Voluntary selective attention can prioritize different features in a visual scene. The frontal eye-fields (FEF) are one potential source of such feature-specific top-down signals, but causal evidence for influences on visual cortex (as was shown for “spatial” attention) has remained elusive. Here, we show that transcranial magnetic stimulation (TMS) applied to right FEF increased the blood oxygen level-dependent (BOLD) signals in visual areas processing “target feature” but not in “distractor feature”-processing regions. TMS-induced BOLD signals increase in motion-responsive visual cortex (MT+) when motion was attended in a display with moving dots superimposed on face stimuli, but in face-responsive fusiform area (FFA) when faces were attended to. These TMS effects on BOLD signal in both regions were negatively related to performance (on the motion task), supporting the behavioral relevance of this pathway. Our findings provide new causal evidence for the human FEF in the control of nonspatial “feature-based” attention, mediated by dynamic influences on feature-specific visual cortex that vary with the currently attended property.

Keywords: concurrent TMS-fMRI, feature attention, frontal eye-fields, top-down control

Introduction

Causal (i.e., interventional) evidence for top-down modulation of visual cortex by frontal eye-fields (FEF) has started to emerge from monkey studies (Moore and Fallah 2004; Ekstrom et al. 2009). Recent human studies have analogously revealed causal influences of FEF on visual cortex, by combining transcranial magnetic stimulation (TMS) with concurrent fMRI (Ruff et al. 2006; Blankenburg et al. 2010) or with concurrent electroencephalography (EEG) (Taylor et al. 2007; Morishima et al. 2009). Morishima et al. (2009) tested specifically the role of human FEF in the control of (nonspatial) “feature-based” attention. By applying TMS over a site close to right FEF, the authors demonstrated that, subsequent to a single TMS pulse, evoked potentials at posterior electrodes differed depending on whether the participants attended faces or moving dot stimuli. The high temporal resolution of EEG allowed demonstration of differential attention-effects on feature-specific posterior EEG components following a TMS pulse within 20–40 ms, suggesting a role for right FEF as an origin for top-down signals affecting processing in posterior regions. However, the exact neural origin of the EEG signals affected by TMS remains less clear due to the technique’s limited spatial resolution. Also, it did not permit a distinction between FEF influences on target and distractor representations. Finally, it was not considered whether right FEF acts alone or in conjunction with left FEF.

To test whether and how signals are propagated from right FEF to distinct feature-processing areas of the visual cortex (and to left FEF) during feature attention, we combined right FEF TMS concurrently with fMRI, while participants attended to motion or faces (or passively viewed the stimuli) in a variant of the task previously used with TMS-EEG (Morishima et al. 2009). Participants were cued to report either the gender of a face or the direction of a moving field of dots that were always superimposed over the face stimulus. We also included a third condition requiring passive viewing of this display, against which to contrast the attention conditions. TMS was applied in a train of 3 pulses starting 40 ms from the onset of the face + motion display, either with high (neurally effective) or low (ineffective, i.e., control) intensity during all 3 conditions (see Fig. 1). The experiment thus comprised 6 conditions in a 3 × 2 factorial design, however, for simplicity of exposition, the dependent variable referred to in the rest of the text is the difference between the 2 TMS intensities (referred to as “TMS effect”).

Materials and Methods

Participants

Sixteen healthy participants (7 females, age range 19–36 years) were all right handed with normal or corrected visual acuity. Following screening for any contraindications to MRI and TMS, all provided informed consent in accord with local ethics clearance.

Stimuli and Procedure

Participants undertook a feature-based visual attention task in the scanner (see Fig. 1), adapted from Morishima et al. (2009). A letter cue appeared for 180 ms on each trial 1100 ms prior to the target display (500 ms) instructing the participant to attend to either the gender of a face (F) and to make a gender judgment (male/female) or to attend to a field of moving dots (M) and to make a judgment on the motion direction (leftward or rightward diagonal), with both types of stimuli superimposed for all conditions (see Fig. 1). In a third neutral condition (cued by the letter “P”), participants were instructed to passively view the same superimposed displays. Participants responded by pressing 1 of 2 keys on an MR-compatible button box or pressed a third key immediately following stimulus offset for the passive condition. Fixation had to be maintained throughout the trial and was monitored by infrared eye tracking (see below).

