



## TMS of supplementary motor area (SMA) facilitates mental rotation performance: Evidence for sequence processing in SMA



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### ABSTRACT

In the present study we applied online transcranial magnetic stimulation (TMS) bursts at 10 Hz to the supplementary motor area (SMA) and primary motor cortex to test whether these regions are causally involved in mental rotation. Furthermore, in order to investigate what is the specific role played by SMA and primary motor cortex, two mental rotation tasks were used, which included pictures of hands and abstract objects, respectively.

While primary motor cortex stimulation did not affect mental rotation performance, SMA stimulation improved the performance in the task with object stimuli, and only for the pairs of stimuli that had higher angular disparity between each other (i.e., 100° and 150°).

The finding that the effect of SMA stimulation was modulated by the amount of spatial orientation information indicates that SMA is causally involved in the very act of mental rotation. More specifically, we propose that SMA mediates domain-general sequence processes, likely required to accumulate and integrate information that are, in this context, spatial. The possible physiological mechanisms underlying the facilitation of performance due to SMA stimulation are discussed.

### Introduction

Visuo-spatial processing is typically studied using variants of the classic Mental Rotation (MR) tasks, in which individuals are required to judge whether two objects, presented at different orientations, are the same or mirror images of each other (e.g., Shepard and Metzler, 1971; Shepard and Cooper, 1982). In these tasks, reaction times (RTs) are usually found to increase as a function of angular disparity between the objects, a phenomenon that has been taken as evidence that individuals mentally rotate the objects as if they were physically rotating them (e.g., Shepard and Cooper, 1982).

The neural basis of MR has received considerable attention in recent years and has been investigated with multiple modalities, including magnetoencephalography (MEG), electroencephalography (EEG), functional magnetic resonance imaging (fMRI), and transcranial magnetic stimulation (TMS) (e.g., Bode et al., 2007; Kawamichi et al., 2007; Milivojevic et al., 2009a; Vingerhoets et al., 2002; Zacks, 2008; Wraga et al., 2005). Although the contribution of parietal regions to MR processes is well-established (e.g., Harris and Miniussi, 2003; Jordan et al., 2001; Parsons, 2003; Zacks, 2008), the involvement of

the motor system, including the primary motor cortex (M1), premotor regions, and the supplementary motor area (SMA), remains instead less clear. Studies have found that M1 plays a role in MR processes (e.g., Ganis et al., 2000; Pelgrims et al., 2011; Tomasino et al., 2005), whereas others did not show any causal involvement of M1 (Sauner et al., 2006), or interpreted M1 activation as epiphenomenal and/or the result of the spread of activation from adjacent and connected regions, such as the premotor regions (Bode et al., 2007; Eisenegger et al., 2007). Thus, M1 would not be essentially involved in MR tasks but would represent a subsidiary area, which is activated only because premotor areas are activated.

The involvement of premotor cortices and SMA has been found more consistently (e.g., Jordan et al., 2002; Lamm et al., 2007; Leek et al., 2016; Kosslyn et al., 1998; Wraga et al., 2005; see Zacks for a review). Nevertheless, their functional contribution to MR tasks is still debated and several hypotheses have been suggested (see, for example, Lamm et al. (2007)). In particular, according to the “motor imagery theory”, activations of these areas may be due to the fact that participants imagine rotating their own hands/body parts to solve the tasks (e.g., Kosslyn et al., 2001; Vingerhoets et al., 2001, 2002; Wraga

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et al., 2005; Zacks, 2008). By contrast, evidence from recent studies suggested that premotor and SMA activations do not reflect motor simulation, but are associated with MR per se (Lamm et al., 2007; Leek et al., 2016; Richter et al., 2000). More specifically, concerning the SMA involvement, the study by Richter et al. (2000) revealed that the duration of the BOLD (Blood-oxygen-level dependent) signal within SMA increased as a function of the time required to mentally rotating the stimuli. Furthermore, Ecker et al. (2006) found that the strongest correlation between the duration of MR operations and the time course of the hemodynamic response functions (HRF) occurred in the pre-SMA. Interestingly, linear relations between BOLD signal and angular disparity were found within pre-SMA: the larger the angular disparity between the stimuli, the greater the pre-SMA activation (Milivojevic et al., 2009a). Finally, a recent study showed that activation of SMA, and more specifically the pre-SMA regions, during MR tasks was associated with domain-general sequential operations in visuo-spatial processing, such as the serial remapping of spatial positions as a function of the changes in stimulus orientation (Leek et al., 2016).

The present study aimed to investigate whether SMA plays a causal role in MR tasks. Therefore, we applied short TMS bursts to the left SMA while participants were engaged in MR tasks involving objects and hands as stimuli. Since previous studies revealed that pictures of hands implicitly trigger the use of motor imagery strategy and, consequently, the activation of motor regions to a greater extent than abstract pictures do (Kosslyn et al., 1998; Vingerhoets et al., 2001), we could make different predictions about the TMS effect. On the basis of the motor imagery theory, we should find an effect of SMA stimulation only in the task involving hand stimuli. By contrast, we should find a TMS effect in tasks involving both the hand and the abstract object stimuli if SMA mediates non-motor, visuo-spatial operations closely related to MR.

In this study we stimulated also the left M1, for two reasons. First, as SMA is connected with M1 (Narayana et al., 2012), a reasonable speculation is that a possible TMS effect observed when stimulating the SMA would be the result of the indirect modulation of M1 activity. Therefore, to control this aspect, both SMA and M1 were stimulated in separate sessions, and the effects compared.

