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Magnetic stimulation of visual cortex impairs perceptual learning

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ABSTRACT

The ability to learn and process visual stimuli more efficiently is important for survival. Previous neuroimaging studies have shown that perceptual learning on a shape identification task differently modulates activity in both frontal-parietal cortical regions and visual cortex (Sigman et al., 2005; Lewis et al., 2009). Specifically, fronto-parietal regions (i.e. intra parietal sulcus, pIPS) became less activated for trained as compared to untrained stimuli, while visual regions (i.e. V2d/V3 and LO) exhibited higher activation for familiar shape. Here, after the intensive training, we employed transcranial magnetic stimulation over both visual occipital and parietal regions, previously shown to be modulated, to investigate their causal role in learning the shape identification task. We report that interference with V2d/V3 and LO increased reaction times to learned stimuli as compared to pIPS and Sham control condition. Moreover, the impairment observed after stimulation over the two visual regions was positive correlated. These results strongly support the causal role of the visual network in the control of the perceptual learning.

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1. Introduction

Observers can voluntarily attend to a location in the visual field, and subsequent stimuli at that location will be recognized more accurately and rapidly (Posner, 1980). Furthermore, visual perception can be improved through specific training, a phenomenon called Visual Perceptual Learning (VPL) (Gibson, 1963). VPL is one of the strongest examples of plasticity in the adult brain and a core feature of visual cognition. VPL might depend on attention (Ahissar and Hochstein, 1993) and allows for more efficient responses to environmental stimuli.

Despite several decades of investigations, neuronal mechanisms of VPL remain debated (Gilbert et al., 2001; Sasaki et al., 2010; Shibata et al., 2014). Neurophysiologic and neuroimaging studies indicated that VPL induces changes of neural activity in visual cortex (Crist et al., 2001; Schoups et al., 2001; Schwartz et al., 2002; Furmanski et al., 2004) and in higher-order brain regions (Chowdhury and DeAngelis, 2008; Law and Gold, 2009) involved in the control of spatial attention (Sigman et al., 2005; Lewis et al., 2009), as well as in their interaction (Liu et al., 2010; Lewis et al., 2009).

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It has been suggested that VPL shifts the critical locus of processing for learned stimuli from higher-order control regions, early on during training, to visual cortex after learning is completed. For example, in human observers, intensive training on a shape orientation identification task causes a shift in the pattern of activation, measured with blood oxygenation level dependent (BOLD) signals in functional magnetic resonance imaging (fMRI), between frontal-parietal regions (so-called dorsal attention network, DAN) and occipital visual regions (Sigman et al., 2005; Lewis et al., 2009). Specifically, in our study (Lewis et al., 2009), frontal and parietal regions (e.g. posterior intra-parietal sulcus, pIPS) known to be involved in the control of visuospatial attention were more strongly active for novel (untrained) stimuli, and attenuated their response for familiar (trained) stimuli. In contrast, occipital visual regions responded more strongly to trained than untrained stimuli. Moreover, learning-induced modulation of visual cortex activity was topographically selective. In fact, since the task required observers to discriminate stimuli at a peripheral location in the left lower quadrant, corresponding activity modulation was recorded in right dorsal visual cortex. In particular, higher activation was observed in both right V2d/V3 and lateral occipital region (LO; Lewis et al., 2009). Finally, response modulations were behaviorally relevant: subjects with higher sensitivity to trained shapes showed stronger modulation in the trained quadrant of visual cortex. Overall these findings support the hypothesis that whereas higher-order frontal and parietal regions are more important early

on in training presumably for directing visuospatial attention and selecting unfamiliar stimuli, attention control becomes less important later on as ‘templates’ of learned shapes are consolidated in visual cortex.

