We tested whether the frontal eye field (FEF) is critical in controlling visual processing in posterior visual brain areas during the orienting of spatial attention. Short trains (5 pulses at 10 Hz) of transcranial magnetic stimulation (TMS) were applied to the right FEF during the cueing period of a covert attentional task while event-related potentials (ERPs) were simultaneously recorded from lateral posterior electrodes, where visual components are prominent. FEF TMS significantly affected the neural activity evoked by visual stimuli, as well as the ongoing neural activity recorded during earlier anticipation of the visual stimuli. The effects of FEF TMS started earlier and were greatest for brain activity recorded ipsilaterally to FEF TMS and contraterally to the visual stimulus. The TMS-induced effect on visual ERPs occurred at the same time as ERPs were shown to be modulated by visual attention. Importantly, no similar effects were observed after TMS of a control site that was physically closer but not anatomically interconnected to the recording sites. The results show that the human FEF has a causal influence over the modulation of visual activity in posterior areas when attention is being allocated.

**Keywords:** EEG, ERP, frontal eye field, orienting, TMS-ERP, transcranial magnetic stimulation

**Introduction**

The frontal eye field (FEF) has a central role in controlling eye movements (Bruce and others 1985; Gaymard and others 1998), but it also plays an important role in perception and attention (Schall 2001). FEF transcranial magnetic stimulation (TMS) affects the detection of targets in arrays of distractors (Muggleton and others 2003). Effects are apparent even when pulses are applied early (40 and 80 ms) after presentation of the visual array (O’Shea and others 2004). FEF TMS can even affect awareness of masked stimuli (Grosbras and Paus 2003). The effects of FEF TMS may result from an alteration of processes intrinsic to the FEF itself, or they may reflect, at least in part, a causal influence that the FEF exerts over perceptual analysis in posterior visual areas of the brain. The present experiment investigated this by combining FEF TMS with event-related potential (ERP) recordings from visual cortical areas.

Imaging experiments have correlated both parietal and FEF activity with "endogenous" attentional orienting (Nobre and others 1997; Gitelman and others 1999; Corbetta and others 2000; Beauchamp and others 2001). It has also been reported that TMS over either region affects attention as judged by the speed of response to target stimuli (Rushworth and others 2001; Grosbras and Paus 2002; Chambers, Payne, and others 2004; Chambers, Stokes, and Mattingley 2004; Smith and others 2005; Thut and others 2005). In the case of the FEF, performance is facilitated when the target stimuli are presented in isolation and when the TMS is applied prior to target onset (Grosbras and Paus 2002; Grosbras and Paus 2003). The causal influence exerted by the FEF over the other areas that are also activated in imaging experiments, however, has been unclear. Simultaneous microstimulation and recording have recently been used to investigate the impact of FEF stimulation on sustained visual responses recorded from neurons with corresponding receptive fields in extrastriate area (V4) in the macaque (Moore and Armstrong 2003; Moore and Fallah 2004), but these procedures have not been used to investigate FEF-extrastriate interactions during the endogenous allocation of attention.

In the present study, we used a noninvasive method of combined stimulation and recording to investigate the role of FEF in voluntary rule-guided endogenous orienting of attention in human subjects. We applied repetitive TMS to the right FEF during the cueing period of a covert attentional task similar to the one used by Grosbras and Paus (2002) while recording the modulation of the ERPs at electrodes over visual cortex (Fig. 1). The right FEF was investigated because previous studies have shown that effects on an attention task are greater when FEF TMS is delivered to the right hemisphere (Grosbras and Paus 2002). The aim was to test whether FEF TMS affected visual activity recorded in the context of the endogenous spatial precueing task and in the absence of any confounding influence of response preparation or execution. Grosbras and colleagues (Grosbras and Paus 2002; Grosbras and Paus 2003) found that FEF TMS that affected overt eye movements also affected covert attention and target detection. The eye movement effect of FEF TMS was therefore also ascertained prior to the attentional task in the present experiments.

Peripheral stimuli were presented to subjects at the end of each trial, and on some trials, a response to the stimulus was required ("target" or "response" stimulus [RS]). At the beginning of each trial, a central arrow ("precue") warned subjects where the stimuli were likely to appear. The precue indicated the correct location for the following stimulus on 80% of trials ("valid" trials), and it indicated the wrong location on 20% of trials ("invalid" trials). A variant of the precueing procedure developed by Eimer and shown to be particularly appropriate for ERP recording was used (Eimer 1994; Griffin and others 2002; Eimer and others 2003). In this procedure, only a small proportion of visual stimuli are RS targets requiring a response, and most stimuli are not targets (i.e., "nonsignal" stimuli [NRS]). Although both valid and invalid precues preceded stimuli, subjects were only required to respond to RSs that followed valid precues. Attentional modulation of ERPs is particularly strong in this paradigm because subjects only ever need to respond to the RS when presented at the precued location and never when at the invalidly precued location. The
subjects are therefore assured that they are never missing anything of behavioral relevance as long as they attend to the cued location. A second advantage is that there are many NRS trials on which the ERP is uncontaminated by processes related to response execution; on such trials, the ERP only reflects visual stimulus processing. All the analyses in the present report focus on these trials.

