TMS Pulses on the Frontal Eye Fields Break Coupling Between Visuospatial Attention and Eye Movements

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1Experimental Psychology, Helmholtz Institute, Utrecht University; 2Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht; 3Faculty of Science, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands; and 4School of Optometry, University of California at Berkeley, Berkeley, California

Submitted 29 March 2007; accepted in final form 12 August 2007

Neggers SF, Huijbers W, Vrijlandt CM, Vlaskamp BN, Schutter DJ, Kenemans JL. TMS pulses on the frontal eye fields break coupling between visuospatial attention and eye movements. J Neurophysiol 98: 2765–2778, 2007. First published August 15, 2007; doi:10.1152/jn.00357.2007. While preparing a saccadic eye movement, visual processing of the saccade goal is prioritized. Here, we provide evidence that the frontal eye fields (FEFs) are responsible for this coupling between eye movements and shifts of visuospatial attention. Functional magnetic resonance imaging (fMRI)–guided transcranial magnetic stimulation (TMS) was applied to the FEFs 30 ms before a discrimination target was presented at or next to the target of a saccade in preparation. Results showed that the well-known enhancement of discriminative performance on locations to which eye movements are being prepared was diminished by TMS contralateral to eye movement direction. Based on the present and other reports, we propose that saccade preparatory processes in the FEF affect selective visual processing within the visual cortex through feedback projections, in that way coupling saccade preparation and visuospatial attention.

INTRODUCTION

It is well known that the brain selectively processes incoming visual images, enhancing relevant parts (Posner et al. 1980). The preparation of eye movements is an important signal driving selective visual processing. Stimuli presented at target locations of saccades in preparation are discriminated better than stimuli a few degrees away (Deubel and Schneider 1996; Dore-Mazars et al. 2004; Hoffman and Subramaniam 1995; Kowler et al. 1995). The primate frontal eye fields (FEFs) might play an important role in controlling both saccade production and visuospatial attention, although at present the exact mechanisms underlying this coupling remain obscure. FEFs are directly involved in saccade production (Bruce and Goldberg 1985; Robinson and Fuchs 1969) and preparation (Everling and Munoz 2000) and contain visually responsive neurons whose response is modulated by spatial attention (Juan et al. 2004; Mohler et al. 1973; Schall et al. 1993). The influential premotor theory of attention poses that shifting visuospatial attention is equivalent to preparing but not executing movements (Rizzolatti et al. 1987). In a more strict interpretation selective visual filtering and saccade programming are implemented in the same brain regions (Corbetta et al. 1998; Kustov and Robinson 1996). However, this interpretation is subject to debate (Awh et al. 2006; van der Lubbe et al. 2006). Alternatively, oculomotor signals driving selective visual processing may originate in the FEF during saccade preparation and subsequently be projected to and integrated in visual filtering elsewhere. Interestingly, monkey V1 neurons increase activity before saccades into their receptive field (Super et al. 2004). Furthermore, electrical stimulation of FEF neurons elicits activation in ipsilateral V4 neurons with receptive fields matching the FEF neurons’ movement field (Moore and Armstrong 2003). FEF electrical stimulation changes luminance discrimination performance (Moore and Fahle 2001, 2004), indicating that these projections affect visual processing. Transcranial magnetic stimulation (TMS) on the human FEF alters ipsilateral occipital EEG signals (Taylor et al. 2006) and enhances visual awareness (Grosbras and Paus 2003; O’Shea et al. 2004; Ruff et al. 2006). Furthermore, TMS on the FEF lowered the threshold of a second TMS pulse on V4 needed for eliciting phosphene, vivid TMS-evoked visual illusions (Silvanto et al. 2006). FEF projections to the visual cortex are possibly instrumental in saccade preparation (Moore 2006), such as the determination of saccade metrics. Yet, conclusive evidence for a specific visuomotor role of these cortico-cortical feedback connections is lacking; the aforementioned FEF stimulation studies merely changed visual processing during fixation and did not investigate a specific visuomotor function. Disturbing known links between eye movement preparation and visual processing might strengthen the assumption that the projections between the FEF and the visual cortex specifically subserve processing of visual information at the saccade target location.

The aim of the present study therefore was to demonstrate an unequivocal role of the FEF in modulating visual processing during saccade preparation. The above-cited cortico-cortical feedback model underlying visuospatial attention control by the FEF would predict a suppression of the link between eye movements and visual processing enhancements at saccade goals when the FEF is stimulated shortly before a discrimination target is presented. This would disturb the hypothesized feedback signal assumed to be sent to the visual cortex during saccade preparation. Therefore functional magnetic resonance imaging (fMRI)–

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guided TMS was applied to the FEFs of human participants 30 ms before discrimination target presentation. TMS coil placement was stereotactically guided to individual FEF locations obtained in an fMRI experiment on the same participants using a pro- and antisaccade task. Exact timing of the pulses was based on estimates of neuronal signal conduction times derived from the macaque animal model and human psychophysiological TMS studies (see Experiment 3 in Methods) and the assumption that the coupling of visuospatial attention and saccade programming is indeed caused by feedback from the FEF to the visual cortex. The time of TMS delivery was chosen such that the current induced by TMS in the FEF reaches the visual cortex at the same time as incoming visual signals from the discrimination target that emerged on the screen.

Note that these assumed conduction times are averages of many measurements, derived from different methods and species. Therefore a short train of three pulses was used centered around 30 ms before discrimination target visibility, to ensure that the intended neuronal process is affected. When TMS-evoked neuronal activity in the FEF is longer lasting than estimated or the neuronal conduction times differ from our estimates it cannot be ruled out that the FEF incorporates both visual attentional processing and saccade control, as proposed by others (Corbetta et al. 1998; Kustov and Robinson 1996). Nevertheless, disruption of the coupling of saccade programming and attentional shifts by TMS on the FEF would demonstrate a crucial role of the FEF in establishing such a coupling.

The visual cortex codes contralateral retinotopic space, the FEFs contralaterally direct saccades (Bruce et al. 1985), and the FEFs project to ipsilateral visual cortex (Moore and Armstrong 2003). Therefore we predict that for saccades directed to the left, right FEF stimulation will result in larger decreases in perceptual enhancements at saccade targets than for saccades to the right, and vice versa.

**METHODS**

**Participants**

Eight right-handed participants with normal or corrected-to-normal vision participated in all three experiments, five male and three female (three of the authors participated). They had no history of mental or neurological illnesses and were screened for implanted metal objects before entering the fMRI experiment according to the UMC Utrecht internal guidelines. The participants were also screened before taking part in the TMS experiment (Keel et al. 2001). The TMS stimulation protocol stayed within the internationally accepted safety limits (Wassermann 1998). Participants gave their written informed consent. During a practice phase, some participants initially reported mildly unpleasant stimulation of the facial muscles, which is known to occur for TMS of frontal brain regions, and caused directly by the TMS pulse. After becoming familiarized with the experience of TMS all participants were able to complete the experiment.

The experimental protocol was approved by the medical ethical committee of UMC Utrecht (Protocol No. 05-020).

**Experiment 1**

In **experiment 1**, eight participants made eye movements to cued objects. Shortly before the saccade was actually executed, a discrimination target was presented at the saccade target location or a location nearby. The experimental paradigm was a replication (as exact as possible) of experiment 2 from Deubel and Schneider (1996), which is a well-known demonstration of the coupling of the locus of visual attention to the target of a saccade in preparation.

