Effects of Parietal TMS on Visual and Auditory Processing at the Primary Cortical Level – A Concurrent TMS-fMRI Study

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Accumulating evidence suggests that multisensory interactions emerge already at the primary cortical level. Specifically, auditory inputs were shown to suppress activations in visual cortices when presented alone but amplify the blood oxygen level-dependent (BOLD) responses to concurrent visual inputs (and vice versa). This concurrent transcranial magnetic stimulation–functional magnetic resonance imaging (TMS-fMRI) study applied repetitive TMS trains at no, low, and high intensity over right intraparietal sulcus (IPS) and vertex to investigate top-down influences on visual and auditory cortices under 3 sensory contexts: visual, auditory, and no stimulation. IPS-TMS increased activations in auditory cortices irrespective of sensory context as a result of direct and nonspecific auditory TMS side effects. In contrast, IPS-TMS modulated activations in the visual cortex in a state-dependent fashion: it deactivated the visual cortex under no and auditory stimulation but amplified the BOLD response to visual stimulation. However, only the response amplification to visual stimulation was selective for IPS-TMS, while the deactivations observed for IPS- and Vertex-TMS resulted from crossmodal deactivations induced by auditory activity to TMS sounds. TMS to IPS may increase the responses in visual (or auditory) cortices to visual (or auditory) stimulation via a gain control mechanism or crossmodal interactions. Collectively, our results demonstrate that understanding TMS effects on (uni)sensory processing requires a multisensory perspective.

Keywords: crossmodal deactivations, interleaved/concurrent TMS-fMRI, multisensory integration, multisensory interactions, right intraparietal sulcus

Introduction

Multisensory integration was traditionally thought to be deferred until later processing stages in higher order association cortices. Recent evidence from neuroanatomy, electrophysiology and functional imaging in humans, nonhuman primates, and other species suggests that sensory inputs interact already at the primary, putatively unisensory, cortical level (Macaluso and Driver 2005; Schroeder and Foxe 2005; Ghazanfar and Schroeder 2006). Specifically, in human functional imaging studies, the effect of inputs from the nonpreferred sensory modality on activations in primary sensory cortices depends on the presence or absence of concurrent sensory inputs from the preferred modality (Laureti et al. 2002; Johnson and Zatorre 2005). For instance, auditory inputs suppressed activations in visual cortices when presented alone but amplified the blood oxygen level-dependent (BOLD) response to concurrent visual inputs (and vice versa). In other words, competitive interactions (crossmodal deactivations) between sensory cortices for unisensory stimulation mutated into cooperative interactions (superadditive response enhancement) for multisensory stimulation (Werner and Noppeney 2010a, 2011).

The neural mechanisms that mediate these “inhibitory” and “excitative” audiovisual interactions at the primary cortical level are currently unclear. Several functional architectures have been proposed such as feedforward thalamocortical, direct connectivity between sensory areas, and feedback from higher order association areas such as the intraparietal sulcus (IPS) or the superior temporal sulcus (Calvert 2001; Schroeder et al. 2003; Beauchamp et al. 2004; Hackett et al. 2007; Driver and Noesselt 2008; Sadaghi et al. 2009). Recent electroencephalography and transcranial magnetic stimulation (TMS) studies have supported thalamocortical and direct mechanisms by demonstrating multisensory interactions at less than 100 ms poststimulus (Foxe et al. 2000; Molholm et al. 2002; Murray et al. 2005; Romei et al. 2007; Cappe et al. 2010; Raji et al. 2010). Yet, given the sluggishness of the BOLD response, functional magnetic resonance imaging (fMRI) activations in primary sensory cortices may reflect a compound of early and late interactions. Indeed, a recent study combining fMRI and effective connectivity analyses (i.e., dynamic causal modeling) suggested that low-level audiovisual interactions may be mediated by both direct/thalamocortical influences and top-down effects from higher order association areas (Werner and Noppeney 2010a). From a cognitive perspective, these top-down effects may also reflect crossmodal modulation of attentional resources (Shomstein and Yantis 2004; Johnson and Zatorre 2005, 2006; Werner and Noppeney 2011). Thus, the IPS with its connectivity to visual or auditory cortices (Hyvarinen 1982; Maunsell and van Essen 1983; Boussaoud et al. 1990; Lewis and Van Essen 2000a) has been implicated in crossmodal attentional selection and switching (Macaluso et al. 2000; Rushworth et al. 2001; Yantis et al. 2002; Macaluso, Eimer, et al. 2003; Pessoa et al. 2009; Santangelo et al. 2009).

