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Article

Concurrent TMS-fMRI and Psychophysics Reveal Frontal Influences on Human Retinotopic Visual Cortex

Christian C. Ruff,[1,2,3,*] Felix Blankenburg,[1,3] Otto Bjoertomt,[1,3] Sven Bestmann,[1,3,4] Elliot Freeman,[1,2] John-Dylan Haynes,[5] Geraint Rees,[1,3] Oliver Josephs,[3] Ralf Deichmann,[3] and Jon Driver[1,2,3]

[1] UCL Institute of Cognitive Neuroscience
University College London
17 Queen Square
London WC1N 3AR
United Kingdom

[2] UCL Department of Psychology
University College London
26 Bedford Way
London WC1H 0AP
United Kingdom

[3] Wellcome Department of Imaging Neuroscience
University College London
12 Queen Square
London WC1N 3BG
United Kingdom

[4] Sobell Department of Motor Neuroscience and Movement Disorders
University College London
Queen Square House
Queen Square
London WC1N 3BG
United Kingdom

[5] Max-Planck Institute for Human Cognitive and Brain Sciences
Stephanstrasse 1a
D-04103 Leipzig
Germany

Summary

Background: Regions in human frontal cortex may have modulatory top-down influences on retinotopic visual cortex, but to date neuroimaging methods have only been able to provide indirect evidence for such functional interactions between remote but interconnected brain regions. Here we combined transcranial magnetic stimulation (TMS) with concurrent functional magnetic resonance imaging (fMRI), plus psychophysics, to show that stimulation of the right human frontal eye-field (FEF) produced a characteristic topographic pattern of activity changes in retinotopic visual areas V1-V4, with functional consequences for visual perception.

Results: FEF TMS led to activity increases for retinotopic representations of the peripheral visual field, but to activity decreases for the central field, in areas V1-V4. These frontal influences on visual cortex occurred in a top-down manner, independently of visual input. TMS of a control site (vertex) did not elicit such visual modulations, and saccades, blinks, or pupil dilation could not account for our results. Finally, the effects of FEF TMS on activity in retinotopic visual cortex led to a behavioral prediction that we confirmed psychophysically by showing that TMS of the frontal site (again compared with vertex) enhanced perceived contrast for peripheral relative to central visual stimuli.

Conclusions: Our results provide causal evidence that circuits originating in the human FEF can modulate activity in retinotopic visual cortex, in a manner that differentiates the central and peripheral visual field, with functional consequences for perception. More generally, our study illustrates how the new approach of concurrent TMS-fMRI can now reveal causal interactions between remote but interconnected areas of the human brain.

Introduction

Activity in human visual cortex does not depend solely on current input from the retina. Neuroimaging has shown that early retinotopic areas (including V1) can exhibit activity changes even when no visual stimulus is present as a result of factors such as directed attention [1–5] or saccades in darkness [6]. The sources for such modulation of occipital sites are debated but are widely thought to include influences from frontal regions [7–11]. Although it has often been suggested that human frontal cortex may modulate activity in posterior sensory cortices in a "top-down" manner [8, 10, 12–16], such influences have rarely been shown directly. New methodological approaches may be required for direct study of any such causal influences between remote but interconnected regions in the human brain.

Here we combined functional magnetic resonance imaging (fMRI) with concurrent transcranial magnetic stimulation (TMS), which is technically demanding to implement in the scanner but is now achievable [17–19]. In this way, we studied directly whether stimulating over a particular region of frontal cortex (human frontal eye-field, FEF) could modulate fMRI activity in remote occipital visual areas V1-V4 and thus tested for causal influences on retinotopic visual cortex.

We applied frontal TMS over the right posterior middle frontal gyrus, just ventral to the junction of superior frontal sulcus and ascending limb of precentral sulcus, in each individual (see red star in Figure 1A for schematic, Figure S1 for TMS site in individual brains, and supplemental text for TMS-localization procedures). This particular frontal site is widely held to correspond to human FEF on the basis of prior neuroimaging [20], electrical stimulation [21], and purely behavioral TMS studies [22–26]. We chose this specific frontal region as the initial target for TMS for three reasons. First, it is often activated in PET or fMRI studies of directed attention [7] or saccade plans [20, 27], and so it might in principle relate to the occipital modulations observed in such paradigms. Second, recent elegant work using invasive FEF microstimulation in monkeys indicates that influences of this frontal site on visual cortex have some