**APPARATUS.** The position of the left eye was measured with the video-based EyeLink II system from SR Research at 500 Hz, using infrared (IR) video oculography. The IR cameras were mounted to a head support. Stimuli were presented with the Matlab psychophysics toolbox (Brainard 1997; Pelli 1997) on a Pentium PC. During the experiment, participants were seated in a dimly lit room. Visual stimuli were presented on a 19-in. iiyama color monitor with an active screen size of 40 × 30 cm, a refresh rate of 100 Hz, and a resolution of 1,024 × 768 pixels. The participant’s head was placed against a head support and chin rest 35 cm in front of a semisilvered mirror. The mirror was tilted 45° from horizontal with the near side being lowest, and the CRT monitor was mounted above the mirror, facing down. This resulted in a virtual image straight ahead of the participant at an effective distance of 72 cm. The setup was designed with the stimulus projection through a mirror to maximize the distance between the screen and the MiniBIRD magnetic position tracker used for frameless stereotaxy (see Experiment 3), thus minimizing magnetic interference between the magnetic tracker and the CRT computer screen.

**BEHAVIORAL PARADIGM AND PROCEDURE.** Before every session the eye position measurements were calibrated by repeatedly presenting nine targets in a 3 × 3 rectangular pattern on the screen until an approximately linear signal was accomplished.

Participants had to perform four sessions of 72 trials. Before the real experiment subjects performed 72 practice trials.

Each trial was preceded by a drift correction, which was performed after 500 ms of stable fixation on a central black fixation cross (0.52 × 0.52°) on a gray background. A random delay between 0 and 500 ms was added after successful drift correction before the trial actually started, to prevent a high predictability of visual stimulus presentation; otherwise, subjects might react in a rhythmic and automatic fashion to the appearing stimuli because the time of stimulus presentation would be exactly predictable.

After drift correction the trial started. See Fig. 1A for an overview of the stimuli and events constituting a trial. In the following text time is always expressed relative to the offset of the arrow cue, the saccade go-signal. At t = −1,200 ms six colored ellipses (0.82 × 0.164°) were presented, three to the left and three to the right of the fixation cross: red, green, and blue, from center to periphery. The green ellipses were positioned at 5° left and right of the fixation cross. The spacing between the centers of neighboring ellipses was 1.36°. The ellipses contained black 8-shaped premasks (1.05 × 0.52°). At −500 ms a colored triangle (0.85 × 0.52°) appeared (replacing the fixation cross) that pointed to the left or right, indicating which ellipse would be the saccade target (ST). The direction of the saccade (left- or rightward) was indicated by the direction of the arrow and the arrow’s color indicated whether the blue, green, or red ellipse was the ST in that visual half-field. At t = 0 the triangle cue disappeared, which served as a go-signal to make the saccade to the ST indicated by the arrow. By 60 ms after the go-signal the discrimination target (DT), an E or a mirrored E, was presented by blanking parts of one of the 8-shaped premask, at either the left or the right central target (= green ellipse). At the other five nontarget locations, the 8-shaped premask was changed in either a 2 or a 5, ensuring the same luminance change at the DT and distracter location. Thus the DT location could either coincide with the saccade target or the saccade could be directed to one of the neighboring ellipses. By 120 ms after they appeared, the E or mirrored E and the distracters disappeared again, leaving six empty colored ellipses on the screen. Because saccades have a typical latency between 200 and 300 ms, the DT was on average presented well before the saccade was initiated (e.g., during movement preparation). Trials with premature anticipatory saccades that landed at the DT
before it disappeared were removed from analysis (see Data Analysis) because, in that case, the DT would be briefly visible in the fovea, dramatically improving performance for reasons other than the hypothesized saccade preparation. After making the saccade, participants indicated without time pressure whether they had observed an E or a mirrored E at the DT locations by pressing a left or right arrow on the keyboard. As soon as a response was given the premasks disappeared and the subjects could initiate a new trial themselves by pressing the space bar. From trial to trial, the discrimination target position, the saccade target, and the target identity were pseudorandomized. Experiment 1 was an exact replication of the original experiment 2 in Deubel and Schneider (1996).

Data Analysis. For each trial, the onset of the first saccade after the go-signal was detected using a velocity and acceleration threshold of 30°/s and 8,000°/s². To prevent a foveation of the target, trials on which participants made a premature saccade (arriving earlier than 20 ms after the discrimination target was extinguished) were excluded. Furthermore, when saccades were landing more than 2.4° away from the target, moving in the wrong direction, starting more than 1.2° away from the fixation cross (ensuring proper fixation), starting >3 s after the start of the trial, or when no key-press response was given, a trial was also excluded from further analysis.

For each participant the proportion of correct key-press responses, the average saccade latency, and the target deviation were calculated. The ratio of correct responses as well as average (per participant) saccadic cue onsets and offsets were tested in repeated-measures ANOVAs for effects of saccade target position (TARGET).

Experiment 2

This fMRI experiment was aimed at localizing the individual FEF in every subject. A powerful FEF activation paradigm and fMRI scanning sequence are beneficial for individual FEF localization in a relatively short scanning session. Because the FEFs seem preferentially activated when making an antisaccade and thus suppressing automatic visually evoked saccades in humans (Ford et al. 2005; Neggers et al. 2005; Raemakers et al. 2002), experiment 2 consisted of a rapid succession of pro- as well as antisaccade blocks. Furthermore, FEFs not only are involved in saccade production (Bruce and Goldberg 1985; Robinson and Fuchs 1969) and preparation (Everling and Munoz 2000), but also contain visually responsive neurons (Juan et al. 2004; Mohler et al. 1973; Schall et al. 1993). Therefore the present paradigm simply contrasts activity during the saccade task blocks (containing activation due to target presentation as well as oculomotor activation) with activity during fixation periods. This paradigm was tested in the scanner in advance of the present study during a short (8-min) pilot study on two subjects and resulted in strong FEF activation detectible in single subjects.

Apparatus. Imaging was performed on a clinical Philips 3T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) equipped with eight independent receiver SENSE coils allowing parallel imaging (Pruessmann et al. 1999). Stimuli were projected (using the Presentation software from Neurobehavioral Systems) on a Plexiglas 1-m-wide screen placed at 2-m distance from the participants and viewed through a mirror mounted on the head coil.

MR Data Acquisition. One thousand functional T2*-weighted blood oxygenation level–dependent (BOLD) volumes were acquired using a new PRESTO-SENSE acquisition scheme (Neggers, Hermans, and Ramsey, unpublished observations). SENSE techniques use parallel coil acquisition to sample MR images very efficiently (Pruessmann et al. 1999), which has been reported to yield very short acquisition times for three-dimensional (3D) echo-shifted EPI (PRESTO) BOLD image acquisition when using SENSE in one phase encoding direction (Golay et al. 2003). Here we accelerated BOLD image acquisition using a PRESTO acquisition scheme with SENSE in two phase encoding directions (2D SENSE; Neggers, Hermans, and Ramsey, unpublished observations), leading to ultrafast fMRI scanning (500 ms/volume). This method has proven to be much more sensitive to signal changes than conventional 2D EPI (Neggers, Hermans, and Ramsey, unpublished observations), which was useful for individual FEF localization. The acquisition parameters were: TR = 21.75 ms; TE = 32.4 ms; FOV(ap, fh, rl) = 224 × 256 × 128 mm; flip angle = 10°; matrix: 64 × 64 × 32 slices; voxel size 4 mm isotropic; 8-channel head coil; SENSE factor = 2 and 1.8 (in the left/right and anterior–posterior phase encoding directions, respectively). One volume was acquired in 500 ms.
After the functional sessions an anatomical T1-weighted scan was acquired (TE/TR 4.69/8.71 ms; flip angle 8°; FOV 224 × 160 × 168 mm; matrix 256 × 256; slice thickness 1 mm; slice gap 0; voxel size 0.875 × 0.875 × 1 mm). The latter anatomical scan was used to coregister the functional volumes to and for neuronavigation during TMS-coil placement (see Experiment 3).