While the above studies have provided invaluable information on the neural mechanisms of VPL, there is actually scarce direct evidence that the learning specific visual regions (i.e. V2d/V3 and LO) are actually mediating perceptual learning. Here we used repetitive TMS (rTMS) in healthy volunteers to test with a causal approach hypotheses that are based on our fMRI findings (i.e. correlative), and specifically the crucial role of visual cortices in shape identification task. Using the same visual paradigm of our mentioned studies (Lewis et al., 2009; Baldassarre et al., 2012), after the intensive training, rTMS was employed to interfere with the activity in right V2d/V3, LO, or pIPS. If VPL is completed and the template of learned shape is formed in the corresponding (i.e. right) visual regions, then we predict that the inactivation of parietal cortex (i.e. pIPS) will not affect the behavioral performance. On the contrary, we expect impairment in detecting familiar shapes after inactivation of both visual cortices (i.e. V2d/V3 and LO). Furthermore, since our previous neuroimaging experiments showed a similar learning-related fMRI modulation for V2d/V3 and LO, we predict a similar impairment in such visual regions.

2. Materials and methods

2.1 Subjects and stimuli

16 right-handed volunteers (age range: 20–30 yrs. old; 8 females) participated in this experiment. A preliminary self-reported questionnaire assessed that they did not present previous psychiatric or neurological history. Participants gave written consent according to the Institutional Review Board and Ethics Committee of the University of Chieti. The computer monitor was placed in front of them at a distance of about 60 cm.

Subjects were trained with daily sessions to attend to the lower left visual quadrant and find the target shape among the distracters while maintaining central fixation. The stimulus array comprised 12 Ts arranged in an annulus of low eccentricity (i.e. 5° radius) and was displayed across the 4 visual quadrants. Of note, with such low eccentricity in our previous study (Lewis et al., 2009) we did not observed significant eye movements. On each trial subjects fixated a central spot for 200 ms (fixation), after which the target shape (an inverted T) was presented at the center of the screen for 2000 ms (target presentation); finally, an array of

12 stimuli, differently oriented Ts (distracters) with or without an inverted T (target), was briefly flashed for 150 ms (array presentation). The target shape appeared randomly in 1 of 3 locations in the left lower (trained) visual quadrant, and never in the three untrained-quadrants. The target shape appeared randomly in 1 of 3 locations in the left lower (trained) visual quadrant, and never in the other three untrained quadrants. Subjects attended to the lower left visual quadrant and indicated the presence or absence of the target shape visual quadrant by pressing a left/right mouse button with their right hand (Fig. 1a). Each block consisted of 45 trials, 36 (80%) that contained the target and 9 (20%) that did not. Training lasted one week, and an average of 100 practice blocks were necessary to reach a threshold of 80% accuracy in at least 12 consecutive blocks of trials (see Fig. 1b for a representative psychophysical curve). Of note, the accuracy of each block was weighted with the rate of false positive (Sigman and Gilbert, 2000; Sigman et al., 2005; Lewis et al., 2009).

When subjects reached criterion, they were asked to perform three blocks of the same task during each TMS condition (i.e. V2d/V3, LO, pIPS, and Sham). Presentation timing was triggered by the TMS train (see below), and the four TMS conditions were run in a counterbalanced order across subjects, who were instructed to respond as accurately and quickly as possible. Reaction times and the accuracy of the response were recorded for behavioral analyzes. Notably, none of the subjects reported discomfort or pain during each stimulation site.

2.2 Procedures for rTMS and identification of target scalp regions

TMS stimulation was delivered through a focal, figure eight coil, connected with a standard Mag-Stim Rapid 2 stimulator (maximum output 2.2 T). Individual resting excitability threshold for right motor cortex stimulation was preliminarily determined following standardized procedure (Rossini et al., 1994). The rTMS train (i.e. 3 pulses) was delivered simultaneously to the central spot ~2 s before the stimuli array with the following parameters: 150 ms duration, 20-Hz frequency, and intensity set at 100% of the individual motor threshold. The parameters are consistent with published safety guidelines for TMS stimulation (Rossi et al., 2009). Of note, previous studies have shown that such stimulation has effect for at least 2 s, thus affecting target processing (Capotosto et al., 2009, 2012a, 2012b).