*Correspondence: c.ruff@ucl.ac.uk

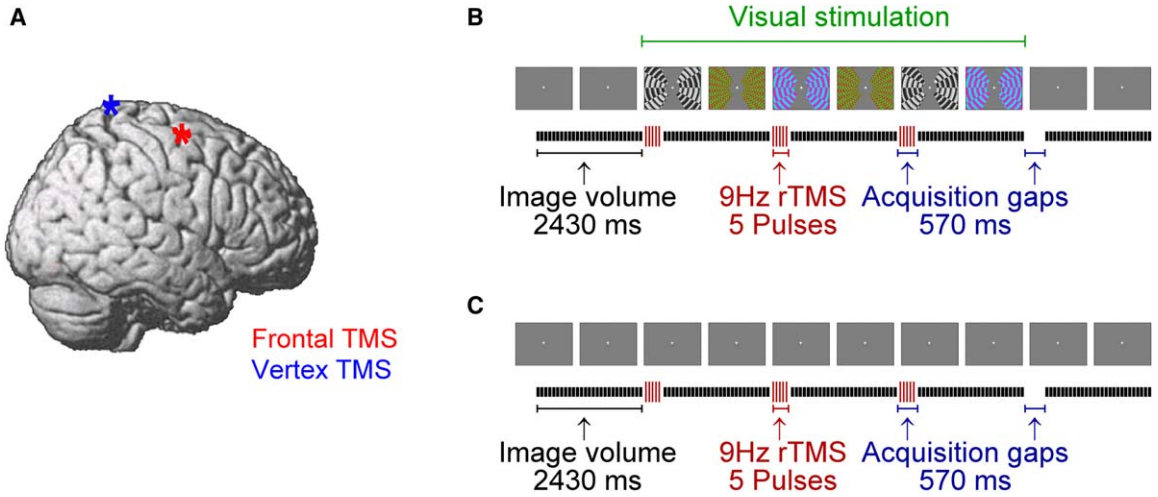

Figure 1. Stimulation Sites and Interleaved TMS and fMRI Protocol

(A) Frontal (red star, over right human FEF) and vertex-control (blue star) TMS sites on a normalized brain template (see Figure S1 for TMS sites on each individual's brain).

(B and C) Schematic timecourse of TMS relative to MR volume acquisition during combined TMS-fMRI. (B) Trials with visual stimuli on the screen during TMS; (C) trials without visual stimuli. For each trial, three TMS trains were delivered in the 570 ms gaps between acquisition of subsequent image volumes, and seven rest scans were included between successive trials. Visual stimuli (when present, as in [B]) remained visible during all three TMS trains and during the acquisition of the three image volumes after the TMS trains.

physiological plausibility in the primate brain at the single-unit level [28]. Finally, TMS to right human FEF can affect some visual judgements behaviorally in both hemifields [22–25, 29]. Here we propose that this might reflect remote influences on activity in retinotopic visual cortex. We tested this directly by measuring human brain activity through the use of blood-oxygenation-level-dependent (BOLD) contrast fMRI in humans. The BOLD signal provides an index of neuronal population activity [30–34] and allowed us to measure any TMS-evoked remote effect on multiple visual areas of the human brain concurrently.

In separate scanning sessions, we applied TMS during fMRI either to right FEF or to a control site at the vertex. We selected the vertex site in order to control for nonspecific effects of TMS because vertex TMS would not be expected to affect visual cortex except by nonspecific means (see Experimental Procedures and Supplemental Data for further rationale). We applied TMS to either site at four different intensities, allowing us to identify any visual brain areas showing activity changes due to the intensity of TMS rather than merely its presence versus absence. Participants had to fixate centrally, with no other task during scanning, to ensure that any remote physiological influences of TMS on activity in visual cortex could not be contaminated by TMS-induced changes in behavior. We administered TMS either while subjects passively viewed a blank display or while they were presented with bilateral moving and changing visual stimuli designed to activate many visual regions (see Figures 1B and 1C). We could thus test whether any TMS influences on activity in visual cortex might depend on the level of bottom-up activation via visual inputs.

We found that increasing the intensity of FEF TMS produced a characteristic pattern of activity modulations in early retinotopic visual areas V1-V4. These activity changes arose in a top-down manner regardless of current visual input, in accord with previous fMRI findings that visual cortex can show activity changes even in the absence of visual stimuli, e.g., during directed attention [3] or saccades in darkness [6]. By contrast, TMS to the control site (vertex) produced no such influences on visual cortex, thus demonstrating the specificity of the FEF TMS effects. Further analyses showed that those effects were not due to eye movements, blinks, or pupil dilation.

The specific retinotopic pattern of fMRI modulations caused in visual cortex by FEF TMS led to a new prediction for perceptual effects that we confirmed in separate psychophysical TMS work outside the scanner. Taken together, our results provide causal evidence that the human frontal eye-field can modulate activity in early retinotopic visual cortex in a manner that differentiates the central from the peripheral visual field, with corresponding consequences for perception.

Results

Concurrent TMS-fMRI

In both fMRI sessions (right FEF or vertex control), we maximized sensitivity for early visual cortex (areas V1-V4) by using an occipital surface coil for fMRI in combination with retinotopic mapping of cortical visual areas for each individual participant. Although TMS does not induce eye movements [22–26], we were careful to assess and eliminate any possible influences on visual cortex from blinks, pupil dilations, or losses of fixation (all measured throughout scanning). We also took considerable care to avoid MR artifacts from concurrent use of TMS (see Experimental Procedures and Supplemental Data).

We used two complementary analysis approaches for the fMRI data. Group analyses of activity across the

