TMS-Adaptation Reveals Abstract Letter Selectivity in the Left Posterior Parietal Cortex

Activation of the left posterior parietal cortex (PPC) has been associated with the encoding of letters independent of visual form. Here we used transcranial magnetic stimulation (TMS)-adaptation to investigate whether this abstract letter selectivity plays a causal role in letter processing. Visual adaptation was used to manipulate the initial activation state of neurons tuned to different letters prior to the application of TMS, after which subjects performed a detection task on letters that were presented in a different case from the adapting letter. After adaptation, TMS applied over the left PPC facilitated the detection of the adapted letter, whereas it had no impact on the detection of nonadapted letters. TMS applied over the right PPC had no significant effect on either type of letter. This interaction between adaptation and the effects of left PPC TMS demonstrates that adaptation modulated neural activity in the left PPC and thus demonstrates abstract letter selectivity in this region. Importantly, as the adapted letter and the target letters were presented in different cases, this finding demonstrates that the left PPC plays a causal role in letter processing independent of visual form.

Keywords: adaptation, letter processing, posterior parietal cortex, transcranial magnetic stimulation

Introduction

Letters are 2-dimensional visual forms that carry phonological information. Neuroimaging studies have consistently implicated the left angular gyrus, located in the posterior parietal cortex (PPC), in their encoding. By investigating how neural activity induced by perceiving nonnative letters changes as a result of learning the corresponding pronunciations, Callan et al. (2005) found that left posterior parietal activity is associated with perceiving visual shapes as letters with phonological information (rather than merely as complex visual forms). Furthermore, left angular gyrus has been shown to play a critical role in the neural network underlying reading, being specifically involved in allowing the transfer between orthographic forms and phonological representation (Pugh, Mencel, Jenner, et al. 2000; Shaywitz et al. 2002; Booth et al. 2003, 2004). In fact, phonological tasks activate the left angular gyrus less in dyslexic than in normal readers (Pugh, Mencel, Jenner, et al. 2000; Pugh, Mencel, Shaywitz, et al. 2000), and an increase in activation of this region has been observed in poor readers following a period of intensive remedial training (Meyler et al. 2008).

These studies have shown that the left PPC is involved in letter processing, but due to the correlational nature of neuroimaging, they do not reveal the causal role of this region in the cognitive process. In the present study, we addressed this issue by using the transcranial magnetic stimulation (TMS)-adaptation paradigm, which can reveal the causal role of the neural tuning in the stimulated region in a given cognitive process (Silvanto and Muggleton 2008; Silvanto et al. 2008). This paradigm is based on the evidence that the behavioral and perceptual effects of TMS are determined by the initial neural activation state; specifically, TMS facilitates the attributes encoded by the less active neural populations within the stimulated region (Silvanto et al. 2007b; Cattaneo and Silvanto 2008). In the TMS-adaptation paradigm, activation states of functionally distinct neural populations are modulated using visual adaptation, and subsequent application of TMS produces a selective facilitation of the attributes encoded by the adapted neurons. This paradigm can reveal neural tuning in the stimulated region: if TMS facilitates the perception of the adapted attribute, this indicates that neurons in the stimulated region were suppressed by adaptation and thus tuned to the adapted attribute.

We have previously demonstrated (Cattaneo et al. 2008), using the TMS-adaptation paradigm, that there is a facilitatory effect of left PPC TMS if an adapting letter is perceptually identical to a test letter. That effect, however, may have been purely based on the visual similarity between adapting and test stimuli rather than their meaning. The purpose of the present study was therefore to examine whether the letter selectivity is abstract; this is a critical issue as it is this abstract nature that differentiates letters from meaningless complex shapes.

In each experimental block, subjects adapted to a single letter that was either a vowel or a consonant. After adaptation, subjects performed a psychophysical task in which they were asked to indicate whether a letter appearing on the screen is a vowel or a consonant. The adapting letters and test letters were presented in different cases (i.e., adaptation to a capital letter followed by discrimination task involving lower case letters or vice versa), enabling us to rule out any effects that may be a function of the physical similarity between the adapting and test stimulus rather than meaning. Before each experimental trial, a single pulse of TMS was applied either over the left PPC or the right PPC with the objective of altering the initial state of the region prior to its activation by the target stimulus. Our results show that TMS applied over the left PPC (but not right PPC) facilitates the detection of the adapted letter, demonstrating abstract letter selectivity in the left PPC.

Methods

Subjects
In all, 13 subjects (5 males and 8 females, mean age 24 years) with normal or corrected-to-normal vision at the University of Pavia took
part in the experiment. Informed consent was obtained from all subjects. The experiment was approved by a local ethical committee of the University of Pavia.

**Letter Adaptation**

The stimuli were presented on a 15-inch (800 × 600 pixels) monitor. Viewing distance was 57 cm. In order to modulate the relative activity levels of letter-selective neurons, subjects were asked to view 1 of the 4 letters prior to the letter discrimination task for 40 s. Each adaptation stimulus was a black letter (appearing on a white background) that was either a consonant or a vowel. The adapting letter could appear either in capital (V, A, E, or F) or in lower case (v, a, e, or f). The height/width of the capital letters were on average 1.3/0.8 degrees of visual angle and that of the lower case letters were 0.7/0.4 degrees of visual angle. The letters appeared in the center of the screen.