Seventy-two gray-scale face stimuli (36 males) were drawn from a face database (FaceGen 3.1; see Oosterhof and Todorov 2008). Face stimuli were cropped to a round shape, blurred (radius 15 Adobe Photoshop [CS4] blur tool) and hair was removed. Motion stimuli were generated such that 750 target dots were moving with a speed of 12°/s diagonally across the screen, either in upper-leftward or upper-rightward direction (mean angle of ±45° [±15°] to vertical plane).
Randomly moving dots (190) were added to introduce 20% noise in the motion direction signal. The total display extended 6° of visual angle and was centered on a black background (∼30° × ∼22° of visual angle).

A short train of TMS (3 pulses at 11 Hz) was delivered on each trial, starting 40 ms after target onset. Pulses were given on each trial either at high intensity (110% of individual resting motor threshold [RMT], see below) or at a much lower intensity (40% RMT), with a 50% probability of occurrence. The high-intensity TMS was expected to be neurally effective whereas the low TMS should have no impact on neural processing but would control for nonspecific TMS effects (such as the acoustic “click” and tactile skin sensation accompanying a TMS pulse).

Each scanning session was comprised of 4 runs with 72 trials each (~14 min per run). Task presentation, TMS triggering, intensity regulation, and relay settings were controlled using the Cogent toolbox (http://www.vislab.ucl.ac.uk/cogent_2000.php).

TMS
Individual RMT were obtained in a separate session prior to scanning, via stimulation over the right M1 “hotspot” for inducing a visible twitch of the first dorsal interosseus muscle in 5 of 10 trials (mean RMT: 72 ± 8% maximal output).

Scalp coordinates for the stimulation sites were first located outside the scanner via theBrainsight Frameless stereotaxic system and software package (Rogue Research, Montreal, Canada), using the native space of each participant’s own T1-weighted anatomical MR image. The target FEF site was identified in the right hemisphere based on anatomical landmarks in the posterior middle frontal gyrus, immediately ventral to the junction of superior frontal sulcus and ascending limb of precentral sulcus as described in earlier work from our laboratory (Ruff et al. 2006). The selected FEF site, after normalizing to MNI space, corresponded to mean XYZ coordinates of 31, 1, 58 (±2, 2, 1 SEM). See Figure 2a for display of a 10-mm sphere centered at these mean MNI coordinates; see also Supplementary Figure 2a for BOLD signals for all 6 conditions separately.

Interleaved TMS-fMRI
Functional images were acquired on a 1.5 T MR system (Siemens Sonata, Erlangen, Germany), with a single channel receive head array.

Figure 1. Schematic example of a single trial. Participants were instructed either to attend the gender of a face (as indicated by “f” cue) or the motion direction (“m” cue) in a field of dots (containing 20% random motion) or passively view the display (“p” cue), with both types of stimuli superimposed on displays that were visually equivalent across all conditions. Short bursts of transcranial magnetic stimulation (TMS) (3 pulses at 11 Hz at either 40% or 110% of RMT) were applied over right FEF commencing 40 ms following target display onset. ITI, intertrial interval (2.5–3.5 volume repetitions [TR] or 8910 ± 1485 ms). CTI, cue-target interval. The TMS parameters were selected based on other recent TMS-fMRI studies (Feredoes et al. 2011; Heinen et al. 2011) and TMS safety guidelines (Rossi et al. 2009).