**FMRI BEHAVIORAL PARADIGM.** The experiment started with a white central fixation cross (size 1 × 1° of visual angle and line thickness 0.1° of visual angle) placed at the center of a black screen. After 500 ms the fixation cross turned into a colored circle (1° visual angle). The colored circle remained visible for 400 ms and constituted the saccadic cue. A red circle instructed the participant to make a prosaccade (toward the peripheral target), a blue circle to make an antisaccade (opposite from the peripheral target with similar amplitude). After 500 ms a peripheral target appeared for 800 ms at 3.8 or 14.8° from the center of the screen on the left or right side. Participants had to make a saccade to this target as soon as possible. Each block contained 10 trials and lasted 20 s, after which a rest block of 20 s followed. During the rest period participants were required to fixate the central fixation target that remained visible on the screen. This sequence was repeated 12 times, accumulating to a total duration of 8 min.

Note that due to the rapid succession of saccades, it is probably no longer possible to demonstrate lateralization of the activated FEF with respect to saccade direction, which would have allowed separation of left FEF and right FEF by function. A rapid succession of saccades (2 s between saccades to peripheral targets, 1 s between target saccades and saccades back to the center) results in largely overlapping hemodynamic responses typically lasting about 12 s before baseline is reached. The main reason for this FMRI experiment was clear FEF activation; the left and right FEF were separated anatomically.

**DATA ANALYSIS.** For the localization of individual FEFs the fMRI data (1,000 T2*-weighted scans) were analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/spm2.html). The functional scans were realigned to the first image to correct for movement of the head, registered to the T1-weighted anatomical image and resliced at 4 × 4 × 4 mm. The resulting images were smoothed with an 8-mm kernel FWHM (full width at half-maximum). The anatomical scan was also segmented using SPM2 segmentation algorithms to create a gray matter probability map, used for the cortical rendering during neural navigation (see Fig. 4). The data were not normalized to a T1 Montreal Neurological Institute (MINI) brain atlas template at this stage because normalization in SPM2 uses nonlinear warping techniques to fit an individual brain to a brain atlas space (Ashburner and Friston 1999). Because the activation maps and anatomical scan were used for TMS coil targeting using rigid body stereotaxy (Neggers et al. 2004) to a real participant’s head (see stereotactic (f)MRI guidance of TMS coil placement), no transformations other than rigid body registration were allowed here.

To detect FEF activation during saccade blocks, a two-regressor general linear model (GLM) model was fitted to the functional images per voxel. The first regressor was a boxcar regressor with a block length of 20 s and 20 s between blocks, convolved with the hemodynamic response function (two superimposed gamma functions) to accurately predict BOLD signal changes during saccade blocks. The second regressor was a constant, modeling baseline. The regression coefficients for each voxel for the first regressor obtained by fitting the GLM to the data were statistically tested against zero using a one-sample t-test, using a P < 0.05 significance threshold [whole brain volume corrected for multiple comparisons according to random-fields theory (Worsley et al. 1996)]. Thus only voxels that were significantly activated (e.g., with T-values above the statistical threshold mentioned earlier) were included in the activation map. The above-cited procedure is a common approach for analyzing block designs in SPM2. Significantly activated voxels could be attributed to either saccadic eye movements or visual target presentation. Still, because the FEF contains both visual and saccadic neurons (Bruce and Goldberg 1985; Juan et al. 2004; Mohler et al. 1973), it can be expected that this test accurately localizes it in individual participants, as previously shown (Neggers et al. 2005; Raemaekers et al. 2002).

Localization of individual FEFs was the main goal of this FMRI experiment. The native space (unnormalized) coordinates of the voxel with the maximum T-value in the statistical T-map within the left and right FEFs were stored for TMS coil guidance in experiment 3. Finally, the T1 anatomical scan and the maximum T-map coordinates were normalized to MNI standard space (the ICBO152 template brain is used to define MNI space) using nonlinear warping as implemented in SPM2, with the sole purpose to calculate the MNI coordinates of the stimulation sites, allowing display and comparisons of the stimulated FEF coordinates between participants and with other studies (e.g., these MNI coordinates are not in any way used for TMS coil placement). These coordinates are displayed for all participants in Fig. 4, B, C, and D.

An additional fMRI analysis was run to see whether there was any difference in fMRI activation for left- and rightward saccades, mostly similar to the analysis mentioned earlier. First, the same block-design analysis as previously described was done on all subjects, and additionally a random effects (RFX) group-level analysis was run, using a one-sample t-test on the individual first-level activation maps (Friston et al. 1999; Holmes and Friston 1998), which is common practice for fMRI group statistics. A cohort of 12 subjects is generally considered a minimum to reliably demonstrate BOLD signal differences using RFX group statistics. Therefore the RFX group analysis was run on fMRI data from the eight participants of the present study and seven additional subjects. These subjects took part in an FMRI experiment that adopted the same FEF localizer paradigm as that in the present experiment 2, whereas it was otherwise unrelated to the present study. A voxel was considered as significant when the t-test was significant at α < 0.05, corrected for multiple comparisons over the entire brain volume, according to random-fields theory. This group analysis provided a map of the FEF that was subsequently used as a region of interest (ROI). Next, a new statistical analysis was run where saccades were modeled as two separate event-related regressors containing the expected hemodynamic response for each left or rightward saccade. Activation amplitude for leftward saccades was subsequently contrasted with rightward saccades, and the individual contrast images of left- versus rightward saccade activation were tested with a one-sample t-test on the second level using RFX statistics corrected for multiple comparisons over the entire brain volume. Also, a more liberal ROI statistical threshold was considered (using small volume correction for multiple comparisons), where the region of interest was the FEF group map of the original block analysis described earlier. These tests would reveal voxels that are differently activated for left- versus rightward saccades (and vice versa), exhibiting the known laterality of the FEF as observed in nonhuman primate studies. Note, however, that the present design with saccades in rapid succession complicates this additional analysis (see earlier reasoning); this analysis was merely added for completeness.

**Experiment 3**

In this experiment it was tested whether TMS on the FEF can influence the coupling of visuospatial attention to the target of a saccadic eye movement, as shown by Deubel and Schneider (1996) and replicated in experiment 1. The same behavioral paradigm as that in experiment 1 was used, while TMS pulses were delivered during each trial.

**APPARATUS.** The equipment used in experiment 3 was identical to that in experiment 1, with the only exception that the TMS coil was placed exactly over the subjects' left and right FEFs using fMRI-guided stereotaxy.

TMS pulses were administered by a Neopulse TMS device (Neotonus, Atlanta, GA) with a squared figure-of-8 iron core TMS coil.
(Epstein and Davey 2002), triggered by the PC running the paradigm through a TTL pulse over the parallel port. In addition to the normal TMS coil also a different (SHAM) coil was used to control for effects secondary to TMS such as auditory stimulation due to TMS coil condenser discharge or slight facial muscle contraction. The SHAM coil contains a metal plate built right below the coil windings, preventing the generated magnetic field from extending into the cortex. Due to the opposite circular currents induced in the metal plate (Lenz’s law) by the TMS pulse a magnetic field is generated that counteracts the magnetic field generated by the coil. This SHAM coil is specially designed to produce similar TMS secondary effects as the real TMS coil, and is an accepted control condition in TMS studies.