Concurrent (or interleaved) TMS-fMRI provides an alternative, technically challenging, causal interventional approach to study the effect that one region exerts over another brain area. Focusing on motor, sensory, and higher order cognitive processing, a number of recent studies have demonstrated an effect of TMS not only on the directly stimulated brain area but also on remote interconnected regions (Baudewig et al. 2001; Sack et al. 2007; Bestmann et al. 2008; Blankenburg et al. 2008; Ruff et al. 2008; 2009; Blankenburg et al. 2010). For instance, application of TMS to right but not left IPS induced functional changes in a widespread right hemispheric frontoparietal system and concurrent impairments of visuospatial processing.
(Sack et al. 2007). More relevant for the aim of the current study, IPS-TMS has also been shown to influence activations in visual and somatosensory cortices in a state-dependent fashion. Even though IPS-TMS increased activation in both visual and somatosensory cortices, this response amplification was observed in different contexts. In the primary visual cortices, IPS-TMS increased activations only in the absence of visual stimulation; it did not influence responses to visual stimulation (Ruff et al. 2008). In contrast, in the somatosensory cortices, IPS-TMS suppressed activations in the absence of somatosensory stimulation yet amplified the response to somatosensory stimuli (Blankenburg et al. 2008). These are surprising and puzzling results. They raise the question whether IPS may influence sensory processing from different modalities in fundamentally different ways.

This study pursued several aims: First, we investigated the influence of IPS-TMS on visual and auditory processing in the same experimental setting and subjects using a random effects approach. This is essential because studies have previously often included only very few subjects, so that differences between somatosensory and visual studies may not necessarily reflect differences between sensory systems but simply result from intersubject variability. Second, we investigated and interpreted the TMS effects not only from the classical unisensory perspective but also within a multisensory framework. Given previous research, we hypothesized that in particular deactivations in sensory cortices may be mediated via crossmodal mechanisms. To address these questions, we investigated the role of top-down influences from the right IPS on the activation profile in the visual and auditory cortices under 3 sensory contexts: visual, auditory, and no stimulation. To control for nonspecific TMS effects, we applied trains of repetitive TMS (rTMS; 1.9 Hz for 20 s) at no, low, and high intensity over right IPS and vertex. We hypothesized that high (vs. low and no) TMS to right IPS would alter the BOLD responses to sensory signals and the activation level in the absence of stimulation in both visual and auditory cortices as our a priori regions of interest.

Materials and Methods

Participants
Twenty participants (7 males; mean age: 25.2 years; standard deviation [SD]: 2.5; 2 left handed) with no history of neurological or psychiatric illness took part in this concurrent TMS-fMRI experiment. Participants had normal or corrected-to-normal vision and reported normal hearing. All participants gave informed consent prior to participation, and the study was approved by the Human Research Ethics Committee of the Medical Faculty at the University of Tübingen.

Experimental Design
The 3 × 3 × 2 factorial design manipulated: 1) sensory context (visual [V], auditory [A], and fixation [Fix]), 2) TMS stimulation intensity (no TMS, low TMS, and high TMS), and 3) TMS location (right IPS and vertex). TMS was applied at no, low, or high intensity either to right IPS or to vertex as a control site. Hence, as shown in Figure 1, the experimental design included 9 conditions for each TMS site amounting to 18 conditions in total.

This design enabled us to investigate the effect of IPS-TMS on auditory (or visual)-evoked activations in auditory (or visual) cortices. Moreover, from the multisensory perspective, we were able to investigate how IPS-TMS affects crossmodal deactivations such as auditory (or visual)-evoked deactivations in visual (or auditory) cortices. Participants were presented with blocks of fixation, auditory, and visual stimulation (block duration: 20 s; Fig. 1). They fixated a white fixation cross presented throughout the entire run in the center of the screen. To maintain participants' attention, they responded to rare auditory (a brief beep, frequency: 700 Hz, duration: 300 ms) and visual (a red fixation cross, duration: 300 ms) targets, which were presented in auditory and visual blocks, respectively.