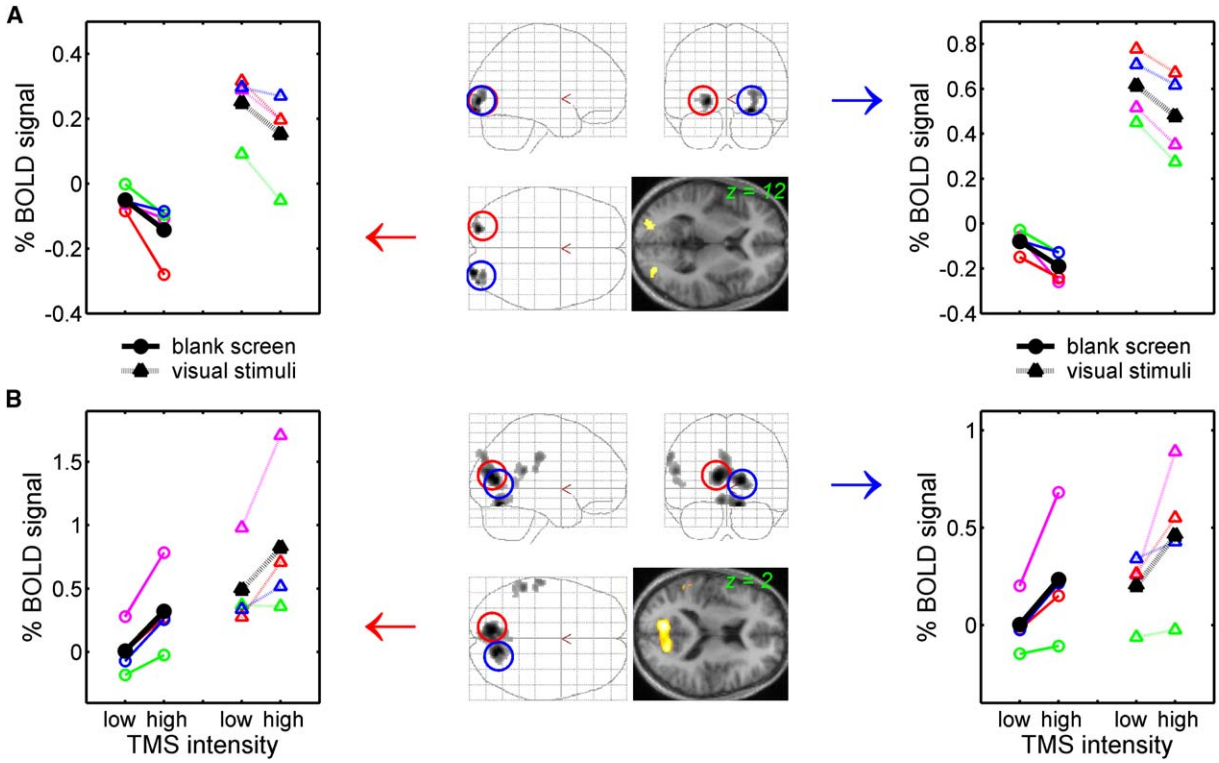

Figure 2. Frontal TMS: Activity Changes in the Group Analysis for Stereotactic Space

The brain displays and associated graphs show (A) significant negative correlations or (B) significant positive correlations of BOLD with frontal-TMS intensity. In the central images these effects are shown as 2D projections of the whole-volume SPM(*T*) onto a transparent schematic of the MNI template brain (used here so that no region is hidden) and as renderings onto a transverse slice of the mean structural scan. All thresholds are set to $T > 3$ and $p < 0.05$ (corrected for multiple comparisons at cluster level). The graphs on either side show single-subject plots of the mean signal intensity (different colors for different subjects, group average in black) in the left-hemisphere regions circled by red in the glass brains (left graphs) or for the right-hemisphere regions circled by blue (right graphs) for the two highest versus two lowest TMS intensities (see also Figure S2 for results at each of the four TMS intensities separately). The plots show that the described effects in the calcarine and occipital-pole regions were consistently present across subjects, both when visual stimuli were present (dotted lines) and when they were absent (solid lines) during TMS. Overall activity in these visual regions was higher with visual stimulation (dotted) than without (solid), but the impact of high versus low intensity of frontal TMS was additive to this.

image volume (EPI images covering occipital cortex and extending into temporal cortex were acquired with the visual surface coil) identified any regions in stereotactic space that reliably displayed activity changes as a function of TMS intensity (or mere TMS presence). We also used standard retinotopic mapping procedures [35] within each individual, in conjunction with cortical flattening [36, 37], to visualize the topography of any TMS effects on early retinotopic areas.

Group Analyses in Stereotactic Space

Group whole-volume analysis revealed two bilateral sets of occipital regions with activity levels related to FEF TMS intensity. A significant negative relationship between BOLD signal and TMS intensity was found in bilateral regions close to the occipital poles (these regions therefore represented central visual locations), with stronger FEF TMS leading to lower activity there (Figure 2A; see also Table S1). The opposite pattern, of significantly higher activity with stronger FEF TMS, was found for bilateral regions in anterior-calcarine sulci (representing the more peripheral visual field; Figure 2B; see also Table S1). These opposite effects on anterior-calcarine sulci versus occipital poles were present in each participant, as shown in plots of mean activity for these regions under high or low TMS intensities (see graphs on either side of Figures 2A and 2B; see also Figure S2). These plots additionally demonstrate that the influence of FEF TMS intensity on these occipital regions was equivalent during the presence or absence of visual stimuli (Figures 2A and 2B), even though overall activity was higher during visual stimulation. No region in the acquired volumes displayed any interaction of frontal-TMS intensity with the presence versus absence of visual stimuli.

By contrast, increased intensity of vertex TMS did not elicit any significant activity changes in visual cortex (the corresponding results in Figure 2 show no significant effects). We formally confirmed this difference between the TMS sites (i.e., frontal versus vertex TMS) during scanning by extracting the mean signal from spherical regions of interest (ROIs, 6 mm radius) centered in the regions that displayed activity changes during FEF TMS (see circles in Figure 2). For both the occipital-pole (central visual field) and anterior-calcarine (peripheral visual field) regions, the modulatory effects of TMS intensity (two highest versus two lowest intensities) were significantly bigger for FEF than for vertex TMS (2×2 repeated-measures ANOVA on the signals from these ROIs; interaction of TMS intensity × TMS site, $p < 0.05$, for each ROI). Pairwise comparisons showed