ERP data sets were recorded when TMS was applied to the FEF and to a control site over the sensorimotor cortex. In addition, an initial data set was recorded to see whether there would still be differences between the valid and invalid ERPs over occipital visual cortex if the data were rereferenced to midline parietal electrode PZ. This step was added to restrict the areas of the brain contributing to the ERP signal to the visual areas under the occipital scalp. This precaution was important because the aim of the subsequent TMS-ERP experiments was to investigate the effect of FEF TMS on such visual areas. Without this precaution, it would be possible that changes elsewhere in the brain, between the reference and recording electrode, would contribute to any ERP signal associated with the application of TMS. To prevent the electroencephalographic (EEG) recording simply being saturated by the TMS, the EEG amplifier was briefly disconnected from the recording electrodes at the time of each TMS pulse (clamping). It was not, however, to examine the effect of TMS on the rarer invalid trials. As explained in Materials and Methods, this is a consequence of ensuring both that the TMS itself did not have any predictive value and that the number of pulses delivered did not exceed the number that might be safely delivered in a single session. The FEF TMS was expected to have its greatest effect on ERPs elicited by stimuli in the contralateral hemisphere. It is not clear what effect TMS should be expected to have on stimuli presented to the ipsilateral hemisphere because most interareal connections in the primate brain are predominantly intrahemispheric (Cavada and Goldman-Rakic 1989). Given the need to limit pulses, it was therefore decided to focus the experiment on examining the effect of TMS in relation to contralaterally presented stimuli.

Materials and Methods

Visual-Spatial Orienting Task

All visual stimuli were white and presented 100 cm in front of the subject on a thin film transistor LCD monitor (refresh rate 75.025 Hz). Subjects viewed peripheral stimuli (Fig. 1) composed of 5 concentric squares appearing briefly (100 ms) and filling 1 of 2 square place holders in the left or right visual field (7° horizontal eccentricity, 1° wide, luminance 20.55 cd/m², background luminance 0.34 cd/m², Michelson contrast 96.7%). They were instructed to respond (R) as quickly as possible to a subset of visual stimuli (20%) designated as Rs, which differed from the NRs by missing one of the inner squares. Central arrow cues (1° wide) preceded the peripheral visual stimulus (500-ms stimulus onset asynchrony) and predicted its location with high probability (80% validity). Subjects were instructed to respond only to Rs that appeared at the cued location (Eimer 1994; Griffin and others 2002; Eimer and others 2003). This manipulation, which permits subjects to commit all their resources to the cued side, has been shown to optimize the magnitude of attentional modulation of the ERP. Cue direction and validity in each trial were determined by a pseudorandom schedule. Eye gaze was monitored using remote infrared eye tracking (iScan, Burlington, MA) to ensure that subjects could perform the task covertly while fixating.

Subjects received training immediately before testing and until successfully completing 50 trials containing at least 3 targets without errors. Subjects then performed blocks (mean 6, range 5–8) of 100 trials (50 left, 50 right). In all experiments, subjects were required to maintain fixation within 1° of the central cross for more than 95% of trials. All subjects met the criterion.

Study Design

Data were collected in separate experiments. Experiment 1 examined the impact of the rereferencing of the ERP data to the midline parietal electrode PZ. It was not clear from previously published data what consequences the use of a PZ reference would have for the shape of the ERP recorded from the critical posterior electrodes. The use of a PZ reference, however, was essential for the subsequent experiments in which TMS was applied over the FEF or a sensorimotor control site between the standard nose reference and the critical recording electrodes on the posterior scalp. With this precaution, it was possible to interpret any TMS-induced changes in ERP recorded from posterior electrodes as changes that were due to neural activity in visual and extrastriate cortex and not elsewhere in the brain, for example, directly under the TMS coil. The effects of TMS over the FEF or the sensorimotor control site were measured in Experiments 2 and 3. The sensorimotor site was chosen as a control because 1) it produced indistinguishable scalp topographies, 2) it was physically closer to the recording electrodes than the FEF site, and 3) it is not thought to be directly anatomically interconnected with posterior visual areas. An additional separate behavioral data set was collected from a small group of subjects (6 subjects, 1 left handed, 2 males, aged 19–21) instructed to respond to Rs even after invalid trials. The behavioral data from these subjects confirmed that valid precues were effective at orienting spatial attention, enabling subjects to process validly cued visual stimuli more efficiently (Fig. 2).

Experiments 2 and 3 each consisted of 2 phases, one for the localization of the TMS site and one for the combined TMS-ERP task. TMS parameters were all within the established safety guidelines (Wassermann 1998) and were approved by the Central Oxfordshire Research Ethics Committee (reference no. C02/092).