TMS coil placement on the subjects’ head surface was stereotactically guided by the individual fMRI activation maps registered to individual MRI anatomical images (see following text for details) that were obtained in experiment 2, ensuring accurate stimulation of the individually and functionally defined regions of interest.

BEHAVIORAL PARADIGM AND PROCEDURE. Before the experiment started the TMS output intensity was determined individually. The motor threshold was determined as the TMS machine output intensity at which stimulation of the cortical thumb-movement area yields visible TMS-evoked thumb twitches for five of ten trials (Schutter and Van Honk 2006). By doing so the TMS intensity could be roughly adjusted for individual differences in magnetic field and electrical cortical conductivity. TMS pulses during the behavioral paradigm were delivered at 110% of the participant’s motor threshold.

After motor threshold determination, the coil was stereotactically guided by fMRI activation maps to the individual FEF location (see subsequent further description), and a few test TMS trials were presented to familiarize the participant with TMS stimulation.

The same behavioral paradigm as that in experiment 1 was used, except that three TMS pulses were delivered during each trial. The pulses were 30 ms apart and the second pulse was delivered at 90 ms after the go-signal, exactly 30 ms before the time the discrimination target was presented for 50% of total target presentation time (120 ms) (see Fig. 2). This is motivated by neuronal signal transmission times between the FEF and the visual cortex, based on our working model assuming that feedback from the FEF to the visual cortex directs visuospatial attention. Without TMS, saccade preparatory signals would (according to this model) arise in the FEF and be projected to the visual cortex to enhance visual processing at the saccade goal. When within the visual cortex these signals from the FEF and incoming visual signals from the retina containing target information arrive simultaneously, a maximal perceptual enhancement of the saccade goal would be achieved. Thus a maximal interference of TMS on the FEF with saccade-induced visual attentional shifts would occur when TMS is applied on the FEF shortly before visual target information arrives in the visual cortex. More exactly, a maximal disturbance of presaccadic attentional shifts is expected when the time from TMS stimulation to target presentation equals the electrical signal transmission from the FEF to the visual cortex minus the transmission time from the retina to the visual cortex. With about 66 ms as an average visual latency for macaque area V1 (Schmolesky et al. 1998), and an estimated conduction time of about 100 ms between the FEF and the visual cortex, this yields 34 ms as the optimal time between TMS and target presentation. We estimated the FEF–visual cortex conduction time at about 100 ms based on the following two studies. First, monkey FEF presaccadic preparatory activation is reported to start about 50 to 60 ms after stimulus presentation in a study also using multiple target configurations (Thompson et al. 2005). The discussed presaccadic activity in V1 is observed on average 156 ms after fixation point offset (Super et al. 2004). When indeed V1 presaccadic activity originates in the FEF, this would imply a conduction time of about 100 ms.

This optimal 34-ms interval between TMS and DT presentation is close to the 30-ms interval used in the present study, and corresponds to values used in other TMS studies aimed at altering visual discrimination performance. In humans only a TMS pulse on the FEF between 20 and 40 ms before a second TMS pulse on V4 reduces the threshold by which phosphenes can be evoked from TMS on V4 (Silvanto et al. 2006). Furthermore, magnetic stimulation of the FEF about 40 ms before presentation of a discrimination target has been reported to be effective in changing perceptual performance (Grosbras and Paus 2003) in humans.

Note that as in experiment 1, a random delay between 0 and 500 ms was added before the trial actually started, to prevent a high predictability of visual stimulus presentation (the trials are identical to those in experiment 1 except for the TMS stimulation). Otherwise, high temporal predictability of discrimination target appearance and TMS stimuli delivery might cause preparatory effects.

The main purpose of the present experiments was to test whether saccadic preparatory processes in the FEF result in improved perceptual discrimination at the target location of the upcoming saccade, through feedback connections to the visual cortex. As described earlier, a rough estimate of the optimal TMS stimulation time, about 34 ms before DT presentation, was deduced from findings on the macaque animal model. However, large uncertainties remain in this estimation, and one single pulse at that time might therefore be offered too early or too late with respect to the FEF neuronal processing of interest to have an effect. Therefore three pulses with an interval of 30 ms, covering a larger interval between the saccade go-signal and the presentation of the DT, were applied, although still centered on the theoretically optimal time. This is assumed to optimize the probability that any preparatory activation in the FEF that can possibly influence processing in the visual cortex would be disturbed (because the neuronal effects of a single TMS pulse last about 30 ms at most; Ilmoniemi et al. 1997). Varying the timing of a single pulse from trial to trial would at this point have resulted in an experiment lasting much too long for a participant to keep performing at the required accuracy because the number of factors and thus required trial repetitions would be multiplied by the number of TMS timing levels. The present experiments therefore focused the statistical power on demonstrating the existence of the putative effect in the first place.

![Figure 2](https://www.jn.org)
Each participant took part in four sessions of 72 stimulation trials. In two sessions, the left FEF was stimulated, and in two other sessions the right FEF. Per hemisphere, in one session TMS was applied with the real TMS coil and in another with the SHAM coil. The order of the sessions was counterbalanced, and after each a 10-min break was introduced.

Performances in TMS trial sessions were compared with SHAM stimulation sessions, rather than interleaving TMS trials with non-TMS trials in one and the same block. When comparing TMS trials with interleaved non-TMS trials one would not know whether effects on a subjects’ performance were caused by the neuronal disturbance caused by TMS, or effects secondary to TMS, such as auditory stimulation or the somatosensory side effects. The SHAM coil mimicks TMS secondary effects. It takes several minutes to mount a different coil to the TMS apparatus making interleaved SHAM and TMS trials—which would theoretically be the ideal option—infeasible.

SHAM coil sessions are an accepted method to control for TMS secondary effects. Furthermore, it is expected that FST stimulation will have an effect for preparation of saccades directed only contralateral to the stimulated FEF, allowing one to contrast performance for contralateral with ipsilateral saccade trials as a control for TMS secondary effects.

STEREOTACTIC fMRI GUIDANCE OF TMS COIL PLACEMENT. Placement of the TMS coil was stereotactically guided with a neural navigator (Neggers et al. 2004) using anatomical MRI and FEF fMRI activation images obtained during experiment 2. The positions of eight anatomical landmarks on the head of the participant (tip and bridge of the nose, the inner-and outer meeting points of the upper and lower eye lids, and the upper adherence of the ears; see Fig. 4A) were measured with a 3D digitizer and mapped to similar positions marked on a computer-generated image of the MRI-derived skin rendering. This mapping was accomplished by estimating the optimal rigid body transformation minimizing the sum-squared difference between the positions of the facial markers in digitizer coordinates and the same positions in MRI coordinates [the exact procedure is described in detail in Neggers et al. (2004)]. Using this estimated rigid body transformation, the position and orientation of the 3D digitizer pen could be rendered on the screen as it hovers over the cortical surface and FEF activation maps. The 3D surface rendering of the skin was directly derived from the T1-weighted anatomical scan. The cortical surface was rendered through a gray matter map segmented from the same T1-weighted anatomical scan (segmentation created by SPM2). The fMRI activation T-maps representing the individual FEFs as obtained in experiment 2 were superimposed (see Fig. 4A). The scalp positions directly overlaying the coordinates of maximum (suprathreshold) fMRI activation within both the left and right FEF were marked with the 3D digitizer pen on a swimming cap the participants were wearing. When multiple activation foci were observed within an individual subject’s FEF, the maximum of the cluster closest to the probabilistic FEF map from the BrainMap database (Nielsen and Hansen 2002) was selected for FEF stimulation. The BrainMap database probabilistic maps are based on a large collection of published fMRI results. This stereotactic fMRI-guided TMS setup is able to target functional cortical representations with an accuracy of about 3 mm (Neggers et al. 2004), which should be sufficient for targeting the FEF accurately because a TMS pulse affects roughly 1 to 2 cm of cortical tissue depending on stimulation intensity (Bohning et al. 2001).