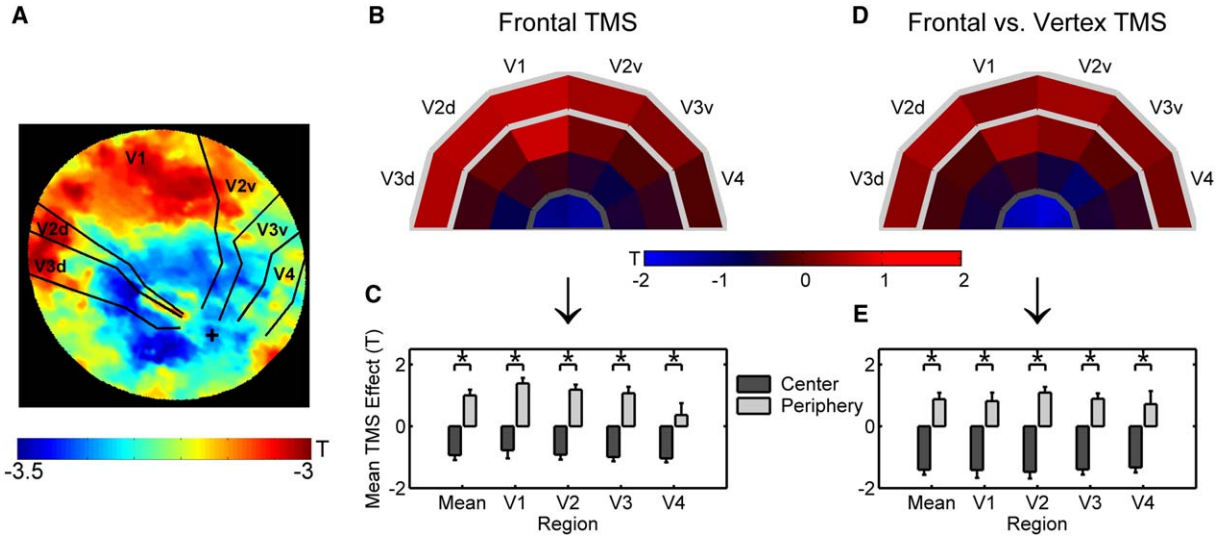

Figure 3. Frontal TMS: Mean Effects for Different Eccentricity Sectors in Retinotopic Visual Areas

(A) An illustrative flatmap of retinotopic visual areas in one subject and hemisphere (see Figure S3 for all others). The flatmaps confirm BOLD-signal increases with stronger frontal TMS in retinotopic representations of the peripheral visual field but BOLD-signal decreases instead for central-visual-field representations around the foveal confluence. The voxel-wise correlation of BOLD with TMS intensity is plotted as a standardized *T* value (in relation to voxel-wise residuals of the model) according to the color bar at bottom. Hot colors indicate positive and cold colors indicate negative correlations with TMS intensity. The foveal representation is indicated approximately by the cross, and borders of all mapped visual areas are indicated by black lines.

(B–E) The correlation of TMS intensity with BOLD (quantified as *T* value) was extracted from each individual flatmap, separately for four different eccentricity sectors in each region (see Supplemental Data). (B) Mean effect of frontal-TMS intensity for each area and eccentricity sector, averaged across flatmaps and voxels within each sector (this measure is conservative, given the larger effects at peak voxels). The most central sector is outlined in dark gray, and the most peripheral sector is outlined in light gray. The effects are color coded according to the scale below. (D) An analogous representation, but now for *differences* between effects of frontal versus vertex TMS. Both (B) and (D) indicate that increased intensity of frontal TMS produced activity increases for peripheral-visual-field representations in V1–V4 ("outer" segments in these graphs) but activity decreases in the most central eccentricity sector. Panels (C) and (E) plot the corresponding mean TMS-induced effect with its standard error ([C] for frontal TMS; [E] for frontal-minus-vertex difference) for the most central and the most peripheral eccentricity sectors when data are averaged across visual areas (leftmost two bars) or separately for areas V1–V4 (data are pooled across dorsal and ventral subdivisions). In all these retinotopic visual areas, increased frontal-TMS intensity produced activity increases for the peripheral sector but activity decreases for the central sector (stars indicate $p < .05$ in paired t tests).

that TMS intensity had significant effects only for FEF TMS (paired t tests, all $p < 0.05$) but not for vertex TMS (all not significant [n.s.]). Finally, the differences in TMS effects *between* the ROIs (occipital poles versus calcarine sulci, i.e., the differential effects for central versus peripheral visual field) were also significantly stronger for FEF than for vertex TMS (2 × 2 repeated-measures ANOVA, interaction of ROI × TMS site, $p < 0.05$). Taken together, these initial group analyses in stereotactic space indicate that TMS intensity over the FEF, but not the vertex, modulated activity in occipital cortex differentially for representations of the peripheral versus central visual field. As discussed below, we confirmed this pattern in further detail by examining individually flatmapped retinotopic visual areas.

Individual Retinotopic Analyses

A topographic pattern of FEF TMS effects on fMRI activity in occipital visual cortex was reliably present in early retinotopic visual areas for all participants and hemispheres (Figure 3A shows one example; Figure S3 shows all). Specifically, we found activity increases with stronger FEF TMS in peripheral visual field representations for each retinotopic visual area (notably, including even V1), whereas activity decreases were located in representations of the central visual field around the foveal confluence. Although individual flatmaps (Figure S3) show minor variations, as typical for such data, the overall pattern was clearly present in each.

We confirmed this consistency by quantifying the pattern across subjects. We divided each of the areas V1–V4 into four sectors representing different eccentricities in the visual field (see Supplemental Data and [38]) and then extracted the inter-participant mean effect of FEF TMS intensity on BOLD signal for each such sector (see Figure 3B, where "inner" segments are less eccentric and "outer" segments are more eccentric). Figure 3C shows the mean effect of FEF TMS intensity for the most peripheral (light bars) and for the most central (dark bars) retinotopic sector in visual cortex. Averaged across areas V1–V4 (leftmost pair of bars in Figure 3C), activity in the peripheral sector was significantly increased by higher-intensity FEF TMS, but activity in the central sector was instead significantly decreased by higher-intensity FEF TMS (t tests, both $p < 0.001$). This same pattern also applied significantly when each retinotopic area was considered individually (Figure 3C; t tests, all $p < 0.05$, except for the trend in the peripheral V4 sector). In direct paired comparisons, the FEF TMS influence was significantly different for the peripheral than for the central sector in all visual areas (Figure 3C; asterisks indicate $p < 0.05$ in paired t tests).