TMS Site Localization

To verify functionally the correct placement of the TMS coil over the FEF, we used a cued prosaccade paradigm similar to that previously used to show an effect of FEF TMS on saccades (Ro and others 1997). Grosbras and colleagues (Grosbras and Paus 2002; Grosbras and Paus 2003) have reported that TMS at the FEF that disrupts overt eye movements also affects covert visual orienting and target detection in a paradigm similar to the present one.

The stimuli were identical to those used in the covert orienting task except that no stimuli appeared after the precue. Subjects were asked to make a saccade as quickly as possible after the precue, in the indicated direction, toward the outer edge of the peripheral place holder. Five TMS pulses (10 Hz, 110% motor threshold) were applied starting 0.2 s after precue presentation using a figure-8 flat coil with an internal diameter of 7 cm (Magstim Rapid machine, Whitland, Wales). Subjects wore earplugs (signal-to-noise ratio 28) when TMS was applied. Subjects performed 40 trials (20 TMS, 20 no-TMS, 20 leftward saccades, 20 rightward saccades). Eye gaze was recorded with an infrared remote eye-tracking system (iScan).

The TMS site was initially localized anatomically using stereotactic registration to the participant’s structural magnetic resonance imaging...
The FEF in the right hemisphere was defined as the intersection of the inferior part of the sensorimotor cortex (Paus 1996). The initial site was accepted if disruption of leftward saccade endpoint latency was observed and adjusted to a new site 0.5 cm anteriorly in the absence of saccade disruption. In most cases (12 of 18), the first site tested was effective. Subsequent analysis confirmed that all effective sites lay within close proximity of one another and of the FEF in Montreal Neurological Institute (MNI) space (mean x = 33, y = -1, z = 54 mm) (Fig. 2). The control site was located 1.5 cm posterior and 1.5 cm medial to right primary motor cortex (M1), which was localized using previously described procedures (Schluter and others 1999; Johansen-Berg and others 2002). Disruption of leftward saccades did not occur with TMS of this site. In all cases, the first TMS site was accepted.

ERP Recording

In Experiment 1, data were collected and analyzed using standard ERP procedures (18 right-handed subjects including 9 females, aged 19–28). EEG was recorded continuously (NuAmps digital amplifiers, Neuroscan, El Paso, TX) from 32 scalp sites using Ag/AgCl electrodes positioned according to the 10–20 international system (AEEGS 1991) (low-pass filter direct current 200 Hz, 500 Hz sampling rate). Additional electrodes were used as ground (FPZ-FZ) and reference site (nose) and to monitor the electrooculogram (EOG) (recorded bipolarily with electrodes placed around the eyes). Data were subsequently band-pass filtered (0.1–40 Hz). ERPs were constructed offline, from epochs starting 550 ms before and ending 500 ms after visual stimulus onset. Epochs were rejected if the voltage exceeded ±50 μV on any channel, if saccades or blinks occurred in the EOG, or if subjects made false alarms. To compare brain activity evoked by visual stimuli after valid versus invalid precues, the epochs were normalized to the period around the onset of the visual stimulus (-20 to 20 ms). This baseline period, chosen to be compatible with the TMS parameters, minimizes the impact of changes in ongoing activity in the prestimulus period (Griffin and others 2002) and does not overlap with any measurable visual components. A minimum criterion of 25 trials per condition per subject was set to ensure high-quality ERP data. Data from 1 subjects were rejected because not enough trials survived artifact rejection.

Analyses focused on the lateral posterior electrodes, where the visual components P1 and N1 were most pronounced and where the effects of spatial orienting were maximal. We compared the mean signal amplitude for valid and invalid trials in successive 50-ms time bins in order to characterize the attentional modulation systematically. Effects of precue validity upon the ERPs were revealed using repeated-measures analyses of variance. The factors tested were validity (valid, invalid) and hemisphere (left, right). Two separate analyses were conducted. One analysis used data referenced to the nose. In a second analysis, data were rereferenced offline to the signal from electrode PZ, producing a measure of attentional modulation that is attributable mainly to changes in local visual cortical activity. The PZ-rereferenced analysis served as a basis to explore how FEF TMS affected local extrastrate activity during attentional modulation.

**Combined TMS-ERP**

The impact of FEF TMS and control site TMS on functional neural processes indexed by ERPs was also measured in the context of the visual-spatial orienting task in Experiments 2 and 3.

