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INITIAL ACTIVATION STATE, STIMULATION INTENSITY AND TIMING OF STIMULATION INTERACT IN PRODUCING BEHAVIORAL EFFECTS OF TMS

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Abstract—Behavioral effects of transcranial magnetic stimulation (TMS) have been shown to depend on various factors, such as neural activation state, stimulation intensity, and timing of stimulation. Here we examined whether these factors interact, by applying TMS at either sub- or suprathreshold intensity (relative to phosphene threshold, PT) and at different time points during a state-dependent TMS paradigm. The state manipulation involved a behavioral task in which a visual prime (color grating) was followed by a target stimulus which could be either congruent, incongruent or partially congruent with the color and orientation of the prime. In Experiment 1, single-pulse TMS was applied over the early visual cortex (V1/V2) or Vertex (baseline) at the onset of the target stimulus – timing often used in state-dependent TMS studies. With both subthreshold and suprathreshold stimulation, TMS facilitated the detection of incongruent stimuli while not significantly affecting other stimulus types. In Experiment 2, TMS was applied at 100 ms after target onset –a time window in which V1/V2 is responding to visual input. Only TMS applied at suprathreshold intensity facilitated the detection of incongruent stimuli, with no effect with subthreshold stimulation. The need for higher stimulation intensity is likely to reflect reduced susceptibility to TMS of neurons responding to visual stimulation. Furthermore, the finding that in Experiment 2 only *suprathreshold* TMS induced a behavioral facilitation on incongruent targets (whereas facilitations in the absence of priming have been reported with *subthreshold* TMS) indicates that priming, by reducing neural excitability to incongruent targets, shifts the facilitatory/inhibitory range of TMS effects.

Key words: TMS, priming, state dependency, reaction times, facilitation.

INTRODUCTION

Behavioral effects of transcranial magnetic stimulation (TMS) have been shown to depend on various factors, such as timing, intensity, and initial activation state of the stimulated region (for reviews, see e.g. Wagner et al., 2007; Sandrini et al., 2011; de Graaf et al., 2014; Romei et al., 2016). Statedependency can be observed, for example, when TMS is applied after psychophysical manipulations such as visual adaptation or priming, TMS differentially affects the perception of features encoded by neural representations that have undergone a state manipulation compared to those that have not (for reviews see Silvanto et al., 2008; Silvanto and Pascual-Leone, 2008). Initial studies examined such effects in the visual cortex, adaptation to features such as color and motion was found to be reduced and even reversed with TMS (Silvanto et al., 2007, see Silvanto et al., 2008). In the context of visual priming, TMS has been shown to reduce priming effects (Campana et al., 2002, 2006) and selectively enhance the detection of non-primed targets (Cattaneo et al., 2008, 2010a,b; Cattaneo, 2010). State-dependent TMS paradigms based on priming and adaptation have become a useful tool for assessing the tuning of neuronal representations in specific brain regions in various cognitive functions, ranging from letter and number selectivity to action observations (e.g. Cattaneo et al., 2009, 2010a,b; Guzman-Lopez et al., 2011; Jacquet and Avenanti, 2015; Kadosh et al., 2010; Perini et al., 2012; Romei et al., 2016; Mazzoni et al., 2017; Ambrus et al, 2017).

However, it is not known whether these state-dependent effects are modulated by stimulation intensity and timing of stimulation. This is an important question from a methodological perspective, as the nature of behavioral effects in conventional ‘‘virtual lesion” TMS paradigms has been shown to be strictly intensity- and timing-dependent. TMS-induced visual masking studies have shown that the intensity level needed to impair the detection of external stimuli is higher than the intensity needed to induce phosphenes. Specifically impairments are generally obtained when suprathreshold TMS is applied over the early visual cortex 80–120 ms after

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Abbreviations: PT, phosphene threshold; TMS, transcranial magnetic stimulation.

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target onset (see e.g. Kammer et al., 2005; Kammer, 2007; de Graaf et al., 2014, for review). While suprathreshold TMS intensities are needed to mask the detection of visual stimuli, qualitatively different types of effects can occur when TMS intensity is below the PT: in this circumstance, TMS can facilitate the detection of near-threshold stimuli (e.g., Abrahamyan et al., 2011, 2015; Schwarzkopf et al., 2011). Such effects occur within the classic TMS-masking time window (Abrahamyan et al., 2011, 2015) and have been explained in terms of low-intensity TMS being akin to adding low levels of random noise to a weak signal; this can push subthreshold activations above perceptual threshold. At higher intensities this does not occur because, when TMS intensity is increased, higher levels of noise are being added and this downs out the signal, leading to behavioral impairment (see e.g. Miniussi et al., 2013; Romei et al., 2016).