Because of the static magnetic field of the MR scanner, the amplitude of the TMS clicks was amplified to 87.1 dB (low TMS intensity) and 97.3 dB (high TMS intensity). To attenuate these differences in auditory stimulation for low and high TMS intensity, we used dampening headphones and created pseudo-TMS clicks by recording the auditory click produced by a TMS pulse. Pseudo-TMS clicks were presented at 1.9 Hz (=frequency of rTMS stimulation) throughout the entire experiment, that is, in auditory, visual, fixation, and baseline blocks. In the TMS blocks, pseudoclicks and TMS pulses were synchronized. Simultaneous recording of pseudoclicks and real TMS pulses confirmed the perfect synchronization of the pseudo-TMS clicks and TMS pulses. Despite all these efforts, the auditory side effects were not completely equated for high and low TMS conditions most likely also because of additional bone conduction.

The activation blocks of 20 s alternated with 20-s baseline periods (Fig. 1B). We manipulated TMS intensity and the sensory stimulation context over blocks and the TMS location across sessions. The sequence of conditions was pseudorandomized and counterbalanced within and across participants. There were 5 runs per TMS stimulation location, each run included 2 blocks of each condition amounting to a total of 10 blocks per condition. In each run, 6 of the 21 condition blocks contained targets, amounting to a total of 3 visual and 3 auditory targets per run.

Stimuli and Stimuli Presentation
The visual stimulus consisted of a periodically expanding and contracting white ring (diameter minimum: 1.7°, maximum: 17.5°). A. Illustration of the concurrent TMS-fMRI protocol for one scan. 1.9 Hz rTMS was applied by delivering a TMS pulse 10 ms after every sixth slice followed by a gap of 100 ms. For a colored version of this figure, see Supplementary Figure S1.
visual angle; width minimum: 0°, maximum: 2.95° visual angle; length of temporal period: 600 ms) presented on a black background with a white fixation cross in the center of the ring. The visual stimulus was presented continuously in blocks of 20 s.

The auditory stimulus was created with Adobe Audition 2.0 by modulating a sinusoidal tone using the pitch bender function. This created an auditory stimulus that was basically equivalent to a sinusoidally frequency modulated pure tone with a carrier frequency \( f_c \) of 375 Hz and a modulation frequency \( f_{\text{mod}} \) of 2.35 Hz. The maximum frequency deviation, \( \Delta f \), equaled 225 Hz. The duration of each brief auditory stimulus was 425 ms. Thirty-six auditory stimuli were sequentially presented with an interstimulus interval of 110 ms in blocks of 20 s.

Visual and auditory stimuli were presented separately using Psychophysics Toolbox version 3 (Brainard 1997; Kleiner et al. 2007) running on MATLAB 7.5 (MathWorks Inc., MA, USA) and a Macintosh laptop running OS-X 10.5.6 (Apple Inc., CA, USA). The visual stimulus was back projected onto a frosted Plexiglas screen using a LCD projector (JVC Ltd., Yokohama, Japan) visible to the participant through a mirror mounted on the MR head coil. Auditory stimuli were presented via the Siemens pneumatic system, where the standard pneumatic headphones were replaced by E-A-RLINK 3A+2005 insert earphones (EST! Medizintechnik AG, Reutlingen, Germany) and damping headphones (3M Occupational Health & Environmental Safety, MN, USA) used to reduce the clicking sound produced by the TMS pulses. Note that both the insert earphones and the damping headphones are made out of plastic and hence cannot have interfered with the fMRI signal. In addition to this passive damping strategy, the effects of the auditory TMS clicks were reduced by camouflaging them with the pseudoclicks. Subjects indicated their responses (i.e., target detection task) using a MR-compatible custom-built button device connected to the stimulus computer.