Second, as mentioned above, there are contradictory results about M1 involvement in MR tasks. Therefore, this study could help determine whether M1 plays a crucial role in MR tasks or is merely a subsidiary area. One question is what the role of M1 during MR is. If M1 supports motor simulation, as proposed by the ‘motor imagery theory’ (Kosslyn et al., 2001; Vingerhoets et al., 2002), we should expect to observe an effect of M1 stimulation specifically in the task that includes hands as stimuli. By contrast, if M1 is not essential for successful MR performance (Eisenegger et al., 2007; Sauner et al., 2006), no TMS effect should be expected on MR task, either with hand or object stimuli.

## Materials and methods

### Participants

Sixteen students of the University of Padua, Italy, took part in the experiment (11 females and 5 males; mean age: 24.4 years (range: 21–30); Educational level: 16 years (range: 14–18)). Participants had normal or corrected-to-normal vision, and were right-handed according to the Edinburgh inventory (Oldfield, 1971). All were healthy, with no history of head injury or neurological, psychiatric, or physical illness and were all checked for TMS exclusion criteria (Rossi et al., 2009). No participant was a professional musician or athlete. They gave informed written consent before participating in the experiment. The study was carried out in accordance with the guidelines of the Declaration of Helsinki and was approved by the ethical committee of the Department of General Psychology, University of Padua.

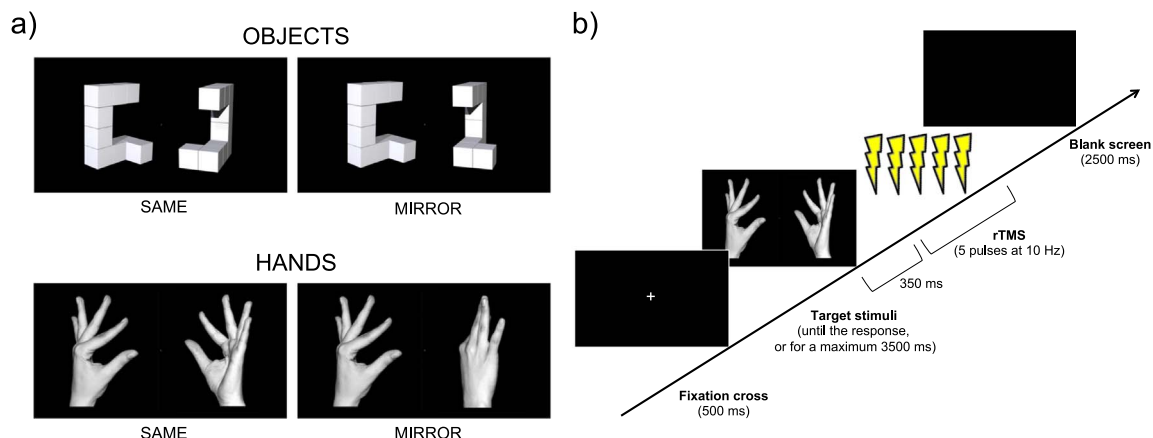
### Stimuli and procedure

Participants were seated in front of a color monitor screen at a distance of about 60 cm. The experiment was run using the E-Prime software system. Two types of MR tasks were designed, involving pictures of objects and hands, and were administered in a counter-balanced order across participants. For each single participant, the order of task presentation was the same among the three TMS sessions. Therefore half of participants always started with the task including object stimuli, whereas the other half started with the task including hand stimuli. In both tasks, pairs of stimuli were simultaneously presented and could appear at different angles of orientation. Participants were asked to verbally report whether the stimulus presented on the right was the same or a mirror version of the stimulus on the left by saying “si” (yes; *same* stimuli) or “no” (no; *mirror* stimuli). Verbal responses were recorded with a digital voice recorder.

The object stimuli were 3-D Shepard and Metzler-like object figures obtained from the dataset by Ganis and Kievit (2014). The objects were white on a black screen and had a natural-looking shading effect. Each object consisted of 7 to 11 cubes and was composed of 4 arms, connected end-to-end in a sequence.

The hand stimuli were 3-D pictures of upright hands, constructed with a digital camera and picture-editing software (Adobe Photoshop C24 Version 11.0). The hands were also white on a black background. As suggested by Ganis et al. (2000), in order to avoid visuo-motor interference, the stimulus on the left side was always a left hand whereas the stimulus on the right side was a left hand in 50% of the pairs and a right hand in the other 50%. Fig. 1 shows some examples of the pairs of stimuli and a schematic timeline of an experimental trial.

Seven different configurations of objects and hands were used to



**Fig. 1.** Stimuli and Timeline. a) Example of object stimuli (upper panel) and hand stimuli (lower task), presented at 150° of angular disparity. The stimuli could be identical (“same”) or mirror images of each other (“mirror”). b) A schematic illustration of the timeline of an experimental trial.

attenuate practice effects. For the hand task, the seven configurations (at 0° of orientation) were the following: three with the back of the hand facing forward, three with the palm facing forward, and one with a lateral view of the hand.

The object stimuli had a size of 26.2°×13.7° in visual angle when viewed from a distance of 60 cm. The hand stimuli were created accordingly. All the stimuli were rotated around the vertical axis. Each task consisted of 56 pairs of stimuli: 7 'same' or 7 'mirror' pairs of stimuli, presented with four possible MR angular disparities (0°, 50°, 100° and 150° of rotation).