Given that behavioral state-dependent TMS effects generally involve facilitation of weak signals (such as enhancement of adapted or non-primed targets for which baseline performance level is relatively low), one might expect these effects to also depend on stimulation intensity, as suggested by the above "noise" explanation of TMS effects. We examined this question using a priming paradigm in which participants were primed to a conjunction of color and orientation, after which participants were required to detect the color of a target grating (these stimuli were adapted from the study by Silvanto et al., 2007). This is a priming variation of the McCollough effect (McCollough, 1965) in which color afterimages induced by a square-wave grating are orientation specific, indicative of the existence of color–orientation channels in the visual cortex. In TMS-priming paradigms, the common finding is that TMS behaviorally facilitates the detection of non-primed attributes while leaving primed items unaffected (e.g. Cattaneo et al., 2008, 2010a,b); the question we addressed here is whether this effect is obtained at both subthreshold and suprathreshold TMS intensities. In Experiment 1, single-pulse TMS was applied at *target onset* (as generally done in TMS-priming studies; e.g. Cattaneo et al., 2009, 2010a, b) over the early visual cortex or Vertex (baseline) at an intensity of either 80% or 120% of the individual's PT. At this time point, information regarding the target stimulus has not yet reached V1/V2 and the TMS effect therefore reflects an interaction between the TMS pulse and residual activation induced by the prime stimulus. The results showed that the state-dependent TMS effect was not modulated by the TMS intensity; with both subthreshold and suprathreshold TMS intensities, TMS facilitated targets incongruent with the prime, as generally found in TMS-priming studies (e.g. Cattaneo et al., 2009, 2010a,b). In Experiment 2, TMS was applied at 100 ms after target onset (classic TMS-masking time window; e.g. Kammer et al., 2005; de Graaf et al., 2014); at this time point V1/V2 is responding to visual input. Only suprathreshold PT facilitated the detection of nonprimed stimuli, with no effect on performance with subthreshold PT intensity. Thus whether TMS was applied before or after target-related information had reached the visual cortex determined the intensity dependency of these results.

EXPERIMENTAL PROCEDURES

Experiment 1

Participants. Twenty-two participants (10 M, mean age = 24 years) with normal or corrected-to-normal vision volunteered to participate in the experiment; of these 17 could perceive TMS-induced phosphenes. All subjects provided informed consent before participating in the study, which had been approved by the local ethics committee. All participants were naive to the aims of the study and were treated according to the guidelines of the Declaration of Helsinki. Prior to participation, each participant was screened for contraindications to TMS.

Stimuli and psychophysical task. Stimuli were presented at a viewing distance of 60 cm on a 16-inch monitor with a display resolution of 1920 × 1080. Stimuli and task were presented using E-prime software (Psychology Software Tools Inc., Pittsburgh, PA, USA). Both stimulus prime and stimulus target consisted of diagonal lines at 45° to the left or right of vertical such that stimuli were made of a series of stripes. These stripes were either black and green (CIE $x = 0.30$, $y = 0.60$, luminance 20 cd/m2) or black and red (CIE $x = ¼ 0.60$, $y = 0.35$, luminance 20 cd/m2) with a stripe width of 0.25° in a stimulus subtending 6° horizontally and 3° vertically (adapted from Silvanto et al., 2007's study). Therefore, four different color–orientation combinations were used, in which prime and target could have: (a) same color and same orientation ("fully congruent" trial); (b) opposite color and opposite orientation ("fully incongruent" trial); (c) same color but opposite orientation ("color congruent" trial); (d) same orientation but opposite color ("orientation congruent" trial). These congruency types appeared with equal frequency within a block. The procedure is shown in Fig. 1. Each trial started with a fixation cross presented in the middle of the display for 500 ms, followed by the presentation of the prime stimulus (appearing for 100 ms) and subsequently by a 300-ms blank screen; after that, the target stimulus appeared in the middle of the screen for 20 ms. The target stimulus was followed by a mask (remaining of the screen till participants' response) composed of black diagonals in both possible orientations and with the gaps filled with green or red with the color for each gap selected at random. A new randomly generated mask was used for each trial. When the mask was presented, participants had to indicate the color of diagonals in the stimulus target display (red or green) by pressing the corresponding key on the keyboard. Both accuracy and response speed were emphasized. Each participant underwent a total of eight experimental blocks, namely two blocks for each stimulation site (V1/V2, Vertex) and for each stimulation intensity (80%, 120% of PT, see below). Each block included 40 trials, 10 for each of the four color–orientation combinations. The order of stimulation sites and intensities was counterbalanced across participants, as well as the orientation–color combinations of the stimuli within each block. Before