Eighteen right-handed subjects (5 females, aged 21–37) participated in the FEF experiment, and 14 right-handed subjects (5 females, aged 21–28) participated in the separate control site experiment. Informed consent was obtained from all subjects before participation. Artifact rejection procedures identical to those used in Experiment 1 meant that data from 12 and 11 subjects in the 2 experiments were retained for full analysis (see below). On some left-cued trials, 5 pulses of TMS at 100-ms intervals were applied during the cue-target interval. No subjects noticed that TMS was only applied on left-cued trials. To prevent TMS itself providing an informative orienting cue, TMS was delivered on both valid and invalid trials in the same proportion (14% of all trials, 28% of left-cued trials). The recommended safety guidelines limited the number of TMS trials overall, and therefore the relative rarity of invalid trials meant that there were only very few invalid trials with TMS (approximately 10 per subject). If a larger number of TMS invalid trials had been included and if there had been a proportionate increase in the number of valid TMS trials (to ensure TMS was delivered at a similar ratio in both cases), the total number of pulses applied would have been unacceptably high. Under these constraints, it was possible to collect sufficient trials to characterize the ERP waveforms for left valid trials with and without TMS, as well as for left invalid trials without TMS. Subjects’ data were only included in the analysis when more than 25 artifact-free exemplars of each trial type were available. On average, 36, 107, and 35 trials were analyzed in the TMS valid, no-TMS valid, and no-TMS invalid conditions, respectively, in the FEF experiment, and on average, 43, 125, and 42 trials were analyzed in the TMS valid, no-TMS valid, and no-TMS invalid conditions, respectively, of the sensorimotor control experiment. As before, care was taken to control for response preparation and execution by only comparing validly cued and invalidly cued stimuli to which subjects did not actually make a response.

Several precautions were taken in order to record ERPs while concurrently applying TMS. First, it was important to ensure that the TMS-induced modulation of the visual ERPs was not just a consequence of applying a TMS pulse between the reference electrode and the
recording electrodes over the posterior visual cortex. Experiment 3, therefore, investigated a sensorimotor control site that was physically closer to the recording electrodes. Despite being physically closer to the visual cortical recording site, the sensorimotor control site is not directly connected with the visual cortical areas, and so stimulation here was not expected to modulate visual cortical activity. Second, rather than using a vertex or midline control site, the use of the sensorimotor site also controlled for any lateralized somatosensory and acoustic effects that might have directed attention to one side of space. Because of their proximity, the sensorimotor and FEF sites produced indistinguishable scalp sensa- tions. Third, further precautions were also taken to ensure that the ERP modulation reflected local changes of visual activity in posterior visual and extrastriate areas. Activity was therefore recorded from electrodes PO7 and PO8, where visual components were largest, and referenced to the nearby midline electrode PZ, which emphasizes the contribution of locally distributed brain activity. Additionally, by using a conditioning preamplifier with externally gated microswitches (modified 1902 preamplifiers and 1401 amplifier, Cambridge Electronic Design, Cambridge, UK), it was possible to disconnect the amplifier from the electrodes for a 2-ms period while recording the time the TMS pulse was applied (Ilmoniemi and others 1997). The disconnection prevented each TMS pulse from saturating the EEG signal or damaging the amplifier. During disconnection from the recording electrodes, the amplifier connected to an internal channel with no signal. The signal received by the amplifier was effectively "clamped." The "clamping" process itself created an artifact, albeit more limited than would be created by the unchecked TMS pulse. The clamping was also applied on no-TMS trials so that differences between TMS and no-TMS trials were unambiguously related to the TMS pulse and not to the clamping. Direct recordings were made without any filtering or alternating current coupling because these may interact with the clamping artifact to produce large ghost harmonics or "aliasing." To prevent buildup of slow drift on the signal, an automated algorithm applied a baseline correction procedure during the intertrial interval.

On the basis of previous pilot experiments, it was possible to be confident of EEG recordings made within 30 ms of each TMS pulse. The TMS train was therefore applied during the cuing period (Fig. 1) starting 50 ms after onset of the precue and ending 50 ms before the presentation of the visual stimuli and well before the first visual ERP component. In this way, it was possible to investigate the visual ERP in the absence of any TMS-induced artifact.

In the combined TMS–ERP experiments, EEG was recorded continuously with Spike2 software (v3.21) from Ag/AgCl electrodes mounted on an elastic cap (Easy Caps, Herrsching-Breitbrunn, Germany). The signal was amplified 10 000 times and digitized at a sampling rate of 1020.4 Hz. Four electrodes were used, and 4 clamping amplifiers were available. Electrodes PO7 and PO8 were used as active recording sites, with PZ as the reference electrode and the right mastoid as the ground. Impedances were kept below 5 kΩ. Horizontal and vertical eye movements and blinks were detected by remote infrared eye tracking (Iscan). Data were processed using Neuroscan (v4.3). Once again, the period around the onset of the visual stimulus (~20 to 20 ms) was used as the baseline. In addition, to determine whether TMS also led to changes in the ongoing tonic neuronal activity measurable at posterior electrodes, epochs were also normalized to the period before TMS (~500 to ~490 ms prestimulus). Artifact rejection criteria were the same as in Experiment 1. Data from 6 subjects in the FEF condition and 3 subjects in the control site condition were rejected.