For both types of task, the sequence of events and stimuli was as follows: A fixation cross was presented on the screen for 500 ms, followed by the target stimuli, which remained visible until the response or for a maximum of 3500 ms. If the participants responded before 3500 ms, a blank occurred until the 3500 ms time interval was reached. Then, a blank screen was presented for 2500 ms. This inter-trial interval was designed to ensure that the TMS pulses were well spaced in time, in accordance with the TMS safety recommendations proposed by Rossi et al. 2009 (see the next paragraph for the details of the TMS protocol).

The experiment consisted of three experimental sessions (one for each TMS site). The session included two blocks (one with object stimuli and one with hand stimuli) of 56 trials each. An initial practice session was administered, comprising two blocks. Each block included 16 trials: 2 stimuli configurations (hands, objects) × 2 match conditions (same, mirror) × 4 angles (0°, 50°, 100°, 150°). The stimuli presented in the practice were not used in the experimental sessions. At the end of the experiment, participants completed a questionnaire where they had to indicate the extent to which they used motor imagery and visual imagery strategies in each of the tasks on a four-point-Likert scale (from "1 = never used" to "4 = always used").

#### Transcranial magnetic stimulation

TMS was applied using a Magstim 200 magnetic stimulator that delivers monophasic pulses with a figure-of-eight coil (70 mm outer diameter). Three sessions were administered, in which TMS was applied over either SMA, M1, or the vertex, but with the coil being angled slightly off the head. In this condition (sham stimulation), the two wings of the figure-of-eight coil touched the scalp at 45° from the plane tangential to the scalp so that it could simulate the acoustic and sensation artifacts of TMS without effectively stimulating brain regions (Correa et al., 2014; Lisanby et al., 2001). The order of the TMS conditions (SMA, M1, sham) was counterbalanced across participants.

To target the left SMA, a site 3 cm anterior to the vertex was marked in the sagittal midline (Cz of the international 10–20-EEG system) of the scalp and then slightly moved 0.5 cm to the left, in accordance with most studies (Hamada et al., 2008; Janssen et al., 2015; Matsunaga et al., 2005). Several studies showed that the optimal position for SMA stimulation was between 2 and 4 cm anterior to the vertex (Cunnington et al. 1996; Terao et al. 2007; Verwey et al. 2002; Serrien et al. 2002; Oliveri et al. 2003), and neuroimaging studies have also identified SMA 2–3 cm anterior to Cz (Hikosaka et al. 1996; Lee et al. 1999). The coil was positioned tangentially to the skull, with the handle perpendicular to the sagittal axis (pointing to the right). This was shown to be the best coil orientation for an optimal stimulation effect of SMA (Janssen et al., 2015).

The identification of the hand area of the left M1 was based on the presence of motor evoked potentials (MEPs) recorded from the right first dorsal interosseous (FDI) muscle. In this condition, the coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° away from the sagittal axis. Participants also wore a swimming cap where we marked the "hotspot" with a colored dot. Moreover, a chin support was used to reduce head movements.

A TMS train (5 pulses at 10 Hz) was applied in each trial 350 ms after the onset of stimulus presentation. This high-frequency paradigm

was chosen based on a previous study that investigated MR of hands and abstract stimuli (i.e., letters) (Pelgrims et al., 2011), whereas the time interval was selected based on MEG and EEG studies exploring the spatio-temporal dynamics of MR (Iwaki et al., 1999; Kawamichi et al., 2007; Schendan and Lucia, 2009). Indeed, the ERP correlates of mental rotation processes were found to occur between 350 and 800 ms post-stimulus onset (e.g., Milivojevic et al., 2009b; Schendan and Lucia, 2009).

The TMS intensity was set at 110% of the resting motor threshold, which was defined as the minimum output intensity leading to 5 MEPs (about 50  $\mu$ V in amplitude) in 10 consecutive trials. The mean stimulation intensity was 58.5% (range: 48–68%) of the output of the stimulator, which is in accordance with international safety guidelines (Rossi et al., 2009).

Electromyographic (EMG) activity was recorded from the left and right FDI muscles using Ag/AgCl surface electrodes, with the active electrode over the muscle belly and the reference electrode over the second phalanx of the index. The ground electrode was positioned over the right wrist. The signal was online amplified, bandpass filtered (10–1000 Hz) and digitalized (sampling rate: 2048 Hz).