Using fMRI-guided TMS to accurately stimulate the FEF is probably superior to using the 10–20 EEG electrode position system or average head size because FEF location is highly variable across individuals (see Fig. 4, B and C). Alternatively, other studies have used individual functional localization of the FEF by mapping (parts of) the scalp with multiple TMS pulses (Ro et al. 1999; Terao et al. 1998), after which one can observe which location significantly delayed saccades. Although this method most likely also constitutes a sufficiently accurate method for individual FEF localization, it is probably more time consuming.

DATA ANALYSIS. The same variables of interest as those in experiment 1 were calculated: the percentage of correct responses, saccade latency, and deviation of the saccade endpoint from the target. In experiment 3, first the same ANOVA as in experiment 1 was performed for the effect of ST location (TARGET) on the aforementioned variables. Furthermore, differences in percentage correct measures between inner and central saccade target location on the one hand and outer and saccade target location on the other hand (i.e., the effect size of discrimination enhancement for coinciding saccade and discrimination target location) were subjected to an ANOVA in experiment 3. The factors TMS- or SHAM stimulation (TMS), ipsi- or contralateral TMS/SHAM stimulation (SIDE) with respect to saccade direction were included. Initially, leftward and rightward saccades with the same target eccentricity were pooled. To investigate whether the observed effects are different for left and right FEF stimulation, a second ANOVA was performed with the stimulated hemisphere (left or right FEF stimulation) as an additional factor (HEMI). Level of significance was set at $P < 0.05$.

RESULTS

Psychophysics: the coupling of saccades and attention (experiment 1)

In experiment 1 the coupling between the locus of maximal visual attention and the prepared saccade was replicated, as demonstrated originally in Deubel and Schneider (1996).

The saccade latency [on average $298 \pm 37$ ms (SD)] and landing position distributions are given in Fig. 3, B and C. Trials with premature saccades (arriving at the ST location before the DT disappeared) were discarded to prevent foreshadowing of the DT and, when saccades landed too far from the ST, a trial was also excluded. In sum 15.8% of trials were removed from analyses for experiment 1 after application of the criteria mentioned in METHODS (see Table 1 for the ratio for all individual subjects). Saccade latencies were not different between any of the ST locations [TARGET: $F(2,14) = 0.94; P = 0.41$]. There was a difference between the average deviation of saccade landing position from the indicated ST location [TARGET: $F(2,14) = 6.731; P < 0.009$]; in particular the landing positions around the inner target location scattered more strongly than around the central and outer target locations ($1.02, 0.66, 0.73^\circ$ visual angle).

As in the experiments reported by Deubel and Schneider (1996), a marked increase in the proportions of correctly discriminated targets was found [TARGET: $F(2,14) = 16.38; P = 0.00021$] for trials where the ST coincided with the DT ($\sim 81.5\%$ correct responses) as compared with noncoinciding DT/ST locations (inner/outer discrimination target $\sim 57.6\%$ correct responses); see Fig. 3A. This is remarkable considering that for noncoinciding locations the saccade was prepared to a location only a few degrees of visual angle away from the DT. Furthermore, the DT was presented shortly before the actual eye movement. This discrimination performance difference is generally considered clear evidence of an allocation of visuospatial attention at the target location of a movement in preparation.

fMRI localization of individual FEF (experiment 2)

The same eight participants also took part in an fMRI experiment aimed at localizing the FEFs for each subject
individually. Here, participants made PRO and ANTI saccades in rapid succession in the scanner in a series of short blocks with fixation periods in between.

SACCADE BLOCKS VERSUS FIXATION. Figure 4A shows a 3D cortical rendering with superimposed suprathreshold FEF activity during the saccade blocks compared with the fixation blocks, for one representative participant in native space (i.e., not normalized to standard MNI space; see METHODS). The craniotopic marker cursors and skin rendering are also visible [the image is generated by our stereotactic software (Neggers et al. 2004)]. The left and right FEFs can be seen, as well as the supplementary eye fields (SEFs) and the areas around the intraparietal sulcus (IPS) that are involved in the generation of eye movements.

The activation maps for all eight subjects that participated in the present study are given in Fig. 4B, projected onto their cortical surface normalized to MNI atlas space to allow comparisons between subjects. The FEFs are clearly activated in all subjects, as are the oculomotor-sensitive areas in the intraparietal sulcus [possible human lateral intraparietal cortex (LIP) homologue]. For some subjects more than one subarea can be distinguished in the FEF area, but this was not replicated in all individuals. The native space coordinates of the maximally activated (suprathreshold) voxel within the left and right FEFs were used for later TMS stimulation. When more than one focus of activation was observed in or near the FEF, the cluster with the strongest fMRI activation was selected. A cluster was considered to be part of the FEF when it was near the FEF probability map from the Volume of Interest (VOI) BrainMap database (Nielsen and Hansen 2002).

In the group statistical map of the FEF (Fig. 4B), based on data of 15 subjects, a more medial and lateral part of the FEF can be seen (and perhaps the SEF extremely medial), as some other studies also observed (Darby et al. 1996). The MNI coordinates of the local activation maxima were: −28, −4, 56; −44, 0, 48 (medial and lateral left FEF); 32, −4, 48, 4, 32 (medial and lateral right FEF).

However, in most individual activation maps (see Fig. 4B) the FEFs were usually a connected strip of several local maxima, and no clearly separated medial and lateral subregions could be readily distinguished.

Figure 4C and D depicts an average MNI normalized brain with the x, y, z coordinates of maximum activation (transformed to MNI brain atlas space, i.e., corrected for gross individual brain shape differences) within the FEFs that were stimulated, for all eight participants. In our stereotactic fMRI image-guided TMS setup (Neggers et al. 2004) we were able to individually determine the site on each participant’s scalp directly overlying his/her individual FEF activation maximum. Note the considerable variation of the coordinates across participants. This intersubject variation is such that considerable differences remain even after taking into account anatomical brain shape differences by normalizing the MR data to MNI space.

<table>
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<tr>
<th>Experiment</th>
<th>Part 1</th>
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<td>0.868</td>
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<td>0.576</td>
<td>0.778</td>
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FIG. 3. A: ratio of correctly identified DTs, as a function of ST location, pooled for target arrays left and right of the fixation cross (the DT always appeared at the left or right central location). Error bars denote SE. B: saccade latency distribution, pooled for all conditions. C: saccade horizontal landing point coordinate distribution for 3 different saccade target eccentricities, pooled for left and right target arrays.
LEFTWARD VERSUS RIGHTWARD SACCADeS. An additional RFX group analysis of the fMRI data was performed, contrasting the hemodynamic responses for leftward with rightward saccades of the eight subjects that participated in the present study as well as seven additional subjects from another experiment (unrelated to the present study) that were measured using the same localizer task. There were no voxels significantly activated in a different manner for leftward and rightward saccades in the whole brain analysis either in a more liberal ROI analysis (using small volume correction for multiple comparisons), where the region of interest was the FEF group activation map from Fig. 4B (based on the original saccade vs. rest statistical contrast).