These retinotopic analyses show that TMS over right human FEF had distinct effects on fMRI activity in

representations of the peripheral versus central visual field in early retinotopic visual areas. This accords with the spatially normalized group analysis presented earlier (Figures 2A and 2B), but the retinotopic analyses (Figure 3) additionally show that this topographic pattern of influences holds for multiple areas of early retinotopic human visual cortex, including even area V1.

Thus far, the retinotopic analyses only considered activity in visual areas during FEF TMS. We next compared this directly to the vertex-TMS scanning data by calculating the *differences* between FEF- and vertex-TMS intensity effects for each eccentricity sector in each retinotopic visual area (Figure 3D). This analysis showed essentially the same pattern as for the FEF TMS data alone because only that TMS site produced the topographic pattern of changed activity in retinotopic visual cortex (again consistent with the group analysis in stereotactic space, where vertex TMS was found to have no effect on occipital cortex). We found significant differences between the influences of FEF versus vertex TMS on the central versus peripheral sectors, both when data were pooled across visual areas and for each region alone (2×2 repeated-measures ANOVAs; $p < 0.05$ for all interactions between the TMS site and the central versus peripheral sector). Figure 3E shows these differences between FEF and vertex TMS-intensity effects for the most peripheral and most central retinotopic sectors. Note that a similar pattern is apparent in Figures 3E (difference of FEF and vertex TMS) and 3C (FEF-TMS effects only). Thus, even when directly accounting for any potential nonspecific effects of TMS (via comparison with the vertex TMS data), we still found that stronger FEF TMS led to significantly increased fMRI activity for sectors representing the peripheral visual field, but to decreased activity instead for sectors representing the central visual field, in every retinotopic visual area (compare light and dark bars for each pair in Figure 3E).

On-line eye-tracking throughout scanning (see Experimental Procedures) measured eye position, blinks, and pupil diameter. None of these factors can explain the observed pattern of FEF TMS effects on retinotopic visual cortex (see Figure S4 and supplemental text). The effects on visual cortex also cannot be plausibly attributed to any possible cross-modal influence of the "clicking" sound or somatosensory impact of TMS. Such nonspecific effects were equivalent for frontal and vertex TMS, with similar activation of auditory and somatosensory cortices by these TMS sites here (see Figure S5 and supplemental text).

In sum, these fMRI results show directly that TMS of frontal cortex, over right human FEF, causally modulated activity in retinotopic visual cortex (V1–V4) in a top-down manner. Stronger FEF TMS led to a specific retinotopic pattern of increased activity for the peripheral visual field but led to decreased activity for central visual-field representations, whereas this pattern was not produced by control TMS to the vertex.

Psychophysical Study

The fMRI results described above suggest a behavioral prediction that we tested in a further psychophysical experiment. We could now predict that TMS to the frontal site (over right FEF) may enhance peripheral vision relative to central vision for both hemifields. Given that early visual areas were modulated by FEF TMS, including even V1, we tested this behavioral prediction by using visual stimuli and a judged property that should involve early visual cortex; namely, the perceived contrast of Gabor patches. We applied TMS to the same frontal (right FEF) or vertex sites as before but now did so during a psychophysical task that required participants to judge which of two concurrent stimuli (one central and one peripheral Gabor patch, the latter presented randomly on the left or right) appeared higher in perceived contrast (see [39] for a similar measure). Central fixation was again ensured with on-line eye tracking. The central patch had a fixed (25%) contrast, whereas the peripheral patch on the left or right varied in contrast via an adaptive algorithm (see Experimental Procedures and Supplemental Data). We derived the point of subjective equality (PSE) between central and peripheral contrasts by fitting psychometric functions to the behavioral data (e.g., see Figure 4B). Separate PSEs were determined for each visual hemifield for TMS at the frontal or vertex site.

We chose these particular stimuli and this task for several reasons. Although extrapolating from fMRI signals to visual perception often requires many caveats, in the specific case of contrast there is already some evidence that BOLD increases in early visual cortex can be associated with increases in contrast perception [40–42]. Moreover, perceived contrast can be enhanced by top-down influences (e.g., by attention [39]), which might extend to the present top-down influences from FEF TMS also. Finally, it is often argued (e.g., [8, 10, 43]) that top-down increases in baseline occipital activity may lend a competitive advantage to corresponding visual stimuli when presented. Based on these findings and suggestions, we predicted that the topography of top-down occipital activity changes found during FEF TMS in our fMRI experiment may lead to enhancements of perceived contrast for peripheral relative to central stimuli.

The psychophysical results accorded with these predictions derived from our fMRI results, indicating that the effects of FEF TMS on activity in visual cortex can have perceptual consequences for vision. Perceived contrast judgements were altered systematically by FEF as compared to vertex TMS, with peripheral stimuli having stronger perceived contrast relative to central stimuli during FEF TMS (see Figures 4B–4C). Moreover, this pattern applied equivalently for either peripheral hemifield, again just as expected from our fMRI results in retinotopic cortex during FEF TMS. This outcome was confirmed in a 2 (frontal or vertex TMS) $\times$ 2 (peripheral patch on left or right) repeated-measures ANOVA of the PSE data, which showed a reliable effect of TMS site ($F(1,6) = 7.69$, $p < 0.05$) but no effect or interaction due to hemifield. Note that this effect corresponded to a lateral shift in the psychometric functions (see example in Figure 4B); although the PSEs differed significantly as a result of TMS site, the slopes of the underlying psychometric functions did not (all terms n.s. in a corresponding ANOVA on slopes). Finally, for completeness we also compared the two TMS conditions (which were run in counterbalanced order) to a no-TMS condition run at the end of each session (see Figure S6).