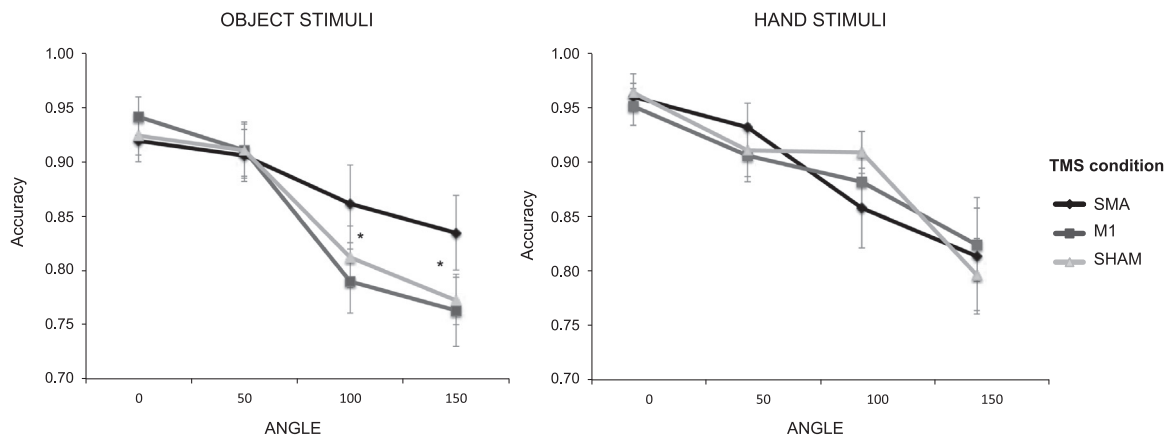
The MEPs were always and only detected during stimulation of M1, whereas no MEP was ever evoked during SMA or the sham stimulation sessions. Notably, the lack of MEPs during the SMA session revealed that no supra-threshold spread of activation to M1 occurred when stimulating SMA. No adverse effects of TMS were reported. In a post-experimental debriefing interview, most of the participants incorrectly guessed which one was the sham stimulation.

#### Data analysis

Proportion of correct responses and mean RTs were measured. RT was defined as the temporal interval between the appearance of the target stimuli and the onset of the vocalization. Verbal responses were recorded by a digital recorder and measured with Audacity software (<http://audacity.sourceforge.net/>). All data satisfied the assumptions of normality and homoscedasticity, according to the Kolmogorov-Smirnov and Mauchly tests, respectively. Only RTs of correct trials were entered into the analysis. The proportion of correct responses and the RTs were analyzed by two separate ANOVAs, including the following within-subject factors: Stimulus (hands, objects), TMS condition (SMA, M1, sham), Match (same, mirror), and Angle (0°, 50°, 100°, 150°). A False Discovery Rate (FDR) correction was applied for multiple comparisons. The effect size was measured by using Partial Eta Squared ( $\eta_p^2$ ).

The analysis of both the accuracy and RTs revealed a significant Angle × Match × Stimulus interaction, which suggests the presence of a different relationship between angle of rotation and behavioral measures in the *same* stimuli relative to the *mirror* stimuli, as well as between the hand and the object stimuli. To better explore this issue, for each participant, the slope of the linear regression for proportion of correct responses and RTs was measured as a function of angle of rotation, separately for the *same* and *mirror* conditions. We then performed two ANOVAs, which included the slope values as dependent variables and Stimulus and Match as independent variables. When appropriate, we carried out one-sample t-tests against zero to verify the presence of a linear relationship between angle of rotation and accuracy/RTs. The value of the slope provides information about the extent to which MR processes are recruited in a task (Shepard and Cooper, 1982).

The analyses were conducted using Statistica (version 8.0) and SPSS (version 23.0).



**Fig. 2.** Proportion of correct responses and mean reaction times (RTs) as a function of TMS condition (SMA, M1, SHAM) and angle of rotation (0°, 50°, 100°, 150°), separately for object and hand stimuli. The error bars represent standard error. The asterisks indicate significant differences.

## Results

### Accuracy

An ANOVA including Stimulus, Match, TMS condition, and Angle was performed on mean proportion of correct responses, revealing a significant main effect of Angle of rotation [ $F(3,45)=26.80$ ,  $p < .01$ ,  $\eta_p^2 = .64$ ]. The other main effects were not significant (all  $ps > .05$ ). The TMS condition  $\times$  Match interaction was significant [ $F(2,30)=3.90$ ,  $p < .05$ ,  $\eta_p^2 = .21$ ], revealing that the TMS stimulation had an effect in the *same* but not in *mirror* stimuli. More specifically, post-hoc comparisons showed that, as compared with both M1 and the sham stimulations, the stimulation of SMA increased performance accuracy in the *same* stimuli (i.e., trials where there was a match between the stimuli on the right and left) ( $ps < .05$ ), but not in the *mirror* stimuli (all  $ps > .05$ ).

Moreover, the Stimulus  $\times$  TMS condition  $\times$  Angle interaction was significant [ $F(6,90)=2.33$ ,  $p < .05$ ,  $\eta_p^2 = .13$ ].

Post-hoc comparisons showed that, in the task with object stimuli, TMS over SMA enhanced the level of performance accuracy in trials with high angular disparity (i.e., 100° and 150°) (Fig. 2). Indeed, in these trials the accuracy of MR performance was significantly higher for SMA stimulation relative to both M1 and sham stimulations (all  $ps < .05$ ). By contrast, in the task with hand stimuli, no difference in accuracy was found as a function of TMS condition (all  $ps < .05$ ) (Fig. 2).

Finally, the Angle  $\times$  Match interaction [ $F(3,45)=3.28$ ,  $p < .05$ ,  $\eta_p^2 = .18$ ] and the Angle  $\times$  Match  $\times$  Stimulus interaction [ $F(3,45)=7.52$ ,  $p < .01$ ,  $\eta_p^2 = .33$ ] were significant.

Post-hoc comparisons investigating the three-way interaction showed that, in the task with object stimuli, the accuracy for the *same* stimuli significantly decreased from 0° trials and 50° trials to 100° trials, and from 100° trials to 150° trials ( $ps < .05$ ), whereas the accuracy for the *mirror* stimuli did not significantly differ across the angle conditions (all  $ps > .05$ ) (Fig. 3). In the hand task, for both the *same* and *mirror* stimuli, the accuracy decreased from 0° trials to 100° trials, and from 100° trials to 150° trials, in which the RTs were slowest ( $ps < .05$ ) (Fig. 3).