Figure 2. TMS effects on BOLD signal in the stimulated right FEF and the left FEF ROI, depend on active attention. (a) The brain image depicts the mean location, averaged across participants, of 10 mm spheres (shown in red) used as ROIs for the targeted right FEF and for the left FEF projected onto a MNI normalized brain (mean MNI coordinates right FEF: 31, 1, 58; mean MNI coordinates left FEF: −31 −3 57). (b) The bars display the TMS-induced difference (high minus low intensity) in BOLD signal extracted from the right FEF ROI for all 3 tasks conditions. TMS affected the BOLD signal for the motion and face tasks, but not for the passive viewing task (see main text). Error bars show ±SEM, and asterisks indicate significant (P < 0.05) differences in post hoc paired t-tests. See Supplementary Figure 2a for BOLD signals for all 6 conditions separately. (c) A similar pattern to right FEF-TMS effects on BOLD activity was found in the left FEF ROI (see also Supplementary Fig. 2b).
T1*-weighted echo planar image (EPI) volumes were acquired every 2.97 s covering the whole brain (TR = 90 ms, TE = 42 ms, 64 × 96 matrix, 33 axial slices, 3 × 3 × 2.5 mm, slice distance of 50%). For details regarding the custom MR sequence to account for artifacts due to presence of the TMS coil, see Ruff et al. (2006); Bestmann et al. (2008); Heinen et al. (2011). A field map scan (double-echo FLASH (gradient recalled echo) sequence with TE1 = 10 ms, TE2 = 12.46 ms; 3 × 3 × 2-mm resolution with 1-mm gap) was also acquired.

In the scanner, the TMS coil was positioned over the marked location with the cable oriented 45° from the vertical midline in a posteromedial direction. A Magstim Super Rapid2 stimulator was used to generate TMS pulses (3 at 11 Hz), together with an MR-compatible, nonferrous figure-of-eight coil (70 mm) (Magstim, Whitland, UK). TMS pulses were always given at fixed time points within the acquisition of subsequent MR slices, thus resulting in fixed interpulse intervals of 90 ms (corresponding to 11 Hz). For further details of the concurrent TMS-fMRI set-up, see also (Bestmann et al. 2008; Heinen et al. 2011) and for details on the relay-based leakage current prevention solution see Weiskopf et al. (2009).

Eye position was monitored with an ASL 504 Remote Infrared Eyetracker (60 Hz; Applied Science Laboratories). Online inspection confirmed good central fixation and post hoc analysis confirmed the occurrence of saccades >1° on only a small proportion of trials (<2%). No significant differences between conditions were observed (F5,11 = 0.09, P > 0.7) and no trials were excluded. However, recorded eye position coordinates were added as regressors to the general linear model to account for and remove any possible small biases associated with eye gaze direction (see below).

Inverse Efficiency (IE) Score Calculation

Reaction times (RT) and accuracy were measured and then combined to yield IE scores (Townsend and Ashby 1982) as mean reaction time divided by proportion correct, thereby including speed and accuracy in one measure (but see Supplementary Fig. 2 for measures of RT and accuracy separately). Three subjects were excluded from behavioral analyses, 1 due to a faulty button response box and the other 2 due to near-chance performance on the motion task.

Data Analysis

Functional MRI Data

Analysis of imaging data was undertaken using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) and Marsbar (http://marsbar.sourceforge.net/). The fMRI data were corrected for any possible TMS artifacts (see also: Ruff et al. 2006; Bestmann et al. 2008; Weiskopf et al. 2009; Heinen et al. 2011) and high-pass filtered (128 s cut-off).

All volumes were realigned to the sixth volume (first 5 were discarded), geometrically unwrapped using the acquired field maps, spatially normalized to Montreal Neurological Institute (MNI) anatomical standard space and spatially smoothed (8 mm). BOLD responses were modeled by convolution with the canonical hemodynamic response function (HRF) and its temporal derivative, using a General Linear Model, including regressors for each of the 6 experimental conditions. In addition, we included 6 head motion measures (translation variables x, y, z and rotational variables pitch, roll, and yaw) and recorded eye-coordinates (x and y) as regressors of no interest (coordinate value averaged across scan).