**TMS Stimulation Sites**

TMS was applied over right IPS as experimental and vertex as a control site. For IPS-TMS, we adopted the Talairach coordinates \((x = 38, y = 44, z = 46)\) as a published activation peak for multisensory motion (Bremmer et al. 2001). Bremmer et al. (2001) identified this region as being commonly activated by visual, auditory, and tactile motion. Since our stimuli also elicited the impression of looming versus receding motion, this multisensory motion area seemed ideal for the purposes of this study. However, please note that these coordinates are close to those reported in numerous studies investigating audiovisual integration (Bushara et al. 1999; Corbetta et al. 2000; Lewis et al. 2000; Calvert et al. 2001; Werner and Noppeney 2011). Furthermore, since these coordinates were also very close to the IPS-TMS location in Ruff et al. (2008), they also enabled a comparison across the 2 studies.

The structural scans of each individual were normalized into Montreal Neurological Institute (MNI) space using unified segmentation. After transforming the Talairach coordinates from Bremmer et al. (2001) into MNI space, individual IPS scalp locations were determined by inverse transforming the new MNI coordinates into native space using the parameters obtained from spatial normalization and computing the intersection between the skull and a perpendicular vector through those coordinates. A posteriori reconstruction of the coil position also allowed us to verify that the individual vertex locations were always anterior to or at (in 1 subject) the intersection of the postcentral gyrus from both hemispheres. Thus, our vertex stimulation site is a well-suited control condition, since it is expected to induce comparable somatosensory and auditory side effects without influencing visual or auditory processing directly (Ruff et al. 2006; Supplementary Material).

**Data Acquisition and TMS Procedures**

A 3-T TIM Trio System (Siemens, Erlangen, Germany) was used to acquire both high-resolution structural images (176 sagittal slices, time repetition \([TR] = 2300 \text{ ms}\), time echo \([TE] = 2.98 \text{ ms}\), time to inversion \([TI] = 1100 \text{ ms}\), flip angle \([\alpha] = 9^\circ\)) and TMS-EPI images. Cartesian k-space sampling, \(TR = 3210 \text{ ms}\), \(TE = 40 \text{ ms}\), flip angle \(= 90^\circ\), \(FOV = 192 \text{ mm} \times 192 \text{ mm}\), image matrix \(64 \times 64 \times 36\) slices acquired sequentially in ascending direction, 3 mm \(\times 3 \text{ mm} \times 3 \text{ mm}\) voxels, slice thickness 2.6 mm, interslice gap 0.4 mm). A total of 298 volume images were acquired for each run.

After each EPI run, a fast structural image (fast low-angle shot \([FLASH]\), 100 slices, \(128 \times 128 \text{ matrix}\), voxel size \(= 2 \times 2 \times 3 \text{ mm}\, TR = 452 \text{ ms}\, TE = 2.46 \text{ ms}\)) was acquired to enable a posteriori reconstruction of the coil position inside the scanner. The TMS coil was marked with water tubes to enable the automatic coregistration of the coil representation in the FLASH images with a preacquired reference image of the coil. In addition, the subject's head in the FLASH images was coregistered to the high-resolution structural scan. Thereby, we were able to determine the coil position inside the scanner with respect to an individual's structural MRI that was also used for neuronavigation.

The EPI sequence was adapted for concurrent TMS-fMRI experiments by introducing gaps of 110 ms after every 425 ms in the GE-EPI sequence. Each gap was introduced to allow the delivery of one TMS pulse 10 ms after each sixth slice acquisition without interference with image quality (Bremmer et al. 2006). Hence, rTMS was applied at 1.9 Hz, that is, every 535 ms (Fig. 1C), using the same coil-holding device as in Moisa et al. (2009). This TMS protocol was employed for 3 reasons. First, a repetition rate of about 2 Hz has previously been shown to induce reliable excitability but only moderate after effects, rendering them ideal for online studies (Arai et al. 2005). Second, the continuous rhythmic TMS pattern lends itself to masking procedures with pseudo-TMS clicks and constant auditory input throughout the entire block. Third, blocks of 2 Hz stimulation have previously been shown to induce significant and constant brain activation throughout the entire duration of the block in a previous concurrent TMS-fMRI experiment (Ruff et al. 2010).