To better explore the relation between angle of rotation and accuracy, we compared the values of linear regression slopes for proportion of correct responses, for the *same* and *mirror* stimuli, for both the object and hand stimuli with an ANOVA. The main effect of Match [ $F(1,15)=9.12$ ,  $p < .01$ ,  $\eta_p^2 = .37$ ] and the Stimulus  $\times$  Match interaction [ $F(1,15)=15.99$ ,  $p < .01$ ,  $\eta_p^2 = .51$ ] were significant. Post-hoc comparison investigating the interaction revealed that the slope was significantly steeper for the *same* stimuli ( $\beta = -.83$ ; SE = .03) than

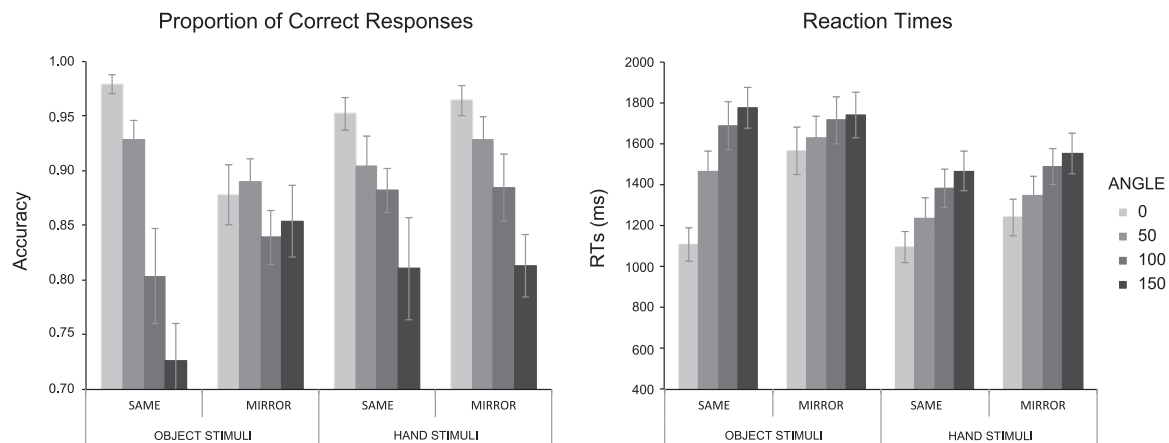
for the *mirror* stimuli ( $\beta = -.13$ ; SE = .16), in the object task ( $p < .05$ ), whereas it did not differ between the *same* stimuli ( $\beta = -.57$ ; SE = .14) and the *mirror* stimuli ( $\beta = -.62$ ; SE = .08) in the hand task ( $p > .05$ ). Notably, when contrasting the values of the slopes against zero (which means no relation between accuracy and angle of rotation), all the slopes were significant ( $ps < .05$ ) except for the slope for the *mirror* stimuli in the task with objects. Taken together, these results indicate that a linear decrease of accuracy as a function of angle of rotation emerged for the *same* stimuli but not for the *mirror* stimuli, in the task with objects, whereas it occurred in both the *same* and the *mirror* condition in the task with hand stimuli.

### RTs

We performed an ANOVA with the same factors – Stimulus, TMS condition, Match and Angle – on the RTs. There were significant main effects of Stimulus [ $F(1,15)=47.5112$ ,  $p < .01$ ,  $\eta_p^2 = .76$ ], with RTs being slower for the object stimuli than for the hand stimuli, Match [ $F(1,15)=13.69$ ,  $p < .01$ ,  $\eta_p^2 = .47$ ], with RTs being slower for the *mirror* stimuli than for the *same* stimuli, and Angle of rotation [ $F(3,45)=86.29$ ,  $p < .01$ ,  $\eta_p^2 = .85$ ]. TMS did not significantly interact with the other factors. However, to ensure that the improvement in the accuracy caused by the SMA stimulation was not accompanied by an increase in the RTs – thus revealing a speed-accuracy trade off – we explored the TMS  $\times$  Match interaction in more depth. TMS over SMA led to significantly faster RTs in MR task relative to the other two TMS conditions, but only for the *same* stimuli (all  $ps < .05$ ). Such results converge with the pattern of accuracy results in revealing a facilitatory effect of SMA stimulation on MR performance. Notably, these results also rule out the hypothesis that the TMS over SMA led to a speed-accuracy trade off. Finally, the Angle  $\times$  Match [ $F(3,45)=14.51$ ,  $p < .01$ ,  $\eta_p^2 = .49$ ] and the Angle  $\times$  Match  $\times$  Stimulus interaction [ $F(3,45)=14.80$ ,  $p < .01$ ,  $\eta_p^2 = .49$ ] were significant.

Post-hoc comparisons were run to analyze the significant Angle  $\times$  Match  $\times$  Stimulus interaction in more depth (Fig. 3). For the *same* stimuli, RTs became significantly slower as a function of the angle of rotation (RTs at 0° < RTs at 50° < RTs at 100° < RTs at 150°;  $ps < .05$ ). On the other side, for the *mirror* stimuli, the only difference was shown between RTs in 0° and 50° trials with RTs in 100° and 150° trials ( $ps > .05$ ) (Fig. 3). For the task with hand stimuli, the *same* and *mirror* stimuli showed a rather similar pattern of RTs as a function of angle of rotation (RTs at 0° < RTs at 50° < RTs at 100°, and were largest at 150°;  $ps < .05$ ) (Fig. 3). Also, for the *same* stimuli, there was a difference between RTs in 100° trials and RTs in 150° trials ( $p < .01$ ).