Regions of Interest

The first regions of interest (ROIs) was targeted right FEF. A 10-mm sphere was placed at the site of each individual’s native space TMS target and masked with a contrast for main task effects (P < 0.001 uncorrected). Left FEF ROI spheres were defined based on the same individual anatomical landmarks as applied to right FEF (but now in the left hemisphere) and again masked for main task effects. Note that this ROI was thus defined independent of any empirically measured impact of TMS. To identify candidate motion-responsive regions (MT+ complex), motion-cued trials were contrasted against face-cued trials regardless of TMS intensity at group level (thresholded at P < 0.05 uncorrected). The resulting activation maps were then inclusively masked by an MNI space anatomical map for MT+ (taken from MarsBar: http://marsbar.sourceforge.net/). The location for left and right MT+ is shown in Figure 3a in the main text. Because the location and extent of the “fusiform face area” (FFA) (Kanwisher and Yovel 2006; Meng et al. 2012) can vary considerably across individuals, a participant-by-participant approach was undertaken for defining this region. For each participant,
face-cued trials were contrasted against motion-cued trials. Responsive voxels ($P<0.05$, uncorrected) were inclusively masked per individual by an anatomical fusiform ROI in MNI space (taken from MarsBar and adjusted, such that it comprised the posterior two-third of the fusiform gyrus). In this way, we were able to identify left and/or right FFA in each participant (15 of 16 for left hemisphere and 12 of 16 for right hemisphere). Compound images combining the ROIs of all participants for left and right FFA are displayed in Figure 3c for illustrative purposes.

The mean percent signal change in each condition from the main experiment was then extracted, using MarsBar, for each ROI, in the following way: for each participant, parameter estimates were scaled to reflect percent signal change relative to the voxel baseline and were averaged across all voxels within the ROIs, and then averaged across trial type.

**Results**

**TMS-Dependent BOLD Effects in Right and Left FEF**

We first tested for effects of TMS on BOLD signals in the stimulated right FEF and in the left FEF by means of region-of-interest (ROI) analyses (see Fig. 2a for spheres centered around the mean ROI coordinates averaged across individuals and Materials and Methods section for applied method). We found positive TMS effects on BOLD signals in the right FEF and left FEF ROI in both active attention conditions, but not for the passive viewing condition (Fig. 2b), leading to a task-by-TMS interaction (right FEF: $F_{2,14}=3.6$, $P<0.05$; pairwise contrasts: face vs. passive $T_{(15)}=2.4$, $P<0.05$, motion vs. passive $T_{(15)}=3.0$, $P<0.05$; left FEF: $F_{2,14}=6.0$, $P<0.05$; pairwise contrasts: face vs. passive $T_{(15)}=2.5$, $P<0.05$, motion vs. passive $T_{(15)}=2.8$, $P<0.05$; see Fig. 2b,c and Supplementary Fig. S2a,b). TMS thus affected local BOLD signals underneath the TMS coil and in contralateral left FEF in a way that depended on attentional context.

**TMS-Dependent BOLD Effects in Feature-Selective Visual Cortex**

**Motion-Responsive MT+**

In each participant, we first identified visual areas sensitive to motion in the left and the right hemisphere and then extracted BOLD signal from these areas for each task condition (see Materials and Methods section). Figure 3a, left panel, shows the MT+ ROIs at a group level. TMS effects on BOLD signal for left and right MT+ did not differ significantly ($T_{(15)}=1.4$, $P>0.2$), and were therefore pooled. TMS effects on MT+ BOLD signal are shown in Figure 3a (right panel) and Supplementary Figure 2c. Unlike FEF (cf. Fig. 2), such BOLD signal changes in MT+ increased only when participants were instructed to make a judgment on the direction of moving dots. In contrast, there was no significant effect of TMS on MT+ activity when participants were either attending to faces or passively viewing the stimuli, leading to a significant task-by-TMS interaction ($F_{2,14}=4.0$, $P<0.05$; pairwise contrasts: motion vs. face $T_{(15)}=2.1$, motion vs. passive $T_{(15)}=3.1$, $P<0.05$).

**Face-Responsive FFA**

FFA ROIs were defined for each participant separately by contrasting attend-face trials with attend-motion trials (with additional anatomical constraints; see Materials and Methods section). Figure 3b (left panel) shows the compound image combining all individual FFA ROIs. The results for TMS effects on BOLD in left and right FFA did not differ significantly ($T_{(10)}=0.28$, $P>0.7$) and were again pooled. TMS enhanced FFA BOLD now only during the condition in which faces were attended, with no such TMS effects observed for the other 2 conditions (task × TMS interaction $F_{2,14}=3.3$, $P<0.05$; pairwise contrasts: face vs. motion $T_{(15)}=2.2$ face vs. passive $T_{(15)}=2.6$, $P<0.05$; see right panel Fig. 3b and Supplementary Fig. S2d).