**Letter Discrimination Task**

After one period of adaptation (lasting 40 s) to a single letter, subjects were presented with a block of 20 experimental trials (i.e., 5 presentations of each of the 4 letters). Test letters presented after adaptation to a capital letter were presented in lower case, and test letters presented after adaptation to a lower case letter were presented in capital. After these 20 trials, the next period of adaptation was carried out, followed by another block of 20 trials. This loop was repeated a total of 8 times for each of the TMS conditions (twice for each of the 4 letters—one adaptation in lower case and another in capital). Each trial began with a black fixation cross appearing in the middle of the screen for 500 ms on a white background, followed by a blank screen for 300 ms, after which the stimulus appeared. Subjects were required to report with button presses whether the stimulus was a vowel or a consonant. The letter remained on the screen until the subject responded. Subjects were asked to respond as quickly as possible. Figure 1 shows the time line of each block.

**Transcranial Magnetic Stimulation**

TMS was delivered by means of a Magstim single-pulse machine (Magstim, Whitland, UK) via a 70 mm figure-of-eight coil. For left and right PPC TMS, stimulation was applied over the electrode locations P3 and P4, respectively; this localization technique has been used in a large number of studies investigating PPC functions (e.g., Walsh et al. 1998; Lewald et al. 2002). TMS was applied using a fixed intensity (70% of machine output) on the basis of many previous studies (O’Shea et al. 2004, 2007; Campana et al. 2006; Pitcher et al. 2007).

**Procedure**

At the beginning of the session, the stimulation sites were localized. Subjects were then given a practice block of 40 trials (without adaptation) to familiarize themselves with the task. For each condition (left PPC TMS, right PPC TMS, No TMS), 8 blocks of adaptation were carried out (2 for each of the 4 letters used in the adaptation; one adaptation was done in capital and the other in lower case; for the time line of each block, see Figure 1). The order of blocks was randomized.

**Results**

As in previous studies using TMS-adaptation (Silvanto et al. 2007b; Cattaneo et al. 2008), performance in the psychophysical task was analyzed as a function of whether the test stimulus was the same or different as the adapting stimulus. Specifically, experimental trials on which the target letter was the same as the adapting letter were classified as “Congruent” and trials on which the target letter was different than the adapting letter were classified as “Incongruent.” As in other TMS studies (e.g., Göbel et al. 2001), reaction times from erroneous responses as well as reaction times longer than 1500 ms were not included in the analysis. Three subjects who did not show a behavioral effect of adaptation in the No TMS condition were excluded from the analysis.

As expected, in the No TMS condition, subjects were significantly slower in detecting the adapted letter relative to the unadapted letters ($t_9 = 2.77; P = 0.02$). The mean detection accuracies for the TMS conditions were No TMS = 97.88%; left PPC TMS = 97.4%; right PPC TMS = 97%.

Figure 2 shows the mean reaction times of congruent and incongruent trials as a function of the TMS condition. A repeated measures analysis of variance (ANOVA) with trial type (congruent and incongruent) and TMS condition (No TMS, left PPC TMS, right PPC TMS) as main factors revealed a significant interaction ($F_{2,18} = 13.34; P = 0.0005$). Pairwise comparisons with the $P$ value adjusted to 0.0125 to adjust for the 4 pairwise comparisons that were carried out (i.e., $P = 0.05/4 = 0.0125$) revealed that left PPC TMS reduced, relative to the No TMS condition, subjects’ reaction times to the adapted letters ($t_9 = 3.52; P = 0.007$). In contrast, RTs to the unadapted letters were unaffected by left PPC TMS ($t_9 = 0.27; P = 0.79$). Relative to the No TMS condition, right PPC TMS did not affect the RTs of either adapted ($t_9 = 1.93; P = 0.09$) or unadapted letters ($t_9 = 0.45; P = 0.66$).