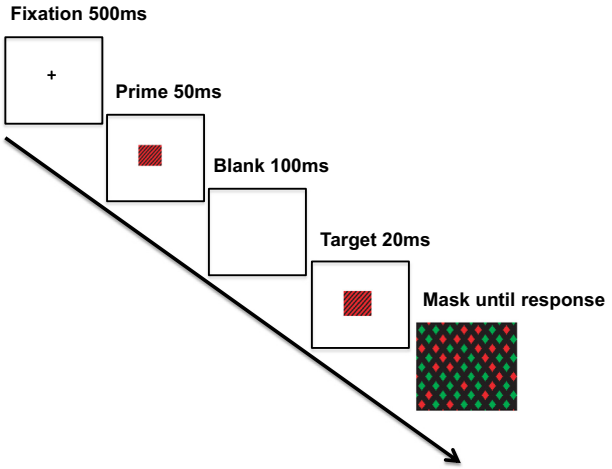

Fig. 1. Timeline of an experimental trial. On each trial, participants were presented with a prime that was either a red–black or green–black grating, tilted either clockwise or counterclockwise. This was followed by a target which could be either fully congruent with the prime (i.e. the same stimulus), fully incongruent (i.e. both color and orientation differed), or partially congruent (either color or orientation matched the prime). Participants had to indicate the color of diagonals of the stimulus target (red or green). In this figure, a fully congruent trial (i.e. prime and target matched for both color and orientation) is depicted. In Experiment 1, Single-pulse TMS was delivered at *target onset* over either V1/V2 region or over the Vertex (baseline) at either 80% or 120% of participant's PT. In Experiment 2, single pulse of TMS was applied at 100 ms after stimulus onset. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the main experiment, participants underwent a block of practice with no TMS (20 trials).

Transcranial magnetic stimulation. TMS was administered using a 70-mm biphasic figure-of-eight coil connected to a Nexstim stimulator (Nexstim Ltd., Helsinki, Finland). The site of stimulation (V1/V2 region) was localized functionally in each participant by means of phosphenes search (see Walsh and Pascual-Leone, 2003, for a detailed description, see e.g. Campana et al., 2002, 2006 for examples). In this method, the coil is initially positioned 2 cm above the inion and its location is subsequently adjusted until foveal phosphenes (overlapping with the target location in the main experiment) are induced. PTs were measured, after dark adaptation, for each participant using a modified binary search algorithm (Tyrrell and Owens, 1998; Thilo et al., 2004). Four participants could not perceive phosphenes and therefore took no further part in the study. The mean PT was 57.5% of the maximum stimulator output. Both phosphene site localization and the main experiment took place in a darkened room. In the main experiment, participants were stimulated at 80% and 120% of their PT. On each trial, a single-pulse TMS was delivered over V1/V2 or Vertex (baseline), concurrently with the onset of the target stimulus, similar to previous TMS-priming studies (Cattaneo et al., 2008, 2010c; Cattaneo, 2010). Vertex was identified as the halfway location between the inion and the nasion and at an equal distance from the left and right inter-trachial notches and was used as control site. We included two Vertex conditions, one in which TMS was applied at 80% and the other with 120%, so that the level of possible auditory artifacts was controlled. During the stimulation, the coil was held with the handle pointing medial to lateral away from the midline and kept in place by the experimenter.

Experiment 2

In Experiment 1, TMS was applied at target onset on each trial, as done in previous TMS-priming studies (e.g. Cattaneo et al., 2008, 2010a,b). In Experiment 2, TMS was applied at 100 ms after target onset, as masking effects with V1/V2 TMS are observed within this time window (see e.g. Kammer et al., 2005, for review).