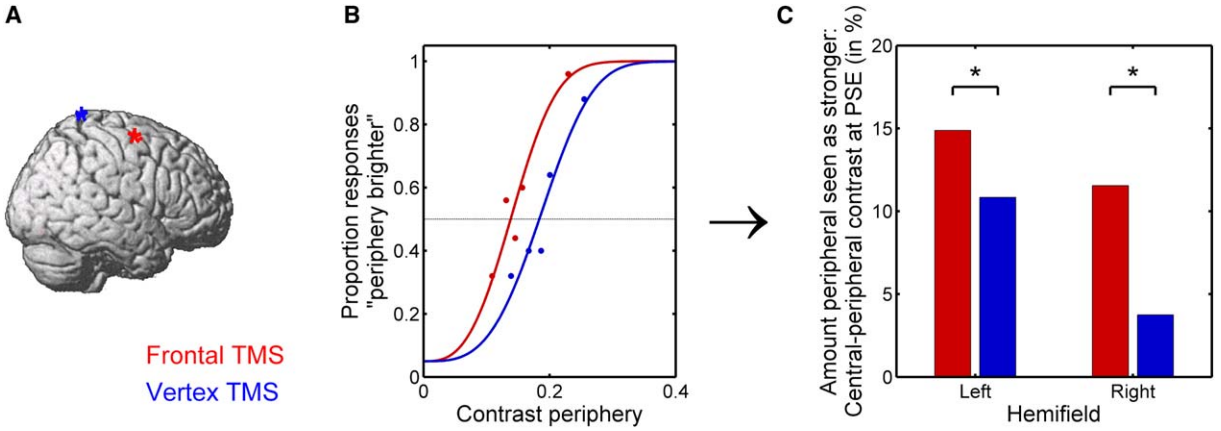

Figure 4. Frontal TMS Enhances Perceived Contrast for Peripheral Relative to Central Visual Stimuli for both Hemifields

(A) Frontal (red star) and vertex-control (blue star) TMS sites, selected according to the same criteria as in the neuroimaging experiments (cf. Figure 1A).

(B) Psychometric curves fitted to the psychophysical data of an illustrative participant (who had also taken part in neuroimaging) for one hemifield when the individual was judging which of two concurrent Gabor patches appeared higher in contrast (either the central patch of fixed contrast or a peripheral patch of varied contrast, unpredictably on the left or right). Separate psychometric functions were obtained with frontal TMS (red curve) or vertex TMS (blue curve) co-occurring with the visual displays in counterbalanced order. The intersection of the dashed horizontal line with either curve indicates the point of subjective equality (PSE) value for the peripheral patch (contrast at which the patch was perceived as equivalent to the fixed central patch) in the corresponding TMS condition; note the lateral shift of the psychometric curve as a result of frontal versus vertex TMS.

(C) Inter-participant mean contrast-value differences between central and peripheral stimuli at the derived PSE (in percent of contrast of central patch) for both TMS conditions and both hemifields. Because of the subtraction of contrast values at the PSE (central minus peripheral contrast value), higher values represent more enhancement of peripheral relative to central perceived contrast. The graph shows that, as compared with vertex TMS (blue bars), frontal TMS (red bars) significantly enhanced peripheral relative to central perceived contrast in both hemifields (stars indicate $p < 0.05$ for main effect of TMS site in ANOVA, in the absence of significant effect or interaction due to hemifield; see also Figure S6 for no-TMS data).

In sum, TMS of right human FEF significantly enhanced perceived contrast for peripheral visual stimuli relative to central stimuli in both hemifields. This accorded with the pattern of peripheral enhancement but central suppression that we observed for early retinotopic visual cortex in the fMRI experiments during TMS of the same frontal site.

Discussion

By combining fMRI with concurrent TMS in the scanner, we found that stimulating a region of frontal cortex (right human FEF) could produce systematic remote effects on fMRI signal in early human retinotopic cortex, including even area V1. These effects could not be attributed to blinks, changes in pupil size, or losses of fixation. The direct comparison with vertex TMS suggests that these effects also did not reflect any nonspecific TMS effects, such as the associated "clicking" sound. Our results thus provide causal evidence that signals originating in human frontal cortex are capable of modulating activity in early human visual cortex, as previously proposed only on much more indirect grounds [8, 10, 12–14].

The present effects of frontal TMS on visual cortex took a specific retinotopic form, with stronger TMS of right FEF increasing fMRI activity for representations of the peripheral visual field but reducing activity for the central field in all retinotopic visual areas. This fMRI pattern led to a novel behavioral prediction that we confirmed with psychophysics by showing that TMS to the same frontal site (versus vertex) enhanced perceived contrast for peripheral relative to central visual stimuli. Although it can be difficult to extrapolate from fMRI effects to visual perception, for the specific case of contrast a relation to fMRI signals in early visual cortex has been established [40–42]. This permitted our new approach of using a pattern of remote activity changes found with concurrent TMS-fMRI to derive (and confirm) a prediction for behavioral effects of TMS.