**Analyses**

Only ERPs to NRIs in the left visual field were examined in all experiments. On such trials the TMS, which was applied to the right cerebral hemisphere in Experiments 2 and 3, was contralateral to the visual stimulus. The TMS and no-TMS valid trials shown are those where the subject was cued to the left and the NRIs appeared on the left. For comparison, the invalid no-TMS trials were those where the subject was cued right and the NRIs also appeared on the left. Only main effects or interactions involving validity were of experimental interest in all 3 ERP recording experiments. Because of the risk of false-positive effects in the multiple interrelated comparisons, results were only considered significant if they persisted over at least 2 successive 50-ms time bins.

**Results**

**Behavioral Effects of TMS**

In the TMS localization task (Fig. 2), overt saccades toward the left peripheral box were significantly delayed with FEF TMS relative to no-TMS (mean latency with FEF TMS = 422 ms, without TMS = 371 ms, \( t_{11} = 3.86, P < 0.01 \)). No delay in saccade latency occurred with TMS of the control site. TMS of the control site decreased group saccade latency (mean control site TMS = 372 ms, without TMS = 402 ms, \( t_{10} = -4.04, P < 0.01 \), compatible with the known alerting effects of TMS (Marzi and others 1998; Walsh and Rushworth 1999). An independent- samples t-test assuming unequal variance confirmed that the effects of TMS over the FEF and control site differed significantly (mean delay FEF TMS = 51 ms, mean facilitation control site TMS = −30 ms; \( t_{17.2} = 5.34, P < 0.01 \)).

Behavioral data recorded during the orienting task from the group of subjects who were asked to respond on both valid and invalid trials (Fig. 2) showed the task oriented attention of spatial attention and could be performed without moving the eyes. Significant reaction time benefits occurred when valid arrow precues predicted the position of target stimuli as opposed to when the cues were invalid (\( t_9 = 2.9, P = 0.033 \), Fig. 2).

Subjects made few overt responses during ERP recording so that it was possible to establish whether the FEF TMS was affecting visual ERPs that were uncontaminated by any motor response selection or execution processes. There were, however, sufficient numbers of trials in both the valid no-TMS and valid TMS conditions, in which contralateral left hemifield stimuli were presented, for a comparison of response times (RTs) to be undertaken. There was no difference in the effect of TMS on RT when applied over either the FEF or the sensorimotor cortex (\( P > 0.1 \)); in both cases, subjects tended to respond more quickly on TMS trials (FEF: \( t_{11} = 2.6, P < 0.05 \); sensorimotor: \( t_{10} = 4.6, P < 0.001 \)). Because previous studies have reported that FEF TMS speeds target detection when it delays overt eye movements, we examined the correlation between the effect of TMS on eye movement in the localization task and RT in the attention task. Although the delay in eye movement was correlated with the decrease in RT for the FEF site (\( r = 0.63, N = 11, P < 0.05 \)), there was no correlation in the case of the sensorimotor cortex (\( r = 0.19, N = 11, P = 0.58 \)). The data point from 1 subject in the FEF experiment was identified as an outlier, both by visual inspection of the scatter plot and by examining the Cook’s Distance measures (>2 standard deviations), and had to be excluded from the analysis.

**ERP Recording**

Experiment 2 validated the use of the PZ reference to investigate the effects of TMS upon locally distributed ERP components over the lateral posterior electrodes PO7 and PO8. Visual components and attentional modulation effects were prominent over the PO7 and PO8 electrodes when either a distal (nose) or proximal (PZ) reference electrode was used. Referencing data from the PO7 and PO8 electrodes to the proximal midline electrode PZ changed the morphology of the waveforms considerably but preserved the time course of the validity effects (Fig. 3, upper 2 panels). The effects of spatial attention during the visual P1 and N1 components (50–200 ms) were concentrated ipsilaterally, as revealed by significant interactions between validity and hemisphere (\( P \) values ≥ 8.67, \( P < 0.05 \)) and by subsidiary effects of validity at PO7.
but not PO8. Main effects of validity or interactions between validity and hemisphere persisted throughout the ERP epoch (F values > 5.53, P < 0.05).

**Combined TMS-ERP Recording**

Similar ERP effects of cue validity were found in the combined TMS-ERP experiments in Experiment 3 as in Experiment 2 (Fig. 3, lower 2 panels). There were significant main effects of cue validity during P1 and N1 time periods (100–200 ms) for the data recorded in both FEF and control site sessions (F values > 5.18, P < 0.05). At P1, the effects of validity were more pronounced ipsilaterally over PO7. The interaction between validity and hemisphere was already significant in the 50- to 100-ms time bin (F values > 7.47, P < 0.05), with larger effects at PO7 than PO8. Extensive later main effects of validity were also observed, lasting from 250 to 500 ms in the FEF TMS experiment (F values > 5.53, P < 0.05) and from 250 to 300 and 350 to 450 ms in the control site experiment (F values > 4.69, P < 0.05; 300–350 ms, P = 0.09).