Participants. 33 participants (12 M, mean age = 23.06 years) with normal or corrected-to-normal vision volunteered to participate in the experiment, of whom 18 perceived TMS-induced phosphenes. All subjects provided informed consent before participating in the study, which had been approved by the local ethics committee. All participants were naive to the aims of the study and were treated according to the guidelines of the Declaration of Helsinki. Prior to participation, each participant was screened for contraindications to TMS.

Stimuli and psychophysical task. This was carried out as in Experiment 1.

Transcranial magnetic stimulation. TMS was carried out as in Experiment 1, with the following differences. TMS was administered using a 70-mm biphasic figure-of-eight coil connected to a Magstim stimulator (Magstim, Wales), rather than using a Nexstim stimulator, as in Experiment 1. On each trial, a single-pulse TMS was delivered over V1/V2 or Vertex (baseline), 100 ms after target onset (rather than at target onset, as in Experiment 1). For the low TMS intensity, we used an intensity of 90% rather than 80%, as the former is most effective in facilitating performance in conventional "virtual lesion" paradigms when applied at this time window (e.g. Abrahamyan et al., 2011). Otherwise the experiment was carried out as Experiment 1 (including localization and behavioral paradigm).

RESULTS

Statistical analyses were carried out on median reaction times of correct responses and accuracy. Only participants who perceived phosphenes were included (as this was a requirement for determining subthreshold and suprathreshold TMS intensities). Data were analyzed as a function of congruency between the prime stimulus and the target – there were thus 4 trial types: congruent trials (i.e., prime and target are identical); incongruent trials (i.e., prime and target differ in both color and orientation); color congruent trials (i.e., target color but not orientation matches the prime) and

orientation congruent (i.e., orientation but not color matches the prime). In the main ANOVA, we examined the impact of TMS on the different trial types as function of prime-target congruency, with TMS intensity (subthreshold/suprathreshold), TMS site (V1/V2/ Vertex) and prime-target congruency (fully congruent, fully incongruent, color congruent, orientation congruent).

Experiment 1

Among the 17 participants who perceived phosphenes, one participant was excluded due to low accuracy (more 2 SD below the participants' mean accuracy), therefore the statistical analyses were carried out on 16 participants. Results for median RTs of correct responses are shown in Fig. 2 (panel A for subthreshold TMS and panel B for suprathreshold TMS). A 2 x 2 x 4 ANOVA with TMS site (V1/V2, Vertex), stimulation intensity (80% of PT, 120% of PT) and prime-target congruency (fully congruent, fully incongruent, color congruent, orientation congruent) was carried out. The analysis showed a significant main effect of prime-target congruency ($F_{(3,45)} = 5.17$, $p = 0.004$, $\eta_p^2 = 0.26$) and a significant interaction TMS site by congruency ($F_{(3,45)} = 4.54$, $p = 0.007$, $\eta_p^2 = 0.23$). The main effect of stimulation intensity was not significant ($F_{(1,15)} < 1$, $p = 0.85$, $\eta_p^2 = 0.00$). No other interactions reached significance (all *ps* > 0.28); most importantly for the present study, there was no significant interaction involving stimulation intensity. The significant interaction TMS site by congruency was analyzed by looking at the simple main effect of TMS site (Vertex vs. V1/V2) within each prime-target congruency condition. The analyses revealed a significant main effect of TMS in the fully incongruent condition ($F_{(1,15)} = 5.31$, $p = 0.036$, $\eta_p^2 = 0.26$), with TMS over V1/V2 facilitating performance compared to TMS over Vertex. In turn, TMS did not affect response latencies in either the fully congruent condition ($F_{(1,15)} = 2.14$, $p = 0.16$, $\eta_p^2 = 0.13$), the color congruent condition ($F_{(1,15)} = 2.48$, $p = 0.14$, $\eta_p^2 = 0.14$), and the orientation congruent condition ($F_{(1,15} < 1$, $p = 0.75$, $\eta_p^2 = 0.01$). These analyses thus show that TMS over V1/V2 selectively facilitated the fully incongruent trials, independently of TMS intensity.

For accuracy scores, a $2 \times 2 \times 4$ ANOVA with TMS site (V1/V2, Vertex), stimulation intensity (80% of PT, 120% of PT) and prime-target congruency (fully congruent, fully incongruent, color congruent, orientation congruent) revealed a significant main effect of congruency ($F_{(3,45)} = 3.81$, $p = 0.016$; $\eta_p^2 = 0.20$).