TMS (experiment 3)

Here, we used the exact same paradigm as that in experiment 1, with the exception that we now applied TMS. Three TMS pulses were delivered over the individual FEFs as localized using fMRI in experiment 2. The pulses were timed such that the second pulse was delivered 30 ms before the DT was presented (see Fig. 2), which is estimated to result in the arrival of a disturbed feedback signal from the FEF in the visual cortex coinciding with the arrival of visual target information from the retina (see Experiment 3 in METHODS for motivation). In four separate counterbalanced sessions, the left or right FEF was stimulated, using a real TMS coil or a SHAM TMS coil. The
data were analyzed as in experiment 1; in addition trials were sorted and analyzed separately for saccades contra- and ipsilateral to the stimulated hemisphere, and for TMS and SHAM sessions.

Excluded trials

Again, trials were discarded from further analysis when saccades were made too early or were not accurate enough (see METHODS). This amounted to 23.8% of the trials. See Table 1 for the percentage of discarded trials for each individual subject for experiments 1 and 3. A two-sample t-test revealed that the number of trials discarded in both experiments was not significantly different \( \text{t}(14) = 1.62; P = 0.13 \).

Averaged over experiments 1 and 3, this number of excluded trials is only to a small degree (8% excluded) due to failure to respond to or inaccurate saccades, and largely due to premature saccades (an additional 19% excluded; the latter numbers do not add exactly up to the average percentages of excluded trials for experiments 1 and 3 because some trials are excluded based on both criteria).

To determine whether TMS altered the number of excluded premature saccades we calculated the number of saccades landing at the target earlier than 20 ms after the discrimination target was extinguished (but passing the other criteria), as well as the total number of trials passing all remaining criteria (e.g., the premature and nonpremature saccades). On average, for ipsi- and contralateral trials in TMS sessions 57.0 ± 5.0 and 56.25 ± 8.0 trials with target saccades were observed, of which 8.6 ± 5.2 and 8.4 ± 5.8 (ipsi- and contralateral) trials had premature saccades. For ipsi- and contralateral trials in SHAM sessions, 59.4 ± 10.9 and 59.9 ± 10.3 trials with target saccades were observed, of which 8.9 ± 10.7 and 9.4 ± 10.0 trials had premature saccades. The ratios between the numbers of premature saccade trials and total saccade trials were subjected to an ANOVA; TMS did not increase or decrease the ratio of premature saccades [TMS: \( F(7,1) = 0.02; P = 0.96 \)], nor as an interaction with saccade laterality with respect to the stimulated FEF [TMS \( \times \) SIDE: \( F(7,1) = 0.21; P = 0.89 \)].

Discrimination performance

The proportion of correctly identified targets is shown in Fig. 5A. As in experiment 1, a marked difference in discrimination performance was observed for trials with coinciding or different DT and ST [TARGET: \( F(2,14) = 33.51; P < 0.00001 \)], demonstrating that saccade planning affects visual discrimination performance at the saccade target. Results show that TMS over the FEF decreased the discrimination performance benefit for coinciding ST/DT target locations contralateral to the stimulated FEF. To test this statistically, repeated-measures ANOVAs were performed on the performance-difference scores for inner versus central and for outer versus center ST location, for each stimulation condition. The increase of the percentage correct responses for coinciding DT and ST locations with respect to noncoinciding locations was less for contralateral TMS compared with contralateral SHAM stimulation, an effect that was absent for ipsilateral TMS compared with SHAM [TMS \( \times \) SIDE: \( F(1,7) = 5.79; P = 0.05 \)]. That is, enhanced visual processing at targets of (prepared) saccades to the right was disturbed only for TMS on the left FEF, and vice versa. This was to be expected because the FEF projects to the ipsilateral visual cortex, and the visual cortex receives signals from the contralateral visual field.

Furthermore, in this experiment the performance increase for central ST locations (=DT location) compared with inner ST locations appeared to be larger than the performance increase for central ST locations compared with outer ST locations [TARGET: \( F(1,7) = 9.42; P = 0.018 \)]. Therefore two separate ANOVAs were computed: one for the perfor-
mance difference between central and inner and one ANOVA for the comparison inner-central and outer-central ST locations, for ipsi- and contralateral TMS and SHAM stimulation. It appeared that the hypothesized effect of contralateral TMS (compared with SHAM) on the performance increase for coinciding DT/ST locations is very clear for the comparison inner-central ST [TMS × SIDE: F(1, 7) = 26.82; P = 0.001], whereas no effect of contralateral TMS was observed for the comparison outer-central ST locations [TMS × SIDE: F(1, 7) = 0.2; P = 0.67].

Effects of stimulated hemisphere

To see whether the difference effects (between discrimination performance at the center ellipse and the other ellipses) of TMS were the same for left or right hemisphere FEF stimulation, in Fig. 6 A and B the discrimination performance is plotted for both hemispheres separately.

Overall, the effects were roughly similar for both stimulated hemispheres, e.g., only discrimination benefits for saccades directed contralaterally to the stimulated FEF are diminished due to TMS. To test this statistically, an additional ANOVA was performed where the data set was expanded to contain observations for both hemispheres (in the previous analyses data were always pooled over the stimulated hemispheres). Again, the TMS stimulation compared with SHAM tended to lower discrimination performance enhancements for coinciding DT and ST locations and saccades contralateral to TMS stimulation [TMS × SIDE: F(1, 7) = 5.09; P = 0.059; both inner-central and outer-central performance differences included]. There appeared to be an interaction (at the statistical trend level) of the latter effect for the stimulated hemisphere [TMS × SIDE × HEMI: F(1, 7) = 4.53; P = 0.071], e.g., the effects of TMS on the FEF on the coupling of discrimination performance to saccade location were somewhat different for right compared with left hemisphere stimulation.

To investigate this further, data for the difference in discrimination performance for inner-central ST locations, showing by far the strongest effects in the previous analyses (with data pooled for left and right hemisphere stimulation), and for outer-central ST locations were subjected to a per-hemisphere analyses. Again, there was a clear effect of TMS in diminishing the discrimination enhancements for coinciding DT–ST locations [TMS × SIDE: F(1, 7) = 21.24; P < 0.005]. Here, no difference was observed between left and right FEF stimulation regarding the effects of TMS in diminishing the discrimination enhancements for coinciding DT–ST locations contralateral to the stimulated FEF [TMS × SIDE × HEMI: F(1, 7) = 1.77; P = 0.23]. For the comparison outer-central ST location, the effect of TMS on performance difference was not significant [TMS × SIDE: F(1, 7) = 2.54; P = 0.16]; neither was the three-way interaction [TMS × SIDE × HEMI: F(1, 7) = 1.98; P = 0.20].

Saccade latency and accuracy

The average saccade latency (262 ± 43 ms) in accepted trials was not changed by TMS stimulation compared with SHAM, neither as a main effect [TMS: F(1, 7) = 0.093; P = 0.77] nor as an interaction with ST position [TMS × TARGET: F(2, 14) = 1.05; P = 0.38] or stimulated side or both [TMS × SIDE: F(1, 7) = 0.214; P = 0.66, TARGET × TMS × SIDE: F(2, 14) = 0.261; P = 0.77]. See also Fig. 5 B for the distribution of saccade latencies for the mentioned conditions. Again, saccades were more accurate for the inner compared with central and outer ST location as reflected in the deviation analyses [TARGET: F(2, 14) = 7.97; P = 0.005], but none of the interactions with TMS or stimulated side reached significance [TARGET × TMS: F(2, 14) = 1.97; P = 0.22, TARGET × TMS × SIDE: F(2, 14) = 0.06; P = 0.95, TARGET × SIDE: F(2, 14) = 0.58; P = 0.59] (see also Fig. 5 C). The latter findings imply that the observed differences in discrimination performance for saccades contralateral to the stimulated FEF cannot be explained as an indirect effect of TMS-induced faster or slower saccade initiation, or more or less accurate saccade execution/planning.