FEF TMS in the cue-target period significantly altered the ERP waveform for left visual stimuli (Fig. 4). An initial analysis using a peristimulus baselining period (20 ms before until 20 ms after onset of the visual stimulus) revealed main effects of TMS from 200 to 250 ms and from 300 to 500 ms (F values > 4.88,
Figure 4. ERPs elicited by valid visual stimuli (presented in the left visual field) with TMS to the right hemisphere (thick lines) and without TMS (thin lines). ERPs are referenced to midline electrode PZ to emphasize visual activity underneath occipital scalp (see Introduction). Top and center panels show data from the control site TMS and FEF TMS conditions, respectively, baselined between –20 and 20 ms postvisual stimulus. Lower panel shows FEF TMS data baselined to the pre-TMS period between 550 and 490 ms before the visual stimulus, to emphasize changes in the ongoing tonic neuronal activity. With either baselining period, FEF TMS is shown to affect the normal attentional modulation of the ERP recorded from visual cortex (see Discussion).

\( P < 0.05 \), with a marginally significant interaction between TMS and hemisphere at 250–300 ms \(( P = 0.05)\) (Fig. 4). Interactions between TMS and hemisphere indicated that larger effects occurred at PO8, ipsilateral to the TMS and contralateral to the visual stimuli. Subsidiary analyses testing for effects of FEF TMS at PO8 and PO7 separately showed that the effects were significant between 200 and 500 ms for the PO8 electrode \((F \text{ values } \geq 4.85, P \leq 0.05)\) but started later at PO7. A trend occurred at 300–350 ms \((P = 0.06)\), followed by significant effects between 350 and 450 ms \((F \text{ values } \leq 5.25, P < 0.05)\). Control site TMS had no significant effects on the ERPs (Fig. 4, top panel).

To assess whether FEF TMS may have influenced ongoing levels of activity in posterior regions, an additional analysis was conducted with the data normalized to the pre-TMS baseline period (Fig. 4, bottom panel). In addition, this analysis should provide a more accurate picture of the time course of the effect of the TMS on the ERP because the baseline for normalization preceded the onset of the TMS. There was a specific effect of right FEF TMS on ipsilateral ongoing activity at PO8. Main effects of TMS and interactions between TMS and hemisphere occurred between 0 and 200 ms after the visual stimulus \((F \text{ values } \geq 6.19, P < 0.05)\), overlapping with the previously identified P1 and N1 components. Subsidiary analyses testing the effects of TMS on PO8 and PO7 electrodes separately showed significant effects of TMS on PO8 activity for all the same time bins \((F \text{ values } \geq 12.05, P < 0.05)\) and no effects on PO7 activity. To track the onset of the effect during the cue-target period, successive comparisons were made between TMS and no-TMS trials after each clamping period. Measures were taken in 20-ms steps, 30 ms after the preceding clamping period and 30 ms before the succeeding clamping period. After each FEF TMS pulse, there was a negative deflection in the PO8 signal, which became increasingly pronounced with each pulse. The effect became significant in the interval after the third pulse and remained significant through the remainder of the cue-target period \((F \text{ values } \geq 6.45, P < 0.05)\). The effects on the contralateral PO7 electrode were weaker and more variable across subjects and did not reach statistical significance.

Discussion

Inducing activity in the right FEF with TMS during the cuing period of a rule-guided covert endogenous attentional orienting task modulated ERPs recorded over visual cortex. The temporal resolution of ERPs and the use of a cognitive task enabled us to demonstrate that FEF TMS altered functional processes related to perception and attention in the visual cortex. The effect was not limited to alterations of ongoing, resting-state, physiological activity. The FEF exerts a causal influence over activity
in visual cortex during the voluntary orienting of visual–spatial attention.

Cueing produced a behavioral benefit (Fig. 2) and modulated visual and later ERP components (Fig. 3) during the endogenous attentional orienting task. Such tasks are described as "endogenous" rather than "exogenous" because a learned rule is applied to orient attention voluntarily, rather than attention being summoned automatically by the appearance of a salient stimulus (Posner 1980). Rule-guided behaviors are important parts of primate and particularly human repertoires (Murray and others 2002). The human FEF is most critical for saccade guidance when endogenous control is required (Henik and others 1994). The ERP results from Experiment 2 also emphasized the local nature of the attentional modulation of visual activity. Attentional modulation of activity recorded by PO7 and PO8 electrodes over posterior visual cortex was still apparent even after reinterpreting the post-midline parietal electrode PZ, which emphasizes locally distributed brain activity and rules out any putative contribution from voltage induced directly by the TMS (Fig. 3).