Neither the main effect of TMS site ($F_{(1,15)} = 3.22$, $p = 0.09$; $\eta_p^2 = 0.18$) nor the main effect of stimulation intensity ($F_{(1,15)} = 2.26$, $p = 0.15$; $\eta_p^2 = 0.13$) reached significance. None of the interactions reached significance (all *ps* > 0.34). Mean accuracies collapsed across TMS conditions were 85% for fully congruent trials, 75% for fully incongruent trials, 85% for color congruent trials, and 82% for orientation congruent trials.

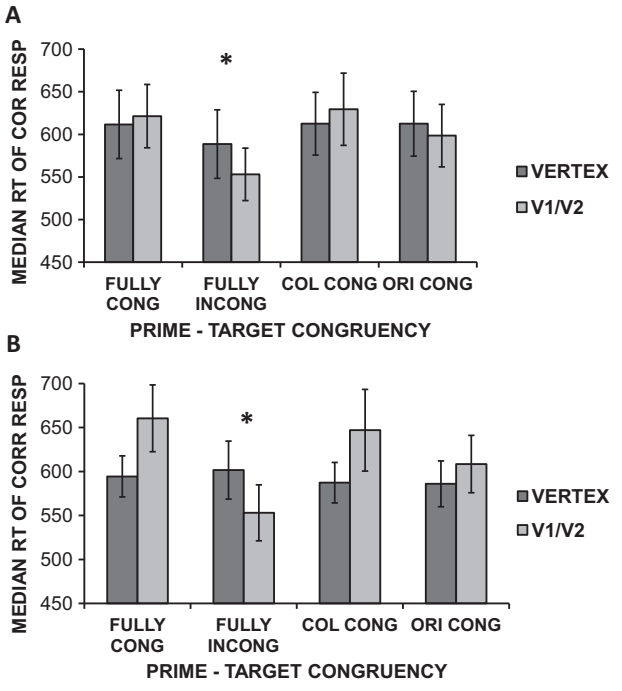

Fig. 2. Mean ($n = 16$) of the median RTs (milliseconds) of correct responses as a function of TMS site and prime-target congruency in Experiment 1 for subthreshold (panel A) and suprathreshold (panel B) TMS. In Experiment 1, TMS was applied at target onset. For both TMS intensities, V1/V2 TMS facilitated performance of fully incongruent trials (relative to Vertex TMS) while having no significant effect on either fully congruent, color congruent or orientation congruent trials. Errors bars indicate ± 1 SEM.

Experiment 2

Among the 18 participants who perceived phosphenes, one participant was excluded due to low accuracy (below 2 SD of the group's mean); therefore the statistical analysis was carried out on 17 participants.

Results for median RTs of correct responses are shown in Fig. 3 (panel A for subthreshold TMS and panel B for suprathreshold TMS). A $2 \times 2 \times 4$ ANOVA with TMS site (V1/V2, Vertex), stimulation intensity (90% of PT, 120% of PT) and prime-target congruency (fully congruent, fully incongruent, color congruent, orientation congruent) as within-subject factors revealed a significant main effect of congruency ($F_{(3,48)} = 3.04$, $p = 0.038$; $\eta_p^2 = 0.16$), a significant main effect of stimulation intensity ($F_{(1,16)} = 6.57$, $p = 0.021$; $\eta_p^2 = 0.29$) and a significant three-way interaction TMS site by stimulation intensity by congruency ($F_{(3,48)} = 7.70$, $p < 0.001$; $\eta_p^2 = 0.32$). No other main effects or interactions were significant (all *ps* > 0.19). The effects of intensity were analyzed in light of the significant three-way interaction. Post-hoc analyses showed that TMS over V1/V2 differently affected responses as a function of intensity compared to Vertex TMS only in fully incongruent trials (TMS site by intensity, $F_{(1,16)} = 7.39$, $p = 0.015$, $\eta_p^2 = 0.32$). Pairwise comparisons on the significant interaction revealed that relative to Vertex stimulation, V1/V2 TMS facilitated performance in this condition but only when applied at 120% of stimulation intensity ($t(16) = 2.63$, $p = 0.018$) whereas no effect was observed when