All participants performed three active rTMS (i.e. V2d/V3, LO, and pIPS) and one inactive TMS (i.e. Sham) conditions corresponding to each stimulation site, applied in different blocks and counterbalanced across subjects. In the “Sham” condition, a pseudo

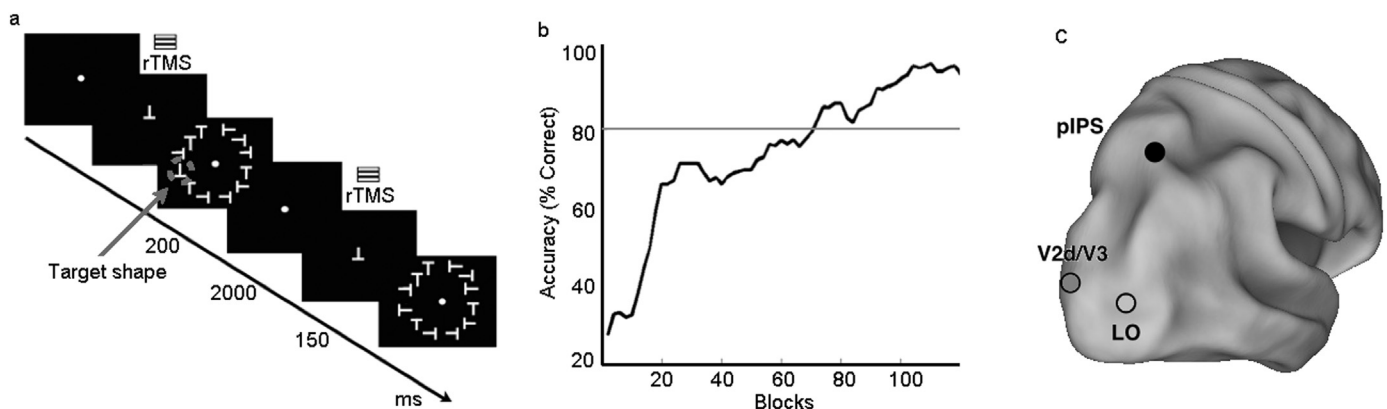


Fig. 1. a) Example of the display sequence in the perceptual learning task. b) Example of a single subject's learning curve. Each block contains 45 trials. The gray line indicates a learning threshold of 80% accuracy in 10 consecutive trial blocks. c) Inflated view of left hemisphere atlas brain with regions of attention and visual networks as in previous work of Lewis et al. (2009). Regions with coordinates are stimulated with rTMS in this experiment and are as follows: right LO: 38, –87, 7 (x, y, z); right V2d/V3: 17, –97, 16; right pIPS: 32, –60, 51.

rTMS was delivered at scalp vertex; it was ineffective due to the reversed position of the coil with respect to the scalp surface (i.e. the magnetic flux was dispersed to air). The location of right V2d/V3, LO, and pIPS was automatically identified on the subject's scalp using the SofTactic navigator system (E.M.S. Italy, www.emsmedical.net), which permits to compute an estimated volume of head MRIs in subjects for whom MRIs are unavailable. The estimated MRIs are calculated with a warping procedure, by acting on a template MRI volume on the basis of a set of points digitized from the subjects scalp. Specifically, it uses a set of digitized skull landmarks (nasion,inion, and two pre-auricular points), and about 40 scalp points entered with a Fastrak Polhemus digitizer system (Polhemus), and an averaged stereotaxic MRI atlas brain in Talairach space (Talairach and Tournoux, 1988). The average Talairach coordinates in the SofTactic navigator system were transformed through a linear transformation to each individual subject's scalp. Such method has an error of about 5 mm over a method in which each subject's own MRI is used for localization (Carducci and Brusco, 2012), thus presenting an error lower than the TMS spatial resolution itself (i.e. 1 cm). This individualized head model preserves the anatomical scalp–brain correlates of a mean MR template, providing an accurate set of estimated MRI data, specific for the subject under examination. This approach has been widely and successfully utilized in previous rTMS studies by our and several other groups using a number of subjects comparable with the present study and investigating disparate cognitive domains (Capotosto et al., 2009; Capotosto et al., 2014; Sestieri et al., 2013; Passeri et al., 2015; Candidi et al., 2011). A mechanical arm maintained the handle of the coil angled at about 45° away from the midline and the center of the coil wings was positioned on the scalp, to deliver the maximum rTMS intensity over each site (individual peak of activation). The coordinates of the different cortical regions were based on our previous