Functional as well as anatomical criteria were used to identify the right FEF in individual subjects. After registering the brain into standard MNI space, it was clear that TMS had been applied over the functional region identified as FEF in a recent meta-analysis (Grosbras and others 2005). Times to complete leftward saccades were significantly delayed after FEF TMS. There was a significant contrast between this pattern of results and the one seen when TMS was applied over a control site in the sensorimotor cortex. In fact, stimulation at the control site was associated with some speeding of the saccade completion; this is probably because TMS at the control site had some general alerting effects (Fig. 2) (Marzi and others 1998; Walsh and Rushworth 1999). The differential results in the 2 conditions rule out generic or artifact-related explanations of the FEF TMS effects.

FEF TMS had a causal impact on visual activity measured with ERPs. The earliest effect of TMS was a sustained negative deflection, becoming significant after the third TMS pulse, during the interval between the cue and the visual stimulus, and remaining until 200 ms after visual stimulus onset. The data were normalized to a pre-TMS baseline period to emphasize ERP shifts occurring after warning cue onset but before visual stimulus presentation (Fig. 4, lower panel). FEF TMS lead to greater negativity in electrode PO8, ipsilateral to the TMS, starting after the third TMS pulse in the 5-pulse train. This negativity remained present in the ERP until 200 ms after visual stimulus presentation and so may be described as an effect on visual processing at the time of attentional modulation of the ERP. The finding that the ERP modulation was predominantly in the hemisphere ipsilateral to the TMS is compatible with a local change in ongoing visual activity subsequent to the warning cue but prior to the visual stimulus (Kastner and others 1999).

The hemispheric specificity of the effects rules out explanations in terms of TMS-induced artifacts. Moreover, the use of the adjacent PZ electrode as a reference emphasizes that the ERP modulation over visual cortical electrodes had a local origin in the same vicinity even though the TMS was applied some distance away over a distinct brain area—the FEF. It should be noted that although an alternative approach based on a current source density analysis might be used to accentuate local changes in voltage, such an approach would still be influenced by any voltage induced underneath the coil. There would therefore be less certainty in attributing TMS-induced changes to processes in cortical areas distant from the TMS coil.

When stimulus-evoked activity was isolated by using the peristimulus period as the baseline, ERPs differed significantly as a function of FEF TMS starting at 200 ms (Fig. 4). This baseline window was used to allow easier comparison of the morphology of the ERPs elicited by the visual stimulus because typically visual ERPs are shown time locked to the presentation of the visual stimulus. Importantly, both baselining methods lead to the same conclusion that FEF TMS affects modulation in visual cortex. It should be noted, however, that using a prestimulus baseline window does not provide an accurate identification of the time of onset of the TMS-induced changes in the visual ERP. Normalizing the ERP to the prestimulus period will give an ERP to the visual stimulus in the standard manner, but it will necessarily remove the effect of the TMS because that effect began to emerge before the baseline period; such a normalization procedure in itself necessarily sets the effect of TMS to zero and so makes it impossible to ascertain the onset of the TMS effect.

It is therefore clear that the TMS is affecting ongoing visual cortical activity even prior to visual stimulation, and it is not just affecting the visual cortex's generation of an ERP. It should equally be noted, however, that the TMS continues to "interact" with the visual cortical activity generated by the visual stimulus, and it is not the case that visual stimulation causes the immediate cessation of the cortical processes that were first started by the TMS. In order to better understand the nature of the interaction between TMS changes in ongoing visual cortical activity and the visual ERP, future studies might manipulate the interval between the cue and the visual stimulus. Future experiments might also investigate the impact of FEF TMS on invalid trials. However, this would require sacrificing either the number of TMS pulses or the strength of cue validity to satisfy safety constraints (see Materials and Methods).

The impact of the FEF TMS is clearly on visual stimulus–related activity rather than on motor preparation or execution processes. Only ERPs to NRSs were analyzed for all 3 trial types, valid no-TMS, valid TMS, and invalid TMS, so any ERP activity is uncontaminated by processes related to motor or premotor brain areas.

It should be noted that the polarity of 1 ERP waveform relative to another cannot be interpreted in terms of a relative increase or decrease in underlying neural activity. The potential difference recorded between 2 electrodes varies with the spatial location of the reference electrode. It is interesting to note, however, that FEF TMS does not lead to an ERP that resembles the one seen on invalid trials when the subject was not attending to the contralateral visual stimulus. This suggests that FEF TMS is not mimicking the effect of inattention in a simple way in this experiment. Grosbras and colleagues, however, have provided evidence that target detection and attention are enhanced when TMS is applied to the FEF just prior to the presentation of isolated visual stimuli (Grosbras and Paus 2002; Grosbras and Paus 2003). These facilitatory effects were elicited from subjects who had been selected because FEF TMS disrupted saccades. There was also some evidence in the present study of a relationship between disruption of overt saccades and facilitation of covert detection.