To better investigate the interaction, an ANOVA on the slope of the linear regression for the RTs was performed, revealing that the effects of Stimulus [ $F(1,15)=7.25$ ,  $p < .05$ ,  $\eta_p^2 = .33$ ], Match [ $F(1,15)=8.31$ ,  $p < .05$ ,  $\eta_p^2 = .35$ ], and Stimulus  $\times$  Match [ $F(1,15)=15.99$ ,  $p < .01$ ,  $\eta_p^2 = .51$ ] were significant.



**Fig. 3.** Proportion of correct responses and mean reaction times (RTs) as a function angle of rotation (0°, 50°, 100°, 150°) and stimulus match (same, mirror), separately for object and hand stimuli. The error bars represent standard error.

< .01,  $\eta_p^2 = .35$ ], as well as the Stimulus  $\times$  Match interaction [ $F(1,15) = 7.26$ ,  $p < .05$ ,  $\eta_p^2 = .32$ ] were significant. Post-hoc comparison investigating the interaction revealed that the slope was significantly steeper for the *same* stimuli ( $\beta = .93$ ;  $SE = .03$ ) than for the *mirror* stimuli ( $\beta = .49$ ;  $SE = .03$ ), in the task using object stimuli ( $p < .05$ ), whereas it did not differ between *same* stimuli ( $\beta = .90$ ;  $SE = .03$ ) and *mirror* stimuli ( $\beta = .87$ ;  $SE = .04$ ), in the task with hand stimuli ( $p > .05$ ).

All the values of the slopes were significantly different from zero ( $ps < .05$ ).

#### Questionnaires

An ANOVA including two variables – Stimulus (objects *versus* hands) and Strategy (visual imagery *versus* motor imagery) – was conducted to compare the scores of strategy frequency for both the object and hand stimuli. Such analysis showed only a significant main effect of Strategy [ $F(1,15) = 55.34$ ,  $p < .01$ ,  $\eta_p^2 = .78$ ], which indicates that visual imagery strategy ( $M = 3.78$ ,  $SE = .12$ ) was more often used compared to motor imagery strategy ( $M = 1.37$ ,  $SE = .20$ ) to support MR performance, regardless of the type of stimulus.

Furthermore, to verify whether participants adopted a motor simulation strategy at least to some extent, we compared the scores of motor imagery against 1 (where in the questionnaire “1” means that such strategy is never used). Given that the score did not significantly differ from 1 either for the object [ $t(15) = 2.05$ ;  $p > .05$ ] or hand stimuli [ $t(15) = 1.00$ ;  $p > .05$ ], this indicates that a motor imagery strategy did not support MR performance in these tasks, at least for this sample of participants.

#### Discussion

The primary goal of this study was to examine the functional contribution of SMA to MR tasks. Our data showed that TMS applied to the left SMA facilitated MR performance in the *same* stimuli but not in the *mirror* stimuli. Also, TMS facilitated performance for the object stimuli, but only in trials with higher angular disparity (i.e., 100° and 150°). Three inferences can be drawn from these results.

First, the evidence that SMA stimulation did not selectively impact performance on the MR task with hand stimuli, but rather had a predominant effect on the task with object stimuli, goes against the motor imagery theory, which suggests that SMA would support motor simulation to solve MR tasks (Wraga et al., 2005; Zacks, 2008).

Second, SMA is involved in more cognitive processes, closely related to MR itself. Indeed, TMS improved performance especially in the *same* stimuli, where MR operations were mostly recruited. This inference is driven by the evidence that linear relationships between

behavioral measures and angle of rotation, which represent a signature of MR operations (Shepard and Metzler, 1971), were observed mainly for the *same* stimuli. More specifically, performance accuracy decreased with increasing angular disparity in the *same* stimuli but not in the *mirror* stimuli of the task with objects. Also, in this task, the slope of linear regression for RTs as a function of angle of rotation was steeper for the *same* than for the *mirror* stimuli. As mentioned above, since such linear relationships between behavioral measures and angle of rotation are an index of the presence of MR operations, this result helps us to interpret the effect of SMA stimulation. The selective effect of TMS to SMA in the *same* stimuli (where MR operations are mostly involved) but not in the *mirror* stimuli suggests that SMA modulates a cognitive process that strictly supports MR per se.

Third, the nature of processes underlying MR was clarified by the evidence that the effect of SMA stimulation was modulated by the amount of spatial orientation information that needed to be processed, being observed only for greater angular disparities between the experimental objects. This finding is consistent with the study by Leek et al. (2016), which showed that the activity in a sub-region of supplementary motor complex (i.e., pre-SMA) was modulated by visuo-spatial transformation during MR. In line with that study, our results suggest that spatial transformation processes are supported by non-motor sequence operations that involve SMA. As proposed in the studies by Leek and collaborators (Leek and Johnston, 2009; Leek et al., 2016), SMA would contribute to sequence processing routines, which would allow spatial mapping between the coordinates of the stimuli, through the computation of vector transformations.