Biphasic stimuli were delivered using a MagPro X100 stimulator (MagVenture, Denmark) and a MR-compatible figure of eight TMS coil (MRI-B88). Unlike TMS over motor and visual cortices, it is not possible to perform a direct measurement (like motor-evoked potentials or phosphenes) of the TMS effects during IPS stimulation. Therefore, one standard approach is to calibrate the intensity of IPS-TMS based on an individual's resting motor threshold. Yet, the existence of a correlation between TMS thresholds for different cortical structures is controversially discussed (Stewart et al. 2001; Boroojerdi et al. 2002; Antal et al. 2004; but see Deblieck et al. 2008; Oliver et al. 2009). Furthermore, individual resting motor thresholds are typically very variable, depending on factors such as posture (Ackermann et al. 1991), mental activity (Izumi et al. 1995; Abbuzzese et al. 1996), or variations in sensory input (Leon-Sarmiento et al. 2005). To minimize the variance of the IPS-TMS effects, we applied TMS at 3 intensities consistently across all subjects. Based on a previous study performed with this coil in this lab, the mean resting motor threshold for this coil was estimated as 55% of the total stimulator output (M Moisa, personal communication). Hence, low and high TMS intensities were set consistently for all participants to 60% and 120% of the mean resting motor threshold for the used coil. This corresponded to 33% (low TMS) and 66% (high TMS) of the total stimulator output. For the no TMS condition, the stimulator output was set to 0%.

Motivated by the experimental choices made in previous studies, low intensity TMS blocks were introduced as an additional control condition that is thought to induce similar side effects as high intensity TMS in the absence of specific TMS effects (Ruff et al. 2006;
Blankenburg et al. 2010). However, our study clearly demonstrates that high TMS induces significantly stronger auditory activations as nonspecific TMS side effects than low TMS. This was the case despite additional masking procedures that were not even employed in previous studies. These findings suggest that low TMS cannot adequately control for nonspecific TMS side effects. Importantly, because of the brain’s multisensory organization, the nonspecific TMS-induced auditory activations can have an effect in both auditory and other sensory cortices, thereby rendering the interpretation of TMS effects challenging not only in auditory but also vertex stimulation.

Conversely, it is difficult to prove that low TMS to IPS does not induce any direct IPS stimulation. In support of subthreshold noneffective IPS stimulation, we observed no significant state-dependent effects for low TMS in our region of interest, when using the statistical thresholds generally applied in this study. In other words, at this threshold of significance, no interactions were revealed between low > no TMS intensity and visual > auditory stimulation ([V > A] low IPS-TMS > [V > A] no IPS-TMS) when imposing the additional constraint of ([V > A] low IPS-TMS > [V > A] low Vertex-TMS). Likewise, the interactions between low > no TMS intensity with 1) visual > fixation or 2) fixation > auditory stimulation were not significant. Nevertheless, classical statistics is in principle not able to prove the absence of an effect. Indeed, at a low uncorrected level of significance (P < 0.05, z = 2.3), we observed an effect in the calcarine sulcus for ([V > A] low IPS-TMS > [V > A] no IPS-TMS). It is therefore conceivable that low intensity TMS may induce very small and unreliable (i.e., variable) suprathreshold effects in IPS depending on the prior activity level of IPS. For instance, subthreshold TMS stimulation of IPS may turn into suprathreshold TMS under auditory or visual stimulation. While our data provide no strong evidence for this mechanism, it is premature to completely ignore these effects.

Given these critical considerations about the putative direct and indirect effects of low intensity TMS, we will identify main- and state-dependent TMS effects using high TMS > no TMS as our main contrast and high TMS > low TMS as an additional statistical constraint at a lower threshold of significance using the inclusive masking option. Extensive image quality tests of our setup (see previous reports: Moisa et al. 2009; Moisa et al. 2010: Supplementary Material) revealed only negligible TMS artifacts on the EPI images. Specifically, these tests scanned for radiofrequency noise induced by the TMS setup, compared only negligible TMS artifacts on the EPI images, and validated the effectiveness of the methods to suppress TMS-induced leakage currents. Furthermore, in the current study, we acquired EPI data with a phantom under different sensory stimulation conditions and TMS stimulation intensities. Comparing each “activation condition” against baseline (height threshold: P < 0.001 uncorrected) yielded only nonsignificant and randomly distributed activation patterns.