perceptual learning study (He et al., 2007) and were as follows: right LO: 38, –87, 7 (x, y, z); right V2d/V3: 17, –97, 16; right pIPS: 32, –60, 51 (Fig. 2a). The chosen coordinates correspond respectively to the epicenters of the one core region of the attention network (pIPS), and two core regions of the visual network (V2d/V3, and LO). Of note, based on this probabilistic approach, the scalp–brain distance for these sites were as follows: 1) right LO (38; –87; 7) $d=17.90$ mm; 2) right V2d/V3 (17; –97; 16) $d=17.00$ mm; 3) right pIPS (32; –60; 51) $d=16.50$ mm. Importantly, rTMS was delivered ~2 s before stimulus array so that the effect was not predominantly on stimulus-evoked activity, which could have been 'masked' by the magnetic stimulation, but either on preparatory or ongoing activity.

2.3 Statistical analyzes

Statistical analyzes were conducted using within-subject ANOVAs for repeated measures. Mauchly's test was used to evaluate sphericity assumption, Green-house-Geisser procedure was used to correct degrees of freedom, and Duncan tests for post-hoc comparisons ($p < 0.05$).

The main statistical design was computed to investigate the causal role of the two visual nodes (i.e. V2d/V3, and LO) in the perceptual learning task. To this aim we carried out an Anova using reaction times (RTs) or percentage of correct responses with Condition (V2dV3, LO, pIPS and Sham) as within-subject factors. To confirm the hypothesis that the two visual nodes are part of a network, we computed a correlation analysis (Pearson test, $p < 0.05$) between reaction times in the three TMS active conditions after subtracting the reaction times in the Sham condition. Of note, for all analyzes were used only RTs of correct responses.

Several control analyzes were computed. To verify that behavioral deficits induced by rTMS did not reflect a cumulative effect,