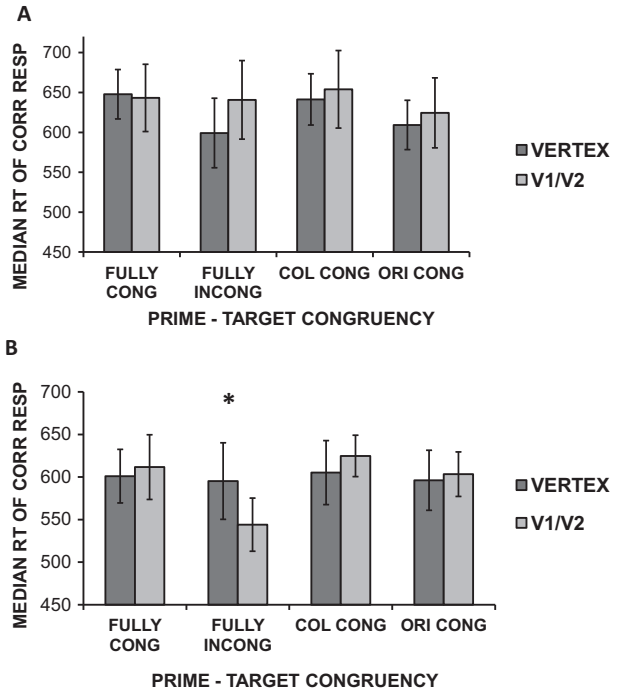

Fig. 3. Mean (n = 17) of the median RTs (milliseconds) of correct responses as a function of TMS site and prime-target congruency in Experiment 2 for subthreshold TMS (panel A) and suprathreshold TMS (panel B). In Experiment 2, TMS was applied 100 ms after target onset. With suprathreshold TMS, V1/V2 TMS facilitated performance of fully incongruent trials (relative to Vertex TMS) while having no significant effect on either fully congruent trials, color congruent or orientation congruent trials. Subthreshold TMS had no effects on any target type. Errors bars indicate ± 1 SEM.

applied at 90% of stimulation intensity $t(16) = 1.42$, $p = 0.18$). In turn, the interaction TMS site by intensity did not reach significance in either fully congruent ($F_{(1,16)} < 1$, $p = 0.65$, $\eta_p^2 = 0.01$), color congruent ($F_{(1,16)} < 1$, $p = 0.87$, $\eta_p^2 = 0.002$), or orientation congruent ($F_{(1,16)} < 1$, $p = 0.82$, $\eta_p^2 = 0.004$) trials.

A repeated-measures 2 × 2 × 4 ANOVA on accuracy scores with TMS site (V1/V2, Vertex), stimulation intensity (90% of PT, 120% of PT) and prime-target congruency (fully congruent, fully incongruent, color congruent, orientation congruent) as within-subject factors revealed a significant main effect of congruency ($F_{(3,48)} = 9.33$, $p < 0.001$; $\eta_p^2 = 0.37$). There were no significant main effects of TMS site ($F_{(1,16)} = 0.91$, $p = 0.35$; $\eta_p^2 = 0.05$) or stimulation intensity ($F_{(1,16)} < 1$, $p = 0.84$, $\eta_p^2 = 0.003$). None of the interactions reached significance (all $ps > 0.09$). Mean accuracies collapsed across TMS conditions were 90% for fully congruent trials, 78% for fully incongruent trials, 82% for color congruent trials, and 83% for orientation congruent trials.

DISCUSSION

Our results show that the induction of state-dependent TMS effects was dependent on stimulation intensity, but only when the stimulated region was responding to target-related information at the time of the TMS pulse. Specifically, in Experiment 1 (where TMS was applied at target onset when V1/V2 is not yet responding to visual input), both sub- and suprathreshold TMS facilitated reaction times to targets incongruent with the prime while not significantly affecting fully congruent or partially congruent stimuli. This selective facilitatory effect of incongruent targets, with no effect on other stimulus types, is consistent with prior TMS-priming studies (e.g. Cattaneo et al., 2008, 2010a,b; Cattaneo, 2010). With single-pulse TMS, the effects observed in the TMS-priming paradigm tend to be on reaction times, as found here (e.g. Cattaneo et al., 2008, 2010; Cattaneo, 2010; we did not observe TMS effects on accuracy, even though a strong priming effect on accuracy was present). In Experiment 2 however, when TMS was applied within the conventional TMS-masking time window (i.e. 100 ms after target onset) at which V1/V2 is responding to visual input, a different pattern was found. Here, TMS applied at subthreshold intensity induced no statistically significant effects, whereas facilitation of incongruent items was observed with suprathreshold intensity. The lack of an effect by subthreshold intensity may reflect lower susceptibility to TMS of neurons responding to visual stimulation.