Our results echo but also extend recent findings from monkey studies. Elegant work by Moore and colleagues has shown that electrical microstimulation of macaque FEF neurons with implanted microelectrodes, at intensities too low to elicit a saccade, can modulate activity in V4 neurons with spatially corresponding receptive fields [28, 44]. At an abstract level, our results accord well with those monkey studies in establishing a *causal* effect of FEF on occipital visual cortex, now for the human brain. However, the studies differ in more concrete details. For instance, we showed that human FEF can influence even the earliest retinotopic visual areas (V1, V2, and V3). Moreover, the present effect of FEF TMS on visual cortex was independent of the concurrent changing and moving visual input, whereas the previous FEF-microstimulation effects on single-unit firing in V4 depended on the visual preferences of the individual neuron and on the preferred static stimulus being present for some time prior to microstimulation [28, 44]. Such differences in the details of our findings and the recent monkey work may be explained by methodological aspects, and one must be cautious in extrapolating from fMRI findings to single-unit findings or vice versa (see also [4] for a discussion of this issue). Here we indexed neural activity

from large populations by using BOLD-contrast fMRI, which may correlate better with local field potentials [31, 33] and synchronized population activity [34] than with spiking output. It has been suggested that BOLD-contrast fMRI may more closely index the input into an area than its local firing rates [30, 32, 45]. For this very reason, fMRI may be particularly sensitive to top-down influences [34, 46], as here. It should also be noted that TMS is very different from microstimulation and will target sizeable neural populations [47]. Most crucially, however, the present findings are fully consistent with other demonstrations that fMRI signal changes in visual cortex can arise without the presence of a visual stimulus (e.g., during directed attention [3, 4]). The present study shows directly that human FEF is a plausible source for such modulations. Moreover, it corroborates a new methodology for studying causal influences between brain areas; unlike the invasive approaches employed in monkeys, this methodology can now be used in humans.

The general point that TMS to frontal cortex can have some remote physiological effects in the human brain was first demonstrated in a pioneering PET study [48], which showed that frontal TMS (again over FEF) could lead to some changes in PET activity for posterior brain regions, such as the parieto-occipital sulcus. Moreover, one recent EEG study reported that TMS over a similar frontal site can change voltage fluctuations recorded from electrodes over posterior scalp positions [49]. Although PET and EEG studies cannot examine retinotopic visual cortex in any detail (because of methodological limitations; see Supplemental Data), here we were able to maximise power for visual cortex (albeit inevitably with less power for more anterior structures such as frontal or parietal cortex) by using fMRI with an occipital surface-coil in conjunction with individual retinotopic mapping. This allowed us to show that TMS of human FEF can affect early retinotopic visual areas, including even V1, with a specific topographic pattern. The new methodological combination of TMS during retinotopic fMRI of visual cortex now opens up many possibilities for future work, including TMS to further frontal or parietal sites in the same or opposite hemispheres.

The specific pattern of FEF TMS influences we found in human visual cortex may have implications for further research on the structure, function, and connectivity of the human FEF. The effects on visual cortex here arose bilaterally (despite right FEF stimulation) and affected even area V1, for which monosynaptic connections with the FEF have not been reported so far in the macaque brain [50, 51]. Although humans might differ from monkeys, we suspect that the FEF-occipital circuits underlying the present effects may be poly- rather than mono-synaptic and might involve intervening frontal [52], parietal [50, 51, 53], or subcortical [54] brain regions. Future extensions of the present method could combine TMS with whole-brain fMRI to examine any roles for intervening regions and pathways and might even test the contribution of transcallosal connections via split-brain patients [55]. However, our main aim here was to characterize any frontal influences on retinotopic visual cortex; we were able to achieve this aim by maximizing our power to detect such effects with an MR surface-coil centered over occipital cortex. Moreover, the bilateral nature of the fMRI effects from right-FEF TMS accorded well with the bilateral psychophysical result we found for the same TMS site, which affected perceived contrast for both visual fields.

It is also noteworthy that the present results revealed distinct effects of FEF TMS on peripheral versus central visual-field representations. This difference may accord with some known anatomical details of macaque FEF, where the central and the peripheral visual field appear functionally differentiated by two neuronal subpopulations. These code for either large saccades and peripheral visual locations or small saccades and more central locations, and they are mainly connected to occipital regions via separate pathways involved in more peripheral or more central vision, respectively [50, 51]. Subdivisions and anatomical connections for human FEF are not as well established as in monkeys, but there are now some initial demonstrations that the peripheral visual field may be represented spatiotopically in human FEF [56], in a patch of cortex readily targeted by TMS. Our results encourage further research into the question of whether the peripheral and central visual field might be separately represented within human FEF, with distinct connections to occipital cortex, in analogy to the macaque brain.

The nature of the fMRI effects on visual cortex as a result of FEF TMS here accorded well with our psychophysical TMS findings, which showed that perceived contrast was enhanced for peripheral relative to central visual stimuli in both hemifields during stimulation of the same frontal site. Such an enhancement for peripheral visual stimuli may conceivably play a functional role during saccade planning and execution or during covert attention to the visual periphery, consistent with the known involvement of the FEF in those situations [7, 10, 27, 57]. Our results may also reconcile seemingly discrepant results from prior, purely behavioral TMS studies that had likewise reported bilateral effects on visual judgments during stimulation of right human FEF. Some of those prior behavioral TMS studies found enhancements of visual judgments [22, 23], whereas others reported impairments instead [24, 25]. Although those prior behavioral studies differed from each other in several methodological details, our fMRI and psychophysical results highlight a previously overlooked factor. The previous reports of visual judgments facilitated by TMS of right FEF had presented visual targets more eccentrically [22, 23] than those reporting behavioral impairments instead [24, 25]; the latter studies used more central targets ($\sim 2^{\circ}$ visual angle). Our fMRI results directly show that TMS of right FEF has opposing effects on representations of the peripheral versus central visual field within retinotopic visual cortex, consistent with the perceptual effects that we found psychophysically.