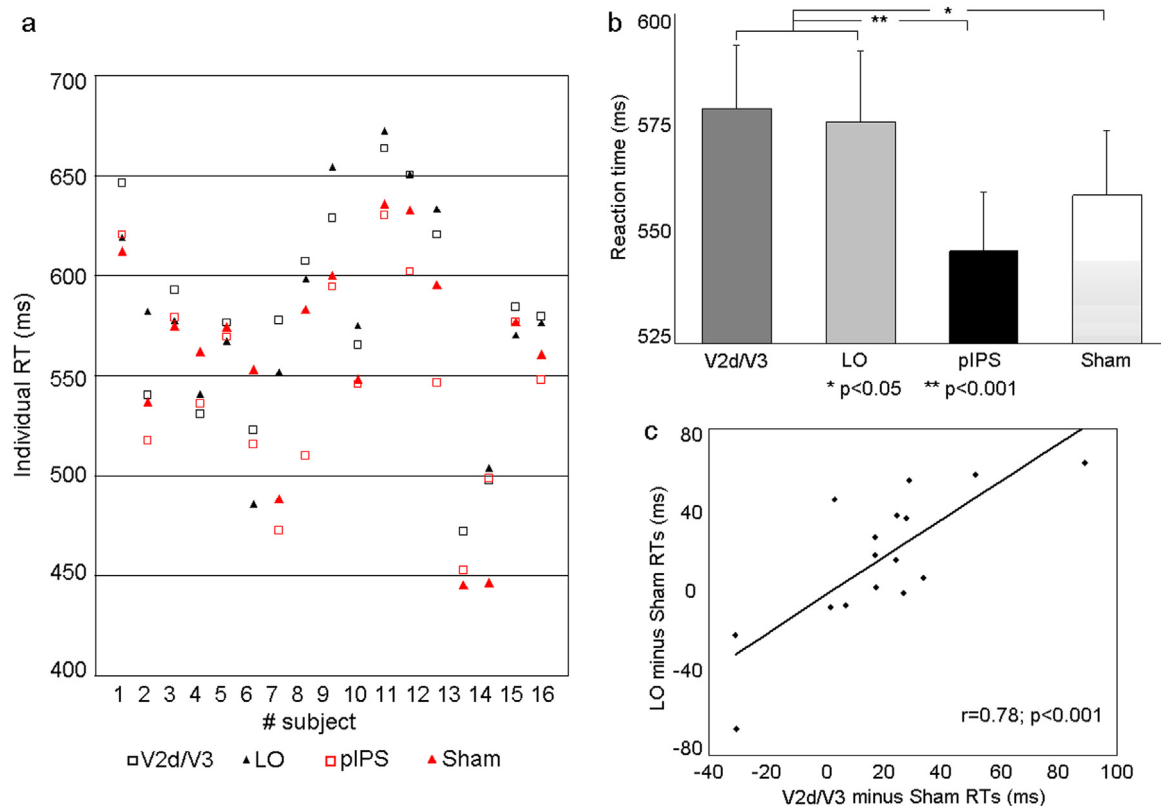


Fig. 2. a) Individual reaction time for each rTMS Condition (V2d/V3, LO, pIPS, Sham). b) Group means (\pm standard error, SE) of the reaction time (ms) for the four rTMS Conditions (V2d/V3, LO, pIPS, Sham). Duncan post-hoc tests: one asterisk ($p < 0.05$), or two asterisks ($p < 0.001$). c) Scatter-plot showing the (positive) linear correlation between reaction times in the two visual nodes (i.e. LO and V2d/V3) after subtracting the RTs of the Sham condition.

we computed the same ANOVA with Condition (V2dV3, LO, pIPS and Sham) and Run (first, second, third) as within-subject factors.

3. Results

3.1 Main analyzes

The results clearly indicated a slowing of response time (RT) during V2d/V3 and LO stimulation as compared to Sham and pIPS stimulation (Fig. 2a and Fig. 2b). This was confirmed by an ANOVA on RTs that showed a main effect of Condition ($F_{3,45}=7.23$ $p < 0.0005$; $\eta^2_{\text{partial}}=0.32$; statistical power=0.97) with slower RTs after both V2d/V3 (580 ms \pm 58 SD) and LO (577 ms \pm 62 SD) as compared to pIPS (548 ms \pm 52 SD; $p < 0.001$) and Sham (560 ms \pm 57 SD; $p < 0.05$). Importantly, no difference were observed between RTs after the two visual regions (i.e. V2d/V3 and LO; $p=0.71$) and between RTs after the active (pIPS) and inactive (Sham) control conditions ($p=0.12$). Of note, in all TMS conditions the behavioral data were normally distributed (Lilliefors test > 0.15). Finally, the same statistical design using Accuracy did not provide any statistically significant difference across conditions. In Table 1 are reported the % of accuracy and the number of false positives (fp) for all TMS conditions with the relative statistical p values ($p > 0.1$).

Interestingly, the behavioral impairment produced by V2d/V3 stimulation (measured as RTs [V2d/V3-Sham]) was positively correlated across subjects to the impairment produced by LO stimulation (RTs[LO-Sham]) ($r=0.78$; $p < 0.001$) (Fig. 2c). Conversely, the effect of rTMS over pIPS was not correlated neither with the interference over V2d/V3 ($p=0.35$) nor with LO ($p=0.25$).