Can these results be explained in the same terms as facilitations observed in conventional TMS studies? As discussed in the Introduction, visual masking effects are generally obtained with intensities above the PT (see e.g. Kammer et al., 2005; de Graaf et al., 2014), whereas behavioral facilitations are found with intensities 80–95% of PT, and particularly when the target stimulus is close to threshold (e.g. Abrahamyan et al., 2011). These effects have been explained in terms of the addition of low vs high levels of noise (by subthreshold vs suprathreshold TMS intensities, respectively) having different outcomes. Addition of low levels of noise can facilitate target detection by pushing near-threshold stimuli beyond perceptual threshold whereas addition of high levels of noise drowns out the signal and thus impairs performance.

When explaining the present effects, one needs to additionally consider that priming modifies the amount of external stimulation required to activate neuronal populations (e.g. Kohn and Movshon, 2003; Kohn, 2007). While a ‘‘primed” representation is more readily activated by a congruent external stimulus (or by a TMS pulse), the non-primed representations are likely to be in an inhibited state and thus less excitable to stimulation. In short, an external stimulus of higher strength is needed to drive the latter neurons (e.g. Gotts et al., 2012). Given the excitability changes induced by priming, the ‘‘noise” model would predict *that the TMS intensity profiles with which impairments and facilitations are observed would be shifted*. Specifically, if a consequence of priming is a reduced excitability of neural representations tuned to stimuli *incongruent* with the prime, then a higher TMS intensity would be needed to obtain the same outcome as in the absence of priming. In the context of the ‘‘noise” explanation, one would thus expect that a higher TMS intensity (relative to what is found in conventional TMS paradigms) would be needed to induce the low levels

of noise with which behavioral facilitations of near-threshold stimuli is obtained. The prediction that follows is that TMS would facilitate non-primed targets in the suprathreshold PT condition but not in the subthreshold PT condition. In Experiment 2, our results are consistent with this – a significant facilitation of targets incongruent with the prime was observed only with suprathreshold TMS intensity. In short, suprathreshold TMS intensity is needed to induce the same behavioral effects which, without priming, are observed with subthreshold TMS – this may reflect reduced susceptibility of TMS of neural representations encoding targets incongruent with the prime which induces a shift in the intensity range of facilitatory vs disruptive effects of TMS.

In Experiment 1, however, the effects were not intensity-dependent. The key difference between Experiments 1 and 2 is that in the former, TMS is applied at target onset – thus information regarding the target stimulus had not yet reached visual cortex when TMS was applied. The situation is thus very different from Experiment 2 where TMS was applied while visual cortex was receiving stimulus-related input, and where behavioral effect reflects an interaction between the target-related activity and the TMS pulse. In Experiment 1, TMS is instead interacting with the residual activation induced by the prime, rather than with the target stimulus per se. One would thus expect a higher susceptibility for TMS than in Experiment 2, given that neurons are not strongly driven by visual input when TMS pulse is applied. As has been observed previously, TMS selectively facilitated performance of incongruent trials in which neurons have been presumably made less excitable to incoming information by the prime (Cattaneo et al., 2008, 2010a,b). The key finding was that this pattern was observed with *both* subthreshold and suprathreshold intensities, consistent with an explanation in terms of higher susceptibility to TMS compared to Experiment 2.

TMS had no statistically significant effect on partially congruent trials (i.e. when only either color or orientation of the target matched that of the prime) – only fully incongruent trials were affected. The lack of TMS effects on these trials is consistent with the idea that color–orientation contingent aftereffects reflect the operation of channels which are tuned for the combination of both features, as originally proposed by McCollough (1965). In other words, given the nature of the stimuli, performance in this task relied on mechanisms which encode both the orientation and color of the target stimulus, rather than neurons which encode either attribute separately.