**TMS Impact on Behavior and Brain-Behavior Relations**

Across participants, right FEF stimulation impaired performance mainly in the (more difficult) motion task (see Fig. 4a). IE scores increased specifically for high-intensity TMS during attention to motion, expressed as a task-by-TMS interaction ($F_{3,10}=5.4$, $P<0.05$) with a disruptive TMS effect on motion ($T_{(12)}=−2.8$, $P<0.05$) but not on face trials ($T_{(12)}=−1.0$, $P>0.3$ ns). Similar effects were observed for proportion correct and reaction time scores separately (see Supplementary Fig. 3).

We next examined whether the participant-by-participant impact of TMS on IE might have a corresponding impact on BOLD signal in each of the ROIs. We found a positive correlation between the TMS effect on BOLD signal in MT+ and motion task performance ($r(12)=0.56$, $P<0.05$; Fig. 4b). Thus, “higher” BOLD signals (due to high vs. low TMS) were associated with impaired performance. A similar relationship was found between the TMS impact on BOLD in the right FEF and performance on the motion task ($r(12)=0.55$, $P<0.05$; Fig. 4c). There were no such brain–behavior relations for the (easier) face task ($P>0.45$).

**Discussion**

We used concurrent TMS-fMRI to test the causal role of the FEF in top-down modulation of neural responses in distinct visual areas during nonspatial feature attention. We found that right FEF TMS affected BOLD responses in posterior motion- and face-responsive visual regions (MT+ and FFA, respectively) in a manner that depended on the attended feature (motion or faces; Fig. 3). Importantly, we found only “target” feature-related effects of FEF TMS (i.e., influences on MT+ but not FFA when attending motion, and vice versa when attending to faces), with no TMS effects in the unattended category ROIs. This indicates that the FEF selectively highlights task-relevant information (Schall and Hanes 1993), possibly by specifically modulating functional connectivity with visual areas processing the target feature. This functional connectivity may reflect selective synchronization of activity patterns between neural populations in the FEF and target feature-processing regions (Wommelsdorf et al. 2007; Fries 2009) facilitating transmission of the TMS-induced signal during attention to the relevant feature.

TMS increased BOLD signal both locally in right FEF and contralaterally in left FEF during the feature-attention conditions, but not during passive viewing (Fig. 2b). The right FEF was thus more influenced by TMS while participants were actively attending to a feature and signals arising here were propagated interhemispherically in this context, akin to previous TMS findings in the motor system (Wassermann et al. 1996; Bestmann et al. 2008). Effects of right FEF TMS on contralateral left FEF have been shown in a previous study, measuring remote BOLD effects following off-line (theataburst) TMS application (Hubl et al. 2008). A recent DTI study demonstrated fiber pathways that connect bilateral FEF via the
supplementary eye fields in humans (Anderson et al. 2012). This pattern of anatomical connections is consistent with the known pathways between these regions in macaque monkeys (Stanton et al. 1995) and may facilitate the signal transmission from right to left FEF. We demonstrate here the involvement of bilateral FEF during feature-based attention. However, to assess whether left FEF plays a causal role in feature attention would require a follow-up study directly targeting this area.

Our findings of context-dependent FEF-TMS effects in anatomically remote regions are consistent with recent monkey studies demonstrating more pronounced modulatory effects on V4 by electrical stimulation in FEF during increased task demands (Premereur et al. 2013). Monosynaptic anatomical connections are known to exist between FEF and MT+ and FEF and temporal occipital cortex in monkeys (Stanton et al. 1995; Ninomiya et al. 2012) and using TMS, functional connectivity between FEF and MT+ has also been demonstrated in humans (Silvanto et al. 2006). The study of Ninomiya et al. (2012) furthermore provides anatomical evidence that top-down modulation from the FEF is not a unitary process but involves segregated neuronal populations (within FEF) and pathways. To our knowledge, there is no anatomical evidence for direct fibers between right FEF to left MT or FFA. Activity in left visual cortex may therefore have been modulated by TMS polysynaptically, possibly via trans-callosal connections between extrastriate visual areas (see Pietrasanta et al. 2012) or a subcortical pathway.