DISCUSSION

The present results demonstrate that the FEF modulates visual discrimination performance during saccade preparation. TMS on the FEF 30 ms before DT presentation decreased the well-known improvement of visual discrimination performance at saccade goals, for saccades directed contralateral to the stimulated FEF. This effect (based on the difference between trials where the saccade target coincided with the DT and trials where they did not) was similar for left and right FEF stimulation, as expected based on the lateralization of and projections between the FEF and visual cortex. However, as can be seen in Fig. 6, A and B, the absolute performance measures for each saccade target individually are somewhat different for left and right FEF stimulation, indicating that the link between attentional processing and eye movements, al-

FIG. 6. Ratio of correct target identifications, as a function of ST locations, for left (A) and right (B) FEF stimulation separately. Black/gray lines denote data from TMS/SHAM blocks, respectively, and squares/circles denote data from contra- and ipsilateral FEF stimulation with respect to saccade direction. Error bars represent SE.
though present in both hemispheres and affected by TMS on the FEF, functions differently in both hemispheres. Saccade latencies were unaffected by FEF stimulation.

Of note, TMS was applied 30 ms before DT presentation. Earliest visual responses in FEF neurons occur after 55 ms (Schmolesky et al. 1998), and direct TMS-induced neuronal responses as measured with EEG last 30 ms at most (Ilmoniemi et al. 1997). The idea that TMS administration 30 ms before DT presentation disturbed the influence the FEFs normally exert over information processing within the visual cortex during saccade preparation (Moore and Armstrong 2003) is therefore compatible with the present findings—that is, the TMS-discrimination target interval was chosen such that the TMS-disturbed signal from the FEF arrives simultaneously in the visual cortex with incoming visual information about the discrimination target. However, some of our assumptions on signal conduction times and duration of TMS-induced neuronal disturbances are rough estimates (see Experiment 3 in METHODS). Effects on the performance in discriminating visual probes after a single TMS pulse on the visual cortex last about 60 ms in a psychophysical study on human subjects (Kammer et al. 2005), where we assume a duration of electrically induced TMS effects in the FEF of 30 ms at most based on TMS-evoked EEG measurements (Ilmoniemi et al. 1997). This might then imply that indeed some attentional filtering could have been done within the FEF itself, due to a considerably longer presence of TMS effects in the FEF than assumed. Furthermore, the electrical conduction times that were used to determine TMS–DT intervals in the behavioral paradigm (see Experiment 3 in METHODS) are derived from available neurophysiological data from another species, the macaque monkey. Finally, it cannot be ruled out that in the FEF the preparation of a saccade is already underway before the saccade go-signal, and then later in the FEF this summed “priming” of a certain location could enhance incoming visual signal processing. This explanation, however, also requires that this prelude activity in the FEF as well as the persistence of the TMS-evoked effects outlive 30 ms, which might not be the case (Ilmoniemi et al. 1997). These discrepancies and uncertainties imply that solely based on the present results, it cannot be ruled out that the link between saccade preparation and visual processing enhancements is also implemented within the FEF itself to some extent. Further research is required to determine the duration of cortically induced TMS effects and the specific timing effects of TMS pulse on the FEF with respect to visuospatial attention shifts. However, combined with the other studies mentioned in the introduction (Moore and Armstrong 2003; Ruff et al. 2006; Super et al. 2004) showing direct influences from the FEF over the visual cortex in the absence of eye movements, the present results are compatible with the idea that feedback from the FEF to the visual cortex causes the coupling of saccade programming and visuospatial attention.

Interestingly, spatial visual attention sometimes deviates from eye movement targets, leading to debates regarding the scope of the premotor theory of attention (Hunt and Kingstone 2003; Juan et al. 2004; Klein and Pontefract 1994). Perhaps also other (occipitotemporal) brain areas project to the visual cortex (De Weerd et al. 1999), constituting top-down biases according to cognitive demand. Still, preparing saccades poses a strong bias on visual processing, although other sources of selectivity may exist. One might argue that visual performance changed for TMS trials because participants were anticipating TMS, subsequently behaving differently or more cautiously. Two observations render this unlikely. First, only performance in trials with saccades contralateral to the stimulated FEF was affected. This corresponds to the lateralization of the FEF for saccade direction and the contralateral retinotopy of the visual cortex. General cognitive effects on discrimination performance of expecting TMS would equally affect ipsi- and contralateral saccade trials. Second, saccade latencies and accuracy were similar for TMS and SHAM blocks, which one would not expect when TMS caused increased arousal.

Furthermore, recent studies on monkeys (Moore and Fallah 2001, 2004) and humans (Grosbras and Paas 2003; O’Shea et al. 2004; Ruff et al. 2006; Silvanto et al. 2006) reported discrimination performance improvements after FEF stimulation. However, we observed a decrease of discrimination performance at saccade targets for TMS trials. Although the aforementioned studies also attribute observed changes to feedback from the FEF to the visual cortex, they did not study visual performance changes induced by saccades, but during fixation, a qualitatively different measure. A putative yet speculative account on the function of connections between the FEF and the visual cortex (Moore and Armstrong 2003; Ruff et al. 2006) is that they subserve saccade preparation. The present findings support this view by demonstrating TMS-induced disturbance of the effects of saccade preparation on visual processing itself. Finally, without saccades to the discrimination targets they would be unperceivable (performance close to chance in experiments 1 and 3 for trials where the ST does not coincide with the DT), another important difference with other studies.

Lateralization of TMS effects

We assumed in the introduction that also for humans, the FEFs are lateralized with respect to saccade direction and project to the ipsilateral visual cortex, and thus correctly predicted that FEF stimulation affects contralateral visual discrimination performance. The reported lateralized visual performance costs when stimulating one FEF are seemingly at odds with the finding that TMS of one FEF during fMRI scanning activates the visual cortex bilaterally (Ruff et al. 2006). However, we observed a clearly lateralized effect of TMS on saccade-enhanced visual processing, which is not the same measurement as BOLD signal changes in the visual cortex. Second, TMS of the FEF might have excited ipsilateral visual cortex through the mentioned ipsilateral cortico-cortical feedback (Moore and Fallah 2004) and, subsequently, the contralateral visual cortex could have been inhibited and/or excited by the ipsilateral visual cortex through transcallosal fiber bundles. In contralateral visual cortex both excitatory and inhibitory transhemispheric connections would result in BOLD signal increases as a result of an increase of cerebral blood flow evoked by GABAergic and glutameric metabolism, explaining the findings of Ruff et al. (2006). Although the link between glutamate excitatory synaptoc neurotransmission and cerebral blood flow dominating the fMRI BOLD signal is increasingly well understood (Raichle 2001), the exact metabolic effects on cerebral blood flow of inhibitory GABAergic synapses are only very recently under investigation. Interest-
ningly, a recent report showed that the BOLD effect contributed by inhibitory synapses might also be positive (Nasrallah et al. 2007).