At a more general level, our findings highlight the fact that TMS not only may affect the targeted cortical site in isolation but can also result in remote physiological effects on interconnected brain regions (as found here for visual cortex after FEF TMS), which may have functional consequences (as found here for visual perception). This could challenge some conventional interpretations of purely behavioral TMS effects; those interpretations have often considered only the targeted brain-site alone. However, this does not limit the utility

of TMS, which can now be used in combination with fMRI, as here, to study influences between brain regions (see also [48, 58]), as well as the causal roles of the targeted site in inducing such effects.

Conclusions

The present results establish that TMS of human frontal cortex, over the right human FEF, can causally modulate functional activity in early retinotopic visual cortex, in a systematic fashion that distinguishes the central and peripheral visual fields, with corresponding perceptual consequences. More generally, our study illustrates how combining TMS and fMRI now allows the direct study of causal functional interactions between remote but interconnected areas of the human brain.

Experimental Procedures

Participants

The same four male participants (29–35 years) took part in both neuroimaging experiments, and there were seven male participants (29–36 years, three of whom also took part in fMRI) in the psychophysical studies. All were right-handed and reported normal vision and no history of neurological or psychiatric illness. They participated with informed consent in accord with local ethics.

Neuroimaging Experiments: Setup and Stimulation

Functional data were acquired on a 1.5 T whole-body scanner (SONATA, Siemens, Erlangen, Germany) with a custom visual surface coil (Nova Medical, Boston, MA) with maximum sensitivity over occipital cortices and extending into temporal cortex. A multi-slice gradient echo EPI sequence was used to acquire BOLD contrast volumes with 27 transverse slices (slice TR 90 ms, 64 × 64 matrix, in-plane resolution: 3 × 3 mm, 2.5 mm slice thickness, 50% spatial gap between adjacent slices, TE = 50 ms). For the TMS-fMRI sessions, a 570 ms gap was included between acquisitions of subsequent volumes (see Figures 1B and 1C) to allow enough time to implement TMS without corrupting MR images. See the Supplemental Data for setup details and all the further technical procedures implemented to avoid MR artifacts during combination of TMS with fMRI.

The same experimental protocol was used for both scanning experiments, except for the TMS site. Each stimulation block comprised three equal-intensity trains of five TMS-pulses (9 Hz, intensity either at 85%, 70%, 55%, or 40% of total output); these were administered in the temporal gap between acquisitions of three subsequent image volumes (see Figures 1B and 1C). In each run (606 volumes), 48 TMS blocks, each interleaved with seven image volumes without any TMS stimulation, were delivered. An equal number of TMS blocks (six) were delivered at each of the four TMS intensity levels, with or without visual stimulation (see Supplemental Data). The run also contained twelve control blocks without any TMS, during which visual stimuli could be present or absent also.

Eye position, pupil diameter, and any blinks were monitored at 60 Hz throughout scanning with an ASL 504 Remote Optics Eye tracker (Applied Science Laboratories, Bedford, MA), via the same mirror used for visual stimulus viewing.

Neuroimaging: Image Processing and Analyses

Data from both sessions (frontal or vertex TMS) underwent identical analyses with SPM2 (http://www.fil.ion.ucl.ac.uk/spm). The first six images of each run were discarded. Images were realigned to the first of the series, corrected for movement-induced image distortions [59], normalised to the MNI stereotactic standard space, and spatially smoothed with a three-dimensional 6 mm full-width-at-half-maximum Gaussian kernel. The voxel-wise effects of the experimental conditions (four TMS stimulation intensities plus no TMS, each with and without visual stimulation) were estimated by multiple linear regression of the voxel time series onto a composite model of the hemodynamic response (see Supplemental Data). Appropriate linear contrasts of the regression parameters for the different conditions were used to assess effects of TMS intensity and presence, at a statistical threshold of $T > 3$ and $p < 0.05$ (corrected for multiple comparisons at the cluster level). All reported peak coordinates correspond to anatomical MNI space, as used in SPM2.

For retinotopic analyses, flattened representations of the SPM(T)s quantifying the correlation of TMS intensity with BOLD signal were plotted onto cortical flatmaps derived by segmentation and cortical flattening in MrGray [36, 37]. The borders of visual areas V1–V4 were determined for each subject by standard retinotopic meridian mapping procedures [35]; see Supplemental Data.

Psychophysical Study

TMS was administered to the frontal or vertex site in separate sets of four blocks (approximately 40 trials per block), and participants judged which of two concurrent Gabor stimuli had higher perceived contrast (see main text, plus Supplemental Data for visual stimulus details). On every trial, a train of 5 TMS pulses was administered using a Magstim Super Rapid stimulator at 10 Hz and 65% stimulator output (corresponding to the maximum TMS intensity in the fMRI experiments as a result of use of a custom MR-compatible TMS coil in the scanner; see Supplemental Data). To rule out order effects for the critical FEF vs vertex comparison, we repeated the procedure on a second day with the opposite order of TMS sites (i.e., AB-BA or BA-AB, counterbalanced between subjects). A training set preceded each session, and each of the two sessions ended with four additional blocks without TMS (these could not be permuted in order but were analyzed for completeness; see Figure S6). We independently adjusted the contrasts of left and right stimuli from trial to trial by using two interleaved adaptive staircases (Modified Binary Search algorithm [60]) in order to probe a contrast range optimally bracketing the point of subjective equality (PSE). For each of the four critical types of trials (left and right hemifield, frontal or vertex TMS), the peripheral PSE contrast was estimated offline by least-squares fitting of a Weibull curve through the obtained psychometric function.

Supplemental Data

Supplemental Data include six figures, one table, and Supplemental Experimental Procedures and are available with this article online at http://www.current-biology.com/cgi/content/full/16/15/1479/DC1/.

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