**fMRI Data Analysis: Preprocessing**

The fMRI data were analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) (Friston et al. 1995). Scans from each subject were realigned using the first as a reference, unwarped, spatially normalized into MNI space, resampled to a spatial resolution of 2 × 2 × 2 mm³, and spatially smoothed with a Gaussian kernel of 8 mm full-width-at-half-maximum. The time series of all voxels were high-pass filtered to 1/128 Hz. The first 3 volumes were discarded to allow for T₁-equilibration effects.

**fMRI Data Analysis: Modeling and Statistics**

The fMRI experiment was modeled using regressors obtained by convolving each activation block with a canonical hemodynamic response function (HRF; n=3), an additional analysis including the canonical HRF and the temporal derivative as 2 basis functions yielded basically equivalent results). In addition to modeling the 9 conditions in our 3 × 3 factorial design separately for each IPS-TMS and Vertex-TMS session, the statistical model included the 6 visual and auditory target blocks and their respective target onsets (after convolving each event-related unit impulse with the HRF) to account for potential attentional differences between blocks with and without targets. The reported statistical comparisons were limited to blocks without targets. Nuisance covariates included the realignment parameters to account for residual motion artifacts.

To allow for a random effects analysis and inferences at the population level, the contrast images (each condition > baseline) were entered in a second level ANOVA (Friston et al. 1999). At the second level, we evaluated the following statistical comparisons.

**Effect of Sensory Context**

Sensory-evoked activations were identified by comparing V (resp. A) > baseline (only no TMS conditions pooled over IPS and vertex). With respect to the deactivations, we were interested only in crossmodal deactivations. In other words, our aim was to identify 1) deactivations induced by auditory stimulation selectively in visual processing areas, that is, areas that are activated by visual stimulation and 2) deactivations induced by visual stimulation selectively in auditory processing areas, that is, areas that are activated by auditory stimulation. Operationally, we hence identified auditory-induced deactivations within the visual activation system by inclusively masking the auditory-induced activations (A < baseline) with the visual-induced activations (V > baseline). Conversely, we identified visual-induced deactivations within the auditory activation system by inclusively masking the visual-induced deactivations (V < baseline) with the auditory-induced activations (A > baseline).

**Effect of TMS Intensity**

The effect of TMS intensity was selectively tested for by comparing high IPS-TMS > no IPS-TMS (pooled across conditions). The effect of TMS intensity can be caused either as a confounding nonspecific side effect of the auditory TMS clicks or via direct “true” TMS effects. To dissociate the activations mediated by the 2 mechanisms, we have employed the following analysis strategy: first, since nonspecific TMS effects should be common to IPS and vertex stimulation, they were identified by inclusively masking the effect of TMS intensity for IPS-TMS (i.e., high IPS-TMS > no IPS-TMS) with 1) high Vertex-TMS > no Vertex-TMS. Furthermore, since high intensity TMS was also shown to induce more auditory activations than low intensity TMS, we additionally inclusively masked with 2) high Vertex-TMS > low Vertex-TMS intensity.

Second, specific effects of IPS-TMS should, in contrast, be selective for high intensity TMS and the IPS stimulation site. Hence, specific TMS effects were identified by inclusively masking the main effect of IPS-TMS with 1) high IPS-TMS > low IPS-TMS and 2) high IPS-TMS > high Vertex-TMS. The application of 2 constraints increases the specificity of our statistical comparison.

**Interaction between TMS Effects and Sensory Context: State-Dependent TMS Effect**

Primarily, we were interested in state-dependent TMS effects, that is, TMS effects that depend on the sensory stimulation context. Since crossmodal deactivations were identified reliably only for auditory stimulation, we selectively investigated whether the TMS effect on the BOLD signal in visual cortices depended on sensory context. Specifically, we investigated whether TMS to IPS modulates visually-induced activations and auditory-induced deactivations in the visual cortex in a different manner.

