMA (Chen *et al.* 2009) and precuneus (Luber *et al.* 2007), it remains technically difficult to target deep midline areas by rTMS. However, we believe that stimulation addressed the desired spots since frameless stereotaxy was used and a variability of time estimates was influenced. This study did not use a dedicated control condition because primary motor or sensory cortices as control sites were unsuitable for our protocol using a supra-threshold stimulation and sham coils do not replicate all the sensory features of active stimulation. Instead, we compared the effects of TBS on two “active” brain sites. Therefore, TMS over SMA and the precuneus might have hypothetically influenced temporal estimates in the same way making it impossible to distinguish a specific effect of brain TMS from its unspecific sensory features. Nevertheless, the main

finding of this study, decreased variability of 10 second interval reproductions, was specific for the SMA condition and likely represents direct effects of TMS on this area.

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