The induced and transmitted TMS signals were directly linked to task performance, shown by a correlation between the TMS-induced “impairment” in performance on the motion task (indexed by increased IE) and increased BOLD both in right FEF and MT+ (Fig. 4b,c). This effect is opposite to what has been described earlier in the study by Ruff et al. (2006). However, in their study, the task was performed separately outside the scanner, while the TMS effects on BOLD were obtained with no task present. Furthermore, the nature of the task was different. The seemingly counter-intuitive effect in the current study could be due to the fact that the task involved specific neuronal populations (coding different motion directions) intermixed within the same visual area. An overall enhancement of BOLD signal by TMS therefore does not necessarily imply an increased specificity between activation patterns of motion direction coding neurons, needed for the

Figure 4. TMS effect on performance and its relation to TMS effects on BOLD in right FEF and MT+ for the motion task. (a) Mean IE scores (see Materials and Methods section) are shown separately for the high- and low-intensity FEF TMS in the motion and face task, with error bars showing ±1 SEM. High TMS led to worse performance (higher IE) on the motion task. Performance on the face task was overall better than on the motion task, and there was no significant effect of effective versus ineffective TMS on the face task. (b) Scatterplot, with regression line, for TMS-induced differences in BOLD signal in MT+, plotted against TMS-induced differences in IE scores for the motion task. Each data point represents one participant. Larger TMS-induced BOLD increases in MT+ were correlated with larger decrements in performance for the motion task. (c) A similar relationship was found in right FEF; that is, larger TMS-induced increases in right FEF BOLD signal correlated with larger performance decrements during the motion task.
discrimination task. On the contrary, due to spatial resolution constraints of TMS, swaths of FEF, including neuronal populations coding for nonrelevant directions may have been activated (introducing neural noise), rather than discrete neuronal populations, thereby impairing discrimination. The content of the TMS-induced signal may thus have been noisy, while propagated along task-relevant pathways. Under normal physiological circumstances however, signals from FEF may enhance salience by specifically modulating motion direction coding neurons activated by the stimulus (Maunsell and Treue 2006). As for the face task, the TMS-induced enhancement in BOLD signal in the FFA may have been too weak to interfere with performance. Possibly because participants were closer to ceiling on this task (so that TMS-induced neural noise has less prominent effects), or it may reflect that in contrast to motion direction discrimination, the face gender discrimination task depends more on global processing than on competitive interactions between different neural populations coding different sensory alternatives (Tanaka and Farah, 1993). Any top-down signal on the FFA from the FEF may thus not have had a similarly disruptive effect on task-relevant fine-grained neural activity patterns in the FFA.

The way in which we defined the FFA ROIs (by attentional modulation) differs slightly from conventional “face localizer” methods described in the literature. It is possible that the attention task in our study relied more on left than right FFA processing. For example, a recent study demonstrated that left FFA involvement is more influenced by task context (e.g., attention) than right FFA (Meng et al. 2012). This may explain why bilateral FFA was not reliably identified in every participant. Alternatively, the precise spatial extent of FFA may have been slightly diluted by any responses to the field of moving dots. However, the average coordinates for our ROIs (−35 −54 −14 for left FFA and 36 −43 −17 for right FFA) were nevertheless well within the bounds reported in the literature for this specific area (Kanwisher et al. 1997).

Taken together, our findings provide causal evidence supporting the emerging view that FEF is not only involved in dynamic, top-down modulation of posterior visual areas during spatial attention, but also during nonspatial feature-based attention (Bichot et al. 1996; Hung et al. 2011; Liu et al. 2011; Zhou and Desimone, 2011). These FEF influences mainly affect the currently relevant (rather than irrelevant) feature, indicating that attentional top-down effects arising from this area may serve to strengthen neural signals coding behaviorally relevant stimulus features.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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