3.2 Control analyzes

To support our main results several control analyzes were carried out. First, we investigated whether the TMS effect on RT was similar for Hits (i.e. presence of the target) and Correct rejection (CR, i.e. absence of the target). To this aim we computed an Anova using reaction times (RTs) with Condition (V2dV3, LO, pIPS and Sham) and Trials (Hits, CR) as within-subject factors. Results confirmed the main effect of Condition as reported in the main statistical design. Moreover, we reported a main effect of Trials ($F_{1,15}=169.87$; $p < .0001$; $\eta^2_{\text{partial}}=0.91$; statistical power=1) showing that the RTs for the CR were significantly slower compared to RTs of the Hits. Interestingly, we did not observe a statistical interaction between Condition and Trials ($p=0.5$) indicating that TMS equally affects behavioral response to both present and absent target. This result provides further evidence that the TMS effects on targeted visual areas are causally linked to the present training.

Second, to test whether TMS equally affects fast and slow responses, we split the whole set of trials of each TMS condition in four parts (i.e. quartiles) from faster to slower responses, and we carried out an Anova using reaction times (RTs) with Condition (V2dV3, LO, pIPS and Sham) and Quartiles (first, second, third, fourth) as within-subject factors. Of note, we did not include in this analysis the Correct Rejections which exhibited longer RTs, as

compared to Hits, as reported above. Results showed the two main effects of Condition (as in the main analysis) and Quartiles ($F_{3,45}=524.42$; $p < 0.001$; $\eta^2_{\text{partial}}=0.97$; statistical power=1). Importantly we did not observe an interaction between Condition and Quartiles ($p=0.67$) thus suggesting that TMS equally affects faster and slower responses.

Third, to assess whether fast and slower learners showed a different pattern of TMS interference, we carried out an Anova where RTs were used as dependent variable and the factors were Group (faster, slower) and Condition (V2dV3, LO, pIPS and Sham). Results did not show the main effect of Group ($p=0.6$) neither the interaction between Group and Condition ($p=0.08$). Moreover, as further control we correlated the number of blocks to reach the criterion with the reaction times in the three TMS active conditions after subtracting the reaction times in the Sham condition. Noteworthy, none of the correlations showed significant result ($p > 0.13$), corroborating the idea that TMS affects slow and fast learners in a comparable way.

Finally, to verify that the behavioral deficits induced by rTMS in parietal and visual regions did not reflect a cumulative effect building up over many trials, but actually reflected interference with preparatory processes on a trial-by-trial basis, we computed an ANOVA with Condition (V2dV3, LO, pIPS and Sham) and Run (first, second, third) as within-subject factors. We checked whether the size of the deficit differed in the first, second, third block of trials, and found no difference.

3.3 Control experiment

To test the causal specificity of the visual cortex only in the representation of perceptually learned visual stimuli, and not with simple visual processing of any object, a new group of subjects ($N=10$, age range: 22–30 yrs. old; 5 females) were asked to perform a new simple visual task. To increase the low accuracy observed at the beginning of the training, in this control experiment no distracters were presented and only the target shape appeared randomly in 1 of 3 locations in the lower left visual quadrant. Subjects attended to this visual quadrant and discriminated the shape of the target (rotated “T”, 80% of total trials, and canonical “T”, 20% of total trials) by pressing a left/right mouse button with their right hand (Sup Fig. 1a). The timing as well as the TMS protocol (150 ms duration, 20-Hz frequency, and intensity set at 100% of the individual motor threshold) was the same of the main experiment. All subjects performed the task during the same four TMS conditions (i.e. V2/V3, LO, IPS and Sham), that were run in a counterbalanced order across subjects, who were instructed to respond as accurately and quickly as possible. Results clearly showed that interference with V2d/V3 and LO did not increase reaction times to simple visual stimuli as compared to pIPS and Sham control conditions (Sup Fig. 1b). This was confirmed by an ANOVA on RTs that showed the lack of the main effect Condition ($F_{3,27}=0.13$ $p=0.94$, $\eta^2_{\text{partial}}=0.01$; statistical power=0.07) with similar RTs after both V2d/V3 (453 ms \pm 39 SD) and LO (457 ms \pm 52 SD) as compared to pIPS (452 ms \pm 51 SD) and Sham (456 ms \pm 36 SD) (Sup Fig. 1c). Notably the accuracy was high in all TMS conditions (V2dV3 96.3% \pm 2.5 SD; LO 95.1% \pm 3.5 SD; IPS 96.7% \pm 2.3 SD; Sham 96.3% \pm 3.1 SD), and no statistically significant difference was observed across conditions. These results support our main conclusion that V2/V3 and LO are causally engaged in the representation of perceptually learned visual stimuli and not in general simple visual processing.