In a second analysis, we classified the data as a function of whether the test stimulus was of the same class (i.e., vowel or a consonant) as the adapting stimulus. The objective of this analysis was to determine whether the state-dependent effects of left PPC TMS extend to trials on which the target letter is the same class (i.e., consonant or vowel) as the target letter. The adapted letter was excluded from this analysis. A repeated measures ANOVA with trial type (congruent class and incongruent class) and TMS condition (No TMS, left PPC TMS, right PPC TMS) as main factors revealed neither a significant interaction ($F_{2,18} = 1.29; P = 0.300$) nor significant main effects of trial type ($F_{1,9} = 0.08; P = 0.787$) or TMS condition ($F_{2,7} = 0.04; P = 0.967$). This shows that the TMS-induced facilitation does not extend to other letters sharing the same class as the adapted letter.
In contrast, left PPC TMS had no effect on the detection of the unadapted letter. Right PPC TMS facilitated, relative to the No TMS condition, the detection of the adapted letter and points to narrow letter tuning in the left PPC. The behavioral effects of adaptation in the No TMS condition were quite weak, with reaction times to adapted letters on average only 17 ms slower than reaction times to nonadapted letters. Despite this weak effect of adaptation, state-dependent effects of TMS were observed, with left PPC TMS reversing this behavioral effect. In the left PPC TMS condition, reaction times to adapted letters were on average 23 ms faster than reaction times to nonadapted letters. The magnitude of this TMS effect in favor of the adapted letter (relative to the nonadapted letter) was therefore approximately 40 ms. As only 5 of our 13 subjects did not show a behavioral effect of adaptation in the No TMS condition, the present study cannot address the issue of whether state-dependent effects of TMS can be obtained without a behavioral effect of adaptation in the No TMS condition. However, previous studies suggest that this may be the case. For example, phosphenes induced from the early visual cortex after color adaptation can appear with the color of the adapting stimulus even after the afterimage induced by the adaptation has faded (Silvanto et al. 2007b). This finding suggests that a perceptual manifestation of adaptation in the baseline measurement is not necessary for obtaining state-dependent effects of TMS; this is plausible as the lack of a behavioral manifestation of adaptation does not imply the absence of neural adaptation.

It might be argued that the first few TMS pulses after adaptation abolished the effect of adaptation, thus returning all neural populations to their baseline state and thus removing the behavioral effects of adaptation. This explanation is unlikely however; if TMS had simply abolished the effects of adaptation, no difference in the detection accuracies of congruent and incongruent letters would have been observed in the left PPC TMS condition. Instead, we found a reversal of the behavioral effects of adaptation: The detection accuracy of the adapted direction was higher than that of the opposite direction. This suggests that the effects of adaptation did last the whole block of trials and that this effect was on each trial reversed by the single pulse of TMS. This view is supported by the finding that the effects of visual adaptation are not abolished by single
pulses of TMS and that the state of adaptation is stable for at least 24 TMS trials after adaptation (Cattaneo and Silvanto 2008), even though repetitive TMS does abolish the effects of adaptation (e.g., Stewart et al. 1999; Theoret et al. 2002). However, those studies were carried out in the context of motion perception with TMS applied over V5/MT, and it may not be accurate to generalize those findings to the non-perceptual domain.

Our results differ from other studies that have not found PPC TMS effects at target onset (e.g., Ashbridge et al. 1997; Kalla et al. 2008). The critical difference between our experiment and these previous studies is that here we manipulated the initial activation state in which neurons in the PPC receive information regarding the target stimulus. The initial activation state is important as any differential activation of different populations of PPC neurons has a major impact on how incoming information is processed. In previous TMS studies, the initial activation state of PPC neurons was not manipulated and thus the TMS effect at target onset could not arise.

Adaptation paradigms may also be useful for modulating the impact and efficacy of theta-burst repetitive TMS protocols. In a previous study (Silvanto et al. 2007a), we showed that if theta-burst TMS is applied during the viewing of a coherently moving visual stimulus, the detection of the viewed direction of motion is not affected by TMS, whereas the detection of the opposite direction is impaired. In other words, the visual attribute presented during TMS was affected less by TMS than other attributes. This seems to contradict our subsequent work on TMS-adaptation in which the adapted attribute is preferentially affected by TMS relative to nonadapted attributes. This discrepancy may be explained by one important difference between the Silvanto et al. (2007a) study and our subsequent work on TMS-adaptation. In the former, theta-burst TMS protocol was applied during the viewing of visual stimuli, with stimulation beginning shortly after the onset of the visual stimulus. At this stage, neurons tuned to the viewed stimulus are presumably more active than other neurons. Therefore, TMS was effectively applied when neurons encoding the presented direction of motion were more active than neurons encoding the opposite direction. The finding that off-line TMS had a bigger impact on the detection of the opposite direction is thus consistent with the view that TMS has a bigger impact on the less active neural populations (cf. Silvanto and Muggleton 2008; Silvanto et al. 2008). In contrast, in our subsequent TMS-adaptation studies, TMS is always applied after adaptation when neurons encoding the adapted attribute are presumably less active than neurons encoding other attributes.

In summary, our results provide a demonstration of the functional significance of letter tuning in the left PPC in letter processing. This is also the first study that has not merely demonstrated the methodological validity of TMS-adaptation but has provided new information on the neural basis of a cognitive function. Future studies combining TMS with neuroimaging techniques such as electroencephalography (e.g., Thut et al. 2003; Fuggetta et al. 2006; Taylor et al. 2007) are required to determine the neural mechanisms of TMS adaptation (for some evidence on the state dependency of TMS effects, see Romei et al. 2008).

Funding
VW was supported by a grant from the MRC.

Notes
The authors wish to thank Dale Steyn for his comments on the manuscript. Conflict of Interest: None declared.

Address correspondence to Juha Silvanto, University of Essex, Department of Psychology, Wivenhoe Park, Colchester CO4 3SQ, UK. Email: juha@essex.ac.uk.

References