Although the transformation of feature coordinates in the MR tasks is performed within a spatial coordinate system, these sequence processes may also be recruited for a broader range of cognitive (not visuo-spatial) tasks. Our hypothesis, indeed, is that SMA is a possible candidate for domain-general sequence processes, such as the information accumulation over time, which is required to implement an internal representation that would guide behaviors/thoughts. Importantly, since the SMA regions were found to play a similar role across different domains, we propose a relatively abstract functional role for SMA regions, which would be associated with domain-general sequence and cumulative processing routines. To support this hypothesis, below we present a brief review of studies showing the involvement of SMA in sequence cumulative processes in a variety of different domains (see Cona and Semenza (in press), for a complete review).

Many studies identified SMA as the neural substrate of the temporal accumulator, which contains sequential temporal information, where the magnitude of any perceived temporal duration is the result of the number of pulses accumulated (see Casini and Vidal (2011), for a review). As such, the accumulator would rely upon sequence routines as well, though in the temporal domain. The first evidence for a

cumulative process in SMA came from EEG studies, which analyzed the contingent negative variation (CNV), an electrical correlate of the accumulation processes (Bendixen et al., 2005; Macar and Vidal, 2002; Macar et al., 1999). These studies observed that the larger the estimated time interval was, the larger the CNV over the SMA was. This result was interpreted as proof that SMA acts as a temporal accumulator (e.g., Macar et al., 1999). Evidence supporting this interpretation can be found in both fMRI data (Coull et al., 2004) and in single unit recordings of awake monkeys (Mita et al., 2009). For example, Coull et al. (2004) found that activity in SMA positively correlated with attention paid to timing. Mita et al. (2009) observed that neurons within the pre-SMA and SMA regions showed a build-up pattern reflecting cumulative processes: the longer the duration to be produced was, the higher the firing rate at the end of the duration was.

Besides the spatial and temporal domain, there is a large consensus that SMA is responsible for sequence processes in motor control, as revealed by several studies of limb or eye movements (see Nachev et al. (2008), for a review). Indeed, SMA regions have been found to be sensitive to many aspects of action sequences (Tanji, 2001). Several studies involving monkeys showed that some neurons in SMA regions respond to the relational order of a movement in a given sequence (Clower and Alexander, 1998; Shima and Tanji, 2000). In humans, studies using positron emission tomography (PET) have shown activations of the SMA regions during motor sequences. Interestingly, such activations have also been found when individuals ‘internally’ simulated the movement sequence without actually executing such actions (Roland et al., 1980). Finally, SMA has been shown to be involved in cumulative processes in other domains, such as grip force scaling (White et al., 2013) and numerical cognition (Arsalidou and Taylor, 2011). Together, these results suggest that the most ‘parsimonious’ explanation of the role of SMA is that this region mediates domain general sequence and cumulative processes engaged in gaining ongoing information, which are then integrated in an internal representation that guides the appropriate action/decision.

An interesting and surprising finding of the current study concerns the TMS-induced improvement of MR performance when SMA was stimulated. Since the physiological nature of the mechanisms underlying the TMS-induced facilitation of cognitive performances is still not well established, we proposed and compared three explanations.

As TMS induces neural noise into a region, it could have interfered with SMA functioning. Therefore, we should assume that, in normal conditions, SMA interferes with MR, perhaps exerting inhibitory control over responses (Chen et al., 2009). Nevertheless, although the SMA could play a role in inhibitory processes (Chen et al., 2009), this does not explain why the facilitation of MR performance was observed only for trials with higher angular disparity and selectively in the object task. If SMA contributed to response inhibition processes, indeed, we should have expected a general, *a-specific*, decrease in response latencies. It is, however, possible that SMA exerts inhibitory influences on brain areas that are involved in MR. In this way, the suppressive influence exerted on these areas would be released by the SMA stimulation.

The second explanation posits that high-frequency TMS applied to regions necessary for task performance can increase cortical excitability in a way that improves performance (Luber et al., 2007). Indeed, TMS at frequencies of 5 Hz or higher was shown to enhance cortical excitability (Berardelli et al., 1998; Peinemann et al., 2000; Siebner et al., 2000). Moreover, our finding is in line with recent studies showing that high-frequency TMS delivered specifically to SMA led to an increase in cortical excitability (Matsunaga et al. 2005; Hamada et al. 2008; Raux et al. 2010). Such local increase, derived from an increase in the amplitude of excitatory post-synaptic potentials (e.g., Iriki et al., 1989), would produce a larger neural response. Nevertheless, a general increase in neural activity might not account for the improvement of a more complex process, such as MR.

The last explanation is that rhythmic TMS at 10 Hz modulated the natural brain oscillations, determining a resonance with alpha activity

(Klimesch et al., 2003; Luber et al., 2006; Thut et al., 2011). In fact, a large and growing body of evidence revealed that rhythmic TMS interacts with brain oscillations in a frequency-dependent manner. This causes a local entrainment of such brain oscillations (Robertson, 2009; Thut et al., 2011) and, in turn, the enhancement in human performance (Hilgetag et al., 2001; Kirov et al., 2009; Reis et al., 2009). In particular, in the study by Sauseng et al. (2009), TMS bursts were applied at 10 Hz (the same frequency used in our work) to promote alpha rhythm. This TMS protocol increased the memory capacity by improving the suppression of irrelevant information in short-term memory. Likewise, Luber et al. (2007) showed that TMS led to a facilitation of working memory performance, but only for specific bands of frequency. Importantly for our study, the work of Klimesch et al. (2003) revealed that TMS bursts delivered at the participants’ individual upper alpha frequency (which is about 10 Hz) over frontal and parietal sites determined an improvement in MR performance. Finally, other studies reported facilitating effects (ignoring those investigating the effects of disinhibition) when the TMS bursts were delivered at a frequency that either equals alpha band (Hamilton and Pascual-Leone, 1998; Wassermann et al., 1999), subharmonics of alpha like at 5 Hz (Boroojerdi et al., 2001; Luber et al., 2007), or harmonics like 20 Hz (Mottaghy et al., 1999; Sparing et al., 2001). Taken together, these studies seem to offer a compelling explanation, and suggest that short TMS trains at 10 Hz might have modulated alpha frequencies, leading to an enhancement in MR performance.