Given the role of IPS in attentional selection, we hypothesized that IPS-TMS would induce a more effective assignment of attentional resources to the stimulated sensory system and conversely withdraw attentional resources from the nonstimulated sensory system. At the neural level, we therefore expected IPS-TMS to jointly amplify 1) activation decreases in visual cortex during auditory stimulation and 2) activation increases during visual stimulation. Hence, we tested for the interaction between visual versus auditory stimulation and high versus no IPS-TMS intensity ([V > A]high IPS-TMS > [V > A]low IPS-TMS). To control for nonspecific TMS effects, we imposed 2 additional constraints using inclusive masking with the following contrasts: 1) the interaction between visual versus auditory stimulation and high versus low IPS-TMS intensity ([V > A]high IPS-TMS > [V > A]low IPS-TMS) and 2) the interaction between visual versus auditory stimulation and high IPS-TMS versus high Vertex-TMS ([V > A]high IPS-TMS > [V > A]high Vertex-TMS).

To dissociate whether these state-dependent TMS effects reflect TMS effects on visually induced activations or auditory-induced deactivations, we tested separately for interactions between TMS intensity and 1) visual > fixation ([V > fix]high IPS-TMS > [V > fix]low IPS-TMS) or 2) fixation > auditory ([fix > A]high IPS-TMS > [fix > A]low IPS-TMS). For each
interaction contrast, we imposed additional constraints (e.g., interaction between V > Fix with 1) high > low TMS and 2) high IPS-TMS > high Vertex-TMS) following the same rationale as described above using inclusive masking.

**Search Volume Constraints**

The effects were tested for 1) within the entire brain and, based on our a priori hypothesis, 2) in the visual and auditory cortices, and 3) motion area hMT+/V5+ as our regions of interest. All regions of interest were defined using the SPM Anatomy Toolbox (Eickhoff et al. 2005). The anatomical mask for the entire visual cortex included 6402 voxels within the bilateral cytoarchitectonic maps BA17, BA18, and hOCC; the anatomical mask for the visual area hMT+/V5+ included 163 voxels in the bilateral cytoarchitectonic maps hOCC; the anatomical mask for the auditory cortex encompassed 973 voxels in the bilateral cytoarchitectonic maps TE 1.0, TE 1.1, and TE 1.2.

Unless stated otherwise, we report activation at $P < 0.05$ corrected at the voxel level for multiple comparisons (familywise error rate) based on Gaussian Random Field theory within the entire brain and in our regions of interest. Additional constraints on statistical effects were imposed using inclusive masks thresholded consistently at 0.01 uncorrected.

**Results**

The data were analyzed in 3 steps. First, we identified stimulus-evoked activations and deactivations in the primary visual and auditory cortices under conditions of no TMS. Second, we tested for the main effect of TMS by directly comparing high and no TMS intensities. Third, we characterized state-dependent effects of TMS in visual cortex by testing for the interaction between sensory context and TMS intensity. The effects were tested for within the entire brain and the visual and auditory cortices as our primary regions of interest.

**Effects of Sensory Context**

**Stimulus-Evoked Activations**

To identify stimulus-evoked activations, we compared sensory stimulation relative to baseline in the absence of TMS stimulation. As expected, visual stimulation induced activations in calcarine sulci and bilateral V5/MT+ and auditory stimulation in bilateral superior temporal gyri (Fig. 2 and Table 1). Visual stimulation also induced significant activations in the right middle frontal gyrus and in the right superior parietal lobule (Table 1).

**Stimulus-Evoked Deactivations**

Deactivations induced by auditory stimulation were identified within the visual activation system by comparing A < baseline masked with V > baseline. As expected, this comparison showed deactivations within the cuneus, specifically the calcarine sulci extending into the lingual gyri (Fig. 2B(i) and Table 1).

Likewise, deactivations induced by visual stimulation were identified within the auditory activation system by comparing V < baseline masked with A > baseline. At an uncorrected level of significance, this comparison revealed deactivations in Heschl’s gyri bilaterally (Fig. 2B(ii) and Table 1). The deactivations within the auditory system were less pronounced than in the visual system, most likely because the auditory system was continuously driven by pseudo- and true TMS auditory clicks.