4. Discussion

We used a causal approach to compare the role of two different visual regions (i.e. V2d/V3 and LO), within the right dorsal visual

Table 1
% of accuracy and the number of false positive (fp) for all TMS conditions with the relative statistical p values.

	V2dV3	LO	pIPS	sham	p value
Acc(%)+SD	92.6+3.8	92.9+3.7	92.7+3.1	91.9+3.8	0.59
# fp+SD	2.9+2.5	3.7+3.3	4.4+3.6	3.8+3.7	0.13

network, and an attention region (pIPS), in the identification of visual shapes learned through Visual Perceptual Learning (VPL). For the first time we show that, following VPL, only activity in topographically appropriate visual regions is causally involved in identifying learned shapes. These findings support the notion that the visual network has a causal role in the control of the perceptual learning.

The present shape identification task represents an entirely new experience for our subjects, and required the development of a new set of stimulus-response associations. Importantly, during training, observers had to: (i) attend to the left lower quadrant and maintain spatial attention; (ii) filter unattended information from the distracters; (iii) develop a perceptual template of the target shape. At the end of the training subjects were able to discriminate the presence/absence of the target shape. Therefore, since response to both Hits and Correct Rejections trials were similarly impaired by TMS over visual regions, our results seem to indicate that the training was affected as a whole. Psychophysical and neuroimaging evidence indicates that while attention is necessary early on in training, after visual expertise is acquired attention is less important. This idea is in line with the strong recruitment of the dorsal fronto-parietal attention network (Corbetta and Shulman, 2002) when subjects attend and identify novel shapes (Sigman et al., 2005; Lewis et al., 2009), and the relative attenuation of activity in these higher order control regions for familiar shapes even when subjects have to pay attention to the same spatial locations (Lewis et al., 2009). In contrast, topographically specific regions of visual cortex (i.e. V2d/V3 and LO) increase their specific activity to the learned shape after the VPL (Sigman and Gilbert 2000; Lewis et al., 2009).

It can be noted that TMS on a specific area may induce not only local but also distant effects on the activity of large-scale brain networks (Eldaief et al., 2011; Cocchi et al., 2015; Andoh et al., 2015) and subsequent behavioral impairment (Ruff et al., 2006). Accordingly, since IPS is hierarchically higher than the targeted visual areas and exhibits a large number of connections, its stimulation may affect both regions functionally and anatomically connected within the DAN as well as the low-level visual areas top-down controlled by IPS during visuo-spatial attention tasks (Bressler et al., 2008). Interestingly, here we did not observe effects on behavioral performance when IPS was stimulated, thus indicating that only the targeted visual areas are causally involved in the discrimination of the present learned shape. Moreover, since several previous findings showed a behavioral impairment after IPS stimulation during attention tasks (Capotosto et al., 2009, 2012a, 2013, 2015), it is unlikely that the present null result for IPS is due to its resiliency to TMS. Furthermore, it can be speculated that the TMS effects on the targeted visual areas should be extended only within the visual network since it exhibits high local efficiency (i.e. high internal connection) and low participations coefficient (i.e. low external connections) (Power et al., 2011).