In summary, a direct causal relationship between activity in FEF and visual cortex is supported by 1) the specificity of the TMS effect to FEF and not the control site despite the control
site’s greater proximity to the recording electrodes (Fig. 4), 2) the hemispheric lateralization of the TMS effects, and 3) the time course of the effects (Fig. 4). The time course is consistent with propagation of the TMS pulse from the FEF along anatomical projections to ipsilateral extrastriate cortex. In the monkey, the FEF is connected to several parietal, temporal, and extrastriate regions (Cavada and Goldman-Rakic 1989; Schall and others 1995; Barone and others 2000), but interareal connections are strongest within a hemisphere. Any distant effects of FEF TMS in the other hemisphere would be mediated by an indirect combination of intrahemispheric interareal connections and interhemispheric homotopic connections.

Activation changes have been reported in striate and parietal cortices when FEF TMS is applied during imaging experiments (Paus and others 1997; Ruff and others 2005). The application of TMS to other areas, such as primary motor cortex, has also been shown to result in activation changes in interconnected brain regions (Paus and others 1998; Bestmann and others 2004). Such previous studies have either not had the same temporal resolution as the current one or not examined whether the distant activity induced by the TMS continues to interact with functional activity in those areas. For example, Lee and others (2003) found that TMS to one area made it less responsive to input from elsewhere. The current study, however, demonstrates how the stimulated area affects the activity in distant and interconnected areas during a cognitive task. Because of the temporal resolution of ERPs, the direction of effect, from FEF to visual areas, is unambiguous.

The behavioral impact of FEF TMS on visual detection and attention is now well documented and depends on the presence of distractors and the timing of the pulses (Grosbras and Paus 2002, 2003; Muggleton and others 2003; O’Shea and others 2004). Although FEF TMS can facilitate the subsequent detection of a single simple stimulus (Grosbras and Paus 2002, 2003), it interferes with the discrimination of a concurrently presented target defined by a conjunction of features in a field of distractors (Muggleton and others 2003; O’Shea and others 2004). The activation of many FEF neurons by TMS may cause a correspondingly broad change in activity across extrastriate areas, which means that the activity induced by a subsequent single visual stimulus is more likely to exceed the threshold for detection. At the same time, such broad changes in activity may hamper discrimination of a target from a field of distractors. With selective microstimulation in the macaque of limited FEF regions, activity increases in extrastriate areas are restricted to neurons with corresponding receptive fields, as are behavioral improvements (Moore and Armstrong 2003; Moore and Fallah 2004).

The FEF TMS effect is consistent with monkey lesion studies showing contralateral visual neglect and visual field defects after unilateral FEF removal (Latto and Cowey 1971). Single-unit and microstimulation studies support a dual role for the macaque FEF not only in oculomotor control but also in perceptual and attentional functions that are independent of eye movements. The FEF neurons do not show strong selectivity for many important aspects of visual stimuli such as color and form, at least prior to extensive practice (Bichot and others 1996). To mediate attentional selection, they may interact with neurons in extrastriate areas where such visual attributes are more widely represented.

During microstimulation experiments in macaques, it is possible to examine the effects of FEF stimulation on visual activity occurring elsewhere in the brain with a very short latency (Moore and Armstrong 2003; Moore and Fallah 2004). At the moment it is difficult to be certain about the verisimilitude of the ERP data recorded in the first 30 ms after TMS application. Despite this limitation, it is important to realize that ERPs, which are thought to reflect the synchronized activity of many neurons, take some time, in the order of tens of milliseconds, to evolve. Even visual ERP components begin, at the very earliest, 50 ms after visual stimulus onset. It, therefore, seems possible to use the technique and to apply the TMS at the time of stimulus onset and still obtain information about the impact of the TMS on visual stimulus–related activity. The present experiments were, however, even more cautious and applied the last TMS pulse prior to the onset of the visual stimulus. Although it remains the case that a number of other manipulations that are important for studying attention are only possible in experiments using animal models (Schall 2001; Moore and Armstrong 2003; Juan and others 2004; Moore and Fallah 2004; Buffalo and others 2005; Rushworth and others 2005), the present results are important because they suggest that some top–down influences upon visual processing, first described in the context of animal models, have a genuine relevance for comprehending the function of the human FEF.

The current finding may be one example of a more general family of top–down modulatory influences exerted by frontal cortex on posterior cortex (Sakai and others 2002). In addition, one may think of the FEF TMS effect in relation to previous findings of TMS of “higher” extrastriate visual areas affecting activity in “lower” primary visual cortex (Pascual-Leone and Walsh 2001; Silvanto and others 2005). However, in the case of the FEF, its influence on posterior visual areas may not only be restricted to top–down modulation but also constitute a bottom-up influence. Anatomical tracing in macaques has shown that the FEF has feed-forward projections to extrastriate areas such as V4 (Barone and others 2000). Single-cell recording has found that FEF neurons respond from 50 ms after visual stimulus onset, so that their latency is similar to area MT and earlier than V4 (Schmolesky and others 1998).

Notes
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