Notably, the finding of a TMS-induced facilitation of performance could drive the development of new treatment protocols for improving visuo-spatial abilities. The study by Hamada et al. (2008) showed that high-frequency TMS applied over SMA improved (albeit modestly) motor symptoms in patients with Parkinson’s Disease, thus indicating SMA as a potential stimulation site for treatment. In light of this, the finding of the current study would extend the list of processes for which SMA stimulation improves performance, revealing that not only motor processes, but also “more cognitive” functions, such as visuo-spatial processes, can benefit from TMS of this cortical area.

A question arising from this study is why SMA stimulation had an impact on MR performance selectively in the task with object stimuli. One possibility is that TMS pulses were administered too late to have an impact on performance in the task with hand stimuli given that such task seems to be easier. This hypothesis is supported by the finding that RTs were shorter for the hand stimuli compared to the object stimuli. Nevertheless, it seems unlikely that SMA involvement is complete before 350 ms from the presentation of the target stimuli (i.e., the onset of TMS pulses), as MR processes were found occurring in a time window between 350 and 800 ms after the stimulus onset (Miliivojevic et al., 2009b; Schendan and Lucia, 2009). Another possibility is that different brain structures mediate MR tasks with hand stimuli. Future studies might include multiple time intervals to address this issue and to better clarify when the SMA is involved, for both the object and hand tasks.

The second goal of the present study was to establish whether M1 has a causal role in MR processes. We found that the pattern of accuracy for M1 stimulation in the task with object stimuli was significantly different from SMA stimulation, but very similar to that observed for sham stimulation. Indeed, no significant difference was observed between M1 and sham stimulation. This result corroborates the conclusion of recent works that M1 does not play an essential role in MR tasks (Eisenegger et al., 2007; Sauner et al., 2006). Kosslyn et al. (2001) proposed a strategy-dependent involvement of M1 in MR. Thus, M1 would be involved only in MR tasks that induce participants to imagine rotating their hands (or rotating stimuli using their own hands). Yet, as revealed by the scores of the questionnaires, participants in our study tended to adopt a visual simulation strategy rather than a motor simulation strategy to execute the task, regardless of the kind of the stimuli. This might be an explanation for the lack of TMS effects when M1 was stimulated.

Finally, the lack of the effect of M1 stimulation on MR suggests that the TMS-induced effects on MR observed during SMA stimulation were not produced by the spread of activation to M1 but were due to the modulation of SMA activity per se. Moreover, no MEP was ever observed during SMA stimulation, suggesting that the spread of activation to M1 was negligible.

### Limitations and conclusions

Some limitations of this study should be mentioned as their acknowledgement can drive future research. Since this study did not use a neuro-navigation system, we cannot surely ascertain which sub-region of SMA (i.e., pre-SMA or SMA-proper) was stimulated. Nevertheless, we could speculate that the pre-SMA was stimulated: in fact neither an indirect activation of M1 nor a slowing down of RTs regardless of the angle of rotation – which might be both accounted for by a modulation of SMA-proper activity (Narayana et al., 2012) – was observed.

Another intrinsic limitation of the TMS technique concerns the spread of stimulation to the adjacent regions and remote connected areas. However, the effect observed in our study seems to be specific to the SMA regions (the pre-SMA in particular) given that the same effect was not observed when adjacent brain regions, like the M1, were stimulated. Furthermore, other regions that could be implicated in MR, such as the Frontal Eye Fields, were far enough away from the site of stimulation. Nevertheless, as for all TMS studies, we cannot exclude that the effect resulted (also) from the stimulation of regions that are anatomically connected to the (pre-)SMA, such as basal ganglia and prefrontal regions, which might play a role in spatial processing as well (Bates and Goldman-Rakic, 1993; Postuma and Dagher, 2006). Therefore, future studies combining TMS with fMRI could be useful to investigate remote causal influences from SMA to putatively interconnected regions (e.g., Bestmann et al., 2003).

Finally, given that most of our participants were female it is possible that we uncovered gender-specific modulation of the TMS effects on MR performance, as women and men have been found to show partially different cortical activation patterns during MR tasks (e.g., Jordan et al., 2002). Both women and men, however, exhibited pre-SMA activations (Jordan et al., 2002).

Despite these limitations, the present study is important because it demonstrated a causal involvement of SMA in MR. More specifically, SMA seems to mediate domain-general sequence processes, which are likely to be required to accumulate and integrate sequential elements into higher-order representations. In this context, SMA would accumulate spatial orientation information, providing a linear metric of space. Finally, the facilitatory effect of SMA stimulation on MR performance suggests that 10 Hz TMS over SMA might be a promising intervention in the treatment of visuo-spatial deficits.

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