Our results do not rule out that interference with these targeted regions may also affect different visual learning, but clearly show that on this particular shape identification task, once VPL is completed, disruption of activity in visual regions impairs behavioral performance. This result is consistent with the interpretation that the processing of learned shapes involves 'template' activity in visual cortex. At the same time, our findings suggest that, upon training achievement, the processing of the familiar shape requires less involvement of attention operations mediated by pIPS, as part of the fronto-parietal network. This is in line with previous fMRI studies showing that the attention network was more activated for untrained as compared to trained shape (Sigman et al., 2005; Lewis et al., 2009), reflecting a higher degree of attention engagement, similar to that occurring during early training. Of note, the performance to novel shape was reported to

be under the chance level (Lewis et al., 2009). Similarly, in the current study the initial accuracy was about 30% and did not allow to causally investigate the role of both visual and parietal regions at the beginning of the training. Nonetheless, the present control experiment ruled out the possibility that the TMS effects over the visual cortex (V2/V3 and LO) reflect interference with simple visual processing, but are specific for the representation of perceptually learned visual stimuli. It may be argued that the lack of the TMS effect in the control experiment may be due to the lower complexity, compared to the main task, as showed by the reaction time difference between the stimuli of the two experiments (roughly 100 ms). Nonetheless, the present control analysis testing whether TMS equally affects fast and slow responses did not reveal any difference, thus it may be assumed that the performance in the control task was not less sensitive to TMS compared to the main task.

The drop in performance in our study may be explained by interference with both preparatory or ongoing activity, and stimulus-evoked activity. The magnetic stimulation was delivered nearly 2 s prior to stimulus presentation hence affecting pre-stimulus processes. However, in previous work, we showed that rTMS delivered with these parameters and timing can produce a modulation of the amplitude of late positive ERP component to the target (Capotosto et al., 2012b). In previous work Chang et al. (2014) showed that rTMS applied to the time of stimulus onset can interfere with VPL of a signal-to-noise detection task. Hence, it appears that interference with both preparatory/ongoing and stimulus-related activity in visual cortex can affect the processing of familiar shapes.

We also report that RTs following magnetic stimulation over V2d/V3 and LO were similarly affected and positively correlated across subjects, but not correlated with IPS stimulation. This finding is consistent with the fMRI modulation during VPL observed in these visual areas in our prior study (Lewis et al., 2009). It is also consistent with the strong functional interaction between these visual regions at rest (Baldassarre et al., 2012). Even though we did not explicitly manipulated the topographic selectivity of rTMS inactivation, for example by stimulating a dorsal (task-relevant) vs. a ventral (task-irrelevant) visual area, the positive correlation is consistent with the idea that VPL modifies activity in visual cortex in retinotopically appropriate locations (Crist et al., 1997; Sigman et al., 2005; Lewis et al., 2009). It could be argued that the positive RT correlation for V2d/V3 and LO stimulation is due to spatial proximity and spread of the rTMS effect. In our opinion this is unlikely. In our previous published work (Capotosto et al., 2009) we have shown dissociated behavioral performance for sites (i.e. frontal eye field and pre-central region) much closer than those considered here. Specifically, the vector distance between frontal eye field and precentral region was 8.7 mm, while the vector distance between V2d/V3 and LO is 24.9 mm. Nonetheless an important control for future studies will be to separately inactivate visual occipital regions not directly recruited in this shape identification task (i.e. MT+) as well as to employ a control learning task (e.g. motion discrimination) to test the causal role of the present targeted visual regions in different paradigms. Furthermore, these future studies should also integrate the behavioral analyzes with the analysis of the eye movements in case of stimuli with higher eccentricity.

To conclude, the present findings show that activity in task-relevant occipital visual regions is causally important for the processing of learned visual shapes. In contrast, activity in parietal cortex presumably related to visuospatial attention is not.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.neuroimage.2016.08.063>.

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