A related problem in interpreting the current findings is that when using fMRI it is surprisingly difficult to find lateralization of the FEF with respect to saccade direction, whereas monkey electrophysiology measures clearly show the FEF should be lateralized (Bruce et al. 1985). In line with this, the present fMRI experiment did not reveal laterality of the FEF according to saccade direction (which was primarily due to the task design; see METHODS). On a speculative account, the general difficulty in finding FEF lateralization using fMRI could be due to the nature of the BOLD signal, a vascular measure, and not so much to the underlying electrical neuronal activity. When a saccade is executed neurons representing this saccade in the contralateral FEF usually activate, whereas neurons in the ipsilateral FEF are often actively inhibited (Schlag et al. 1998), most likely through interhemispheric inhibitory connections. As mentioned earlier, the metabolism of GABAergic inhibitory connections might evoke increases in cerebral blood flow but still inhibit contralateral FEF neurons, causing bilateral fMRI activation but contralateral electrical neuronal activation.

Possible underlying pathways between FEF and visual cortex

The pathway by which the FEF influences visual cortical neurons is subject to debate. Direct projections between FEF and visual cortical neurons exist (Schall et al. 1995). However, activity of V4 neurons could be changed by subthreshold microstimulation of the FEF only when V4 neurons were driven by stimuli in their receptive field, implying that a direct and monosynaptic effect is unlikely (Moore and Armstrong 2003). Furthermore, human subjects do not report phosphenes during cortical FEF stimulation (Godoy et al. 1990; Penfield and Rasmussen 1950), which can be evoked from V4 (Ray et al. 1998). Rather then using a direct projection to the visual cortex, signals from FEF saccade neurons might reach the visual cortex through a dorsal network of visuomotor areas (Moore and Armstrong 2003) including the intraparietal sulcus (LIP in monkeys). LIP is connected to visual cortical areas and FEF saccade neurons (Stanton et al. 1995). Furthermore, it cannot be ruled out that FEF signals reach the visual cortex through known projections to the superior colliculus in the midbrain (Sommer and Wurtz 2000), from where these signals can be relayed to extrastriate areas. How FEF signals actually reach the visual cortex therefore requires more research. For example, lesion studies on monkeys selectively affecting one of the three mentioned pathways might elucidate which pathway to the visual cortex is primarily responsible for coupling saccade preparation and visuospatial attention. Furthermore, TMS on the IPS in human subjects using the same paradigm as that in the present study and various TMS–target intervals could indicate whether the influence of the FEF on the visual cortex is relayed through the parietal lobe.

As suggested earlier, the FEF might also influence the visual cortex through the superior colliculus (SC). Furthermore, the pathway from the FEF to the SC might induce presaccadic modulation of visuospatial attention within the SC itself, and not exclusively in the visual cortex. That is, the SC also receives, besides signals from the FEF, input from the visual cortex (Fries 1984).

Saccade latency and TMS of the FEF

Although saccade latencies were unaffected by TMS, the results can still be reconciled with other studies that did observe TMS effects on saccade latency under specific circumstances. TMS on the FEF 60 ms before saccade initiation delays prosaccades (Priori et al. 1993). Antisaccades were delayed when stimulating the FEF 100 ms after target presentation (~165 ms before saccade onset), but not at 80 or 120 ms (Terao et al. 1998). Prosaccades at that target–TMS interval were unaffected. Possibly, the narrow time window in which TMS is effective in delaying saccades reflects buildup of a signal directly driving saccades rather than the earlier saccade preparation/anticipation-related signals (not necessarily leading to saccades), known to exist in the FEF (Everling and Munoz 2000). In the present study TMS was on average delivered 170 ms before saccade onset, earlier than the 60 ms delaying prosaccades (Priori et al. 1993). We therefore might have stimulated the FEF during an early preparation phase (while the FEF is active at a level not directly driving a saccade) rather than motor command buildup. Furthermore, the interpretation of our results would be complicated when saccades were delayed by TMS. That is, the altered discrimination performance of visual targets could then either be directly attributable to TMS effects on cortical neuronal processing related to attentional processing as hypothesized or be indirectly caused by a delayed initiation of saccades (and associated attentional shift).

Individual localization of the FEF using fMRI

The fact that TMS on the FEF shortly before saccade initiation (Priori et al. 1993) can delay saccades allows for another way to individually localize the FEF, as an alternative to using fMRI. Some studies searched for a localized region on the scalp, where saccades were effectively delayed with TMS, and used these regions for FEF stimulation in subsequent experiments, yielding robust effects of TMS (e.g., Grosbas and Paus 2003). In the original experiments describing the FEF, however, separate classes of visual, visuomotor, and saccade motor neurons were observed within the FEF (Bruce and Goldberg 1985; Mohler et al. 1973). The present study therefore localized the FEF using fMRI measurements during a pro- and antisaccade task, eliciting both visual- and motor-related activation. In our opinion, this FEF localization technique is therefore close to the original definition of the FEF based on single-cell responses to saccades and visual stimuli for nonhuman primates. Both localization methods would most likely result in the accurate stimulation of at least parts of the FEF, depending on how one defines the FEF, and further research is required to demonstrate which localization method has the greatest impact on discrimination performance when used for TMS. The method yielding the largest effects of TMS on visual attention shifts would depend on where the projections from the FEF to extrastriate areas originate—at visual, visuomotor, or motor neurons in the FEF. Here it was assumed that at least visual and visuomotor neurons in the FEF must be affected to disturb the signals projected back to the visual cortex. It would
be hard to compare which method is “correct” in localizing the FEF, as discussed earlier, both methods use a functionally somewhat different definition of what the FEF actually is. Furthermore, our fMRI stereotactic localization method was validated with an fMRI thumb-movement experiment and subsequent TMS-EMG measurements, and was estimated to have an average spatial error ≤4 mm (Neggers et al. 2004), which should be sufficiently accurate for TMS pulses affecting tissue within a range of 1–2 cm (Bohning et al. 2001; Neggers et al. 2004).

The function of FEF control over visual attention shifts

The functional role of the proposed FEF influence on visual cortical processing as demonstrated in recent studies is still subject to debate. In general, saccades are needed to direct the fovea to interesting parts of the visual environment. However, the fovea’s diameter corresponds to only about 1° of visual angle. A certain accuracy when making saccades is thus required. Therefore “preparation” of saccades requires determination of saccade metrics (amplitude/direction) with appropriate precision. When the intention to make a saccade arises it would be helpful to obtain enhanced target representations by improved processing of visual cortical neurons representing the target area. That is, small or detailed targets (e.g., letters) require more precise foveation than large or homogeneous targets. In the present experiments we were actually able to disrupt a known link between eye movements and visual perception by stimulating the FEF, above and beyond the influence that FEF stimulation can reportedly exert on visual cortical activity and visual discrimination during fixation (Grosbras and Paus 2003; Moore and Fallah 2001, 2004; O’Shea et al. 2004; Ruff et al. 2006). This strengthens the idea that direct or indirect projections from the FEF to the visual cortex actually subserve saccade preparation.

In sum, our results indicate a central role for the FEF in directing visuospatial attention across the visual field during saccade preparation. Together with other recent evidence on the influence of the FEF over the visual cortex, our findings suggest that cortico-cortical feedback from the FEF to the visual cortex implements the coupling between saccade preparation and visual attentional shifts. However, our predictions depended on the validity of assumptions regarding neuronal signaling times and the persistence of TMS effects, and thus alternative mechanisms of how the FEFs control visuospatial attention cannot be fully ruled out.

The present findings might be a manifestation of a general system of cortico-cortical feedback channels from (pre-)motor structures to the visual cortex, acting as motor-induced top-down signals improving aspects of visual cortical processing relevant for actions in preparation.

ACKNOWLEDGMENTS

We thank M. Raemaekers for useful advice regarding the design of the fMRI study and N. Ramsey for helping with the development of the new fMRI pulse sequence.

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