How can these results be linked to neural effects of TMS? Moliadze et al. (2003) found that a single-pulse of TMS applied over cat's visual cortex produces a pattern of results that is intensity-dependent. Specifically, weak stimulation (< 50% of maximum TMS intensity) caused an early facilitation of spontaneous and visually induced activity up to 200 ms after the TMS pulse, followed by a late inhibition. In turn, higher TMS intensities increasingly evoked an early suppression of activity up to 200 ms. The early suppression of activity by higher TMS intensities has been linked to disruptive behavioral effects of TMS (e.g. Kammer et al., 2005). This is because it has been consistently shown that relatively high TMS intensities are needed to induce behavioral impairments (e.g. Kammer et al., 2005; Kammer, 2007). The early suppression of neural activity underlying behavioral effects of (high-intensity) TMS is also consistent with the timing of TMS-induced behavioral masking of visual information. Such masking effects (induced by applying TMS over V1/V2) typically occur when TMS is applied at time windows of 80–120 ms after target onset but not at later time windows (e.g., Kammer, 2007; de Graaf et al., 2014). The timing of this masking effect indicates that an early neural effect must underlie them. In short, both the timing and intensity dependency of TMS-induced visual masking suggest a link to the early suppression of neural activity reported by Moliadze et al. (2003). In contrast, lower TMS intensities (which have been shown to induce an early facilitation neural firing; Moliadze et al., 2003) can have a facilitatory impact on behavior (cf. Abrahamyan et al., 2011, 2015; Schwarzkopf et al., 2011). These facilitations occur within the same time window as TMS-induced impairments (Abrahamyan et al., 2011, 2015).

In short, facilitatory and inhibitory effects of TMS on behavior operate at distinct intensity levels – and these seem to correspond to the early period of neural facilitation or inhibition induced by low- and high-intensity TMS, respectively. With respect to the present results, the key issue is the following: state manipulations such as priming, *by changing neural excitability*, would be expected to shift the TMS intensities with which behavioral facilitations and impairments are induced. For example, priming reduces excitability of neural representations incongruent with the prime and therefore a higher TMS intensity is needed to drive neural activity than without priming. Therefore, when TMS is applied at an intensity which normally impairs behavior, for incongruent neuronal representations this intensity might fall within the intensity range in which facilitations are observed. In short, priming is perhaps akin to turning down the TMS intensity for incongruent items – thus changing suppressive high-intensity TMS to facilitatory low-intensity TMS. This explanation can also be applied to the puzzling findings of TMS behaviorally facilitating adapted neuronal representations (e.g. Silvanto et al., 2007) – as adaptation reduces neuronal excitability, high-intensity "suppressive" TMS might behave like low-intensity "facilitatory" TMS. This explanation is admittedly speculatory and the link between behavioral and neural facilitations and suppressions requires direct study.

Finally, it is worthwhile to discuss some of the methodological differences between the two studies. In Experiment 1, subthreshold stimulation was carried out with an intensity of 80% of PT, whereas 90% PT was used in Experiment 2. We used a slightly higher intensity in Experiment 2, as facilitations by TMS in standard "virtual lesion" paradigm are largest at this intensity (Abrahamyan et al., 2011). Could the difference between the subthreshold intensities in the two experiments have influenced our pattern of results? In Experiment 1, state-dependent TMS effects were observed with both

sub- and suprathreshold intensities, demonstrating a lack of modulation by intensity – it is unlikely that a different pattern of results would have been present with an intermediate stimulation intensity of 90% of PT. In Experiment 2, given that the TMS effects disappeared when intensity was reduced from 120% of PT to 90% of PT, it is unlikely that they would have been present with a further reduction of TMS intensity. A second methodological difference is that the two experiments were carried out in different laboratories. The key issue is that performance was quite well matched between the two experiments, with the mean overall accuracy of the incongruent stimuli being 75% and 78% respectively – thus the "signal strength" of the incongruent items did not differ between the experiments. Thirdly, while TMS machines by different manufactures were used in the two experiments, in both studies stimulation intensity was calibrated to PTs.

Implications for understanding mechanisms of state-dependent TMS effects

When TMS is applied during the classic masking time window, in conventional TMS paradigms (i.e. in the absence of state-dependent manipulations) *subthreshold* TMS facilitates detection of weak signals. When priming is introduced and TMS applied at the same time window, facilitations of targets incongruent with the prime are obtained with *suprathreshold* but not with subthreshold TMS. Thus brain state manipulations such as priming appear to shift the facilitatory/inhibitory ranges of TMS, by reducing neural excitability (and thus susceptibility to TMS) of neural representations incongruent with the primed items. Therefore, in a sense, inhibiting neural representations via priming or adaptation is akin to turning down the TMS intensity: higher intensity is needed to obtain the same behavioral outcome.

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