**Effect of IPS-TMS: High > No TMS—Specific Direct and Nonspecific Indirect TMS Effects**

As expected, high versus no IPS-TMS revealed significant activations in the auditory cortices. This main effect of TMS could reflect either auditory stimulation by the TMS clicks as confounds (=nonspecific indirect TMS effect) or true top-down modulatory effects from TMS-IPS stimulation (=specific direct TMS effects). To dissociate the contributions of these 2 mechanisms to the auditory activations, we imposed additional constraints using the inclusive masking option (see Materials and Methods).

**Specific Direct TMS Effects**

True IPS-TMS effects should be selective and enhanced for 1) high > low IPS-TMS and 2) high IPS > high Vertex-TMS. Imposing these 2 additional constraints revealed activations in the left superior temporal gyrus extending into the left rolandic operculum (Fig. 3A and Table 2). Even though the activations were left lateralized, at a lower threshold of significance ($P < 0.05$ uncorrected), they were also observed in the right hemisphere (Fig. 3A). Imposing simultaneously 2 statistical constraints using the inclusive masking option renders our statistical results more specific.

The presence of high IPS-TMS > high Vertex-TMS effects in the auditory cortices of both hemispheres (in the absence of any significant effects for high Vertex-TMS > high IPS-TMS) suggests that they are mediated via top-down effects induced by IPS-TMS rather than being a result of unbalanced auditory TMS inputs to the 2 ears.

**Nonspecific Indirect TMS Effects**

Nonspecific TMS effects due to auditory confounds should be present for both IPS and vertex stimulation. Hence, they should be revealed when masking the main effect of TMS (i.e., high vs. no IPS-TMS) with 1) high > no Vertex-TMS and 2) high > low Vertex-TMS. Indeed, imposing these additional constraints revealed again significant activations in the left Heschl’s gyrus and in the bilateral superior temporal gyri extending to the rolandic operculum that were partially overlapping with the activations attributed to true TMS effects (Fig. 3A and Table 2).

Similar activations in auditory cortices were also obtained when masking high IPS-TMS > low IPS-TMS with 1) high > no Vertex-TMS and 2) high > low Vertex-TMS indicating that low TMS does not control for auditory and somatosensory side effects. Hence, from a unisensory perspective, low TMS cannot be considered a good control condition (as previously suggested) to evaluate remote TMS effects on auditory processing or activations within the auditory cortex, even when extensive measures are applied to control for the auditory TMS side effects as in the current study. More importantly, from a multisensory perspective, it does not form a valid control condition for any type of uni- or multisensory experiment, since activations in auditory cortex can have pronounced nonlinear influences on processing in other sensory systems (see Discussion).

**Interactions between TMS Intensity and Sensory Context: State-Dependent TMS Effects**

To investigate whether IPS-TMS jointly amplified visual-induced activations and auditory-induced deactivations within the occipital cortex, we tested for the interaction between V > A and TMS intensity (high > no TMS). To control for TMS side effects, we imposed 2 additional constraints: a significant interaction 1) between V > A and high > low TMS and 2) between V > A and high IPS > high Vertex-TMS (for further details, see Materials and Methods). These interaction contrasts jointly revealed effects in the cuneus that were located in Brodmann area 18 based on
cytoarchitectonic probability maps (Fig. 3B and Table 2). As shown in the parameter estimate plots, IPS-TMS amplifies the activations to visual stimuli and the deactivations to auditory stimuli (Fig. 3B). However, the response suppression in the visual cortex during TMS stimulation was comparable for 1) auditory and fixation conditions and 2) IPS- and Vertex-TMS stimulation sites. Indeed, the interactions between A < Fix and 1) TMS intensity or 2) TMS site were not significant.

In contrast, while Vertex-TMS also suppressed activation during visual stimulation, IPS-TMS increased the visual-induced activations relative to fixation as confirmed statistically in a significant interaction between V > Fix and TMS intensity (as well as TMS site) (Table 2). Collectively, these results suggest that IPS-TMS selectively enhances the response to visual stimuli in the visual cortex. By contrast, the suppressive TMS effects during auditory stimulation and fixation are more likely to be caused by the TMS clicks as side effects that are common to vertex and IPS-TMS sites rather than true neural TMS effects.

**Eye Monitoring (Outside the Scanner)**

To ensure that the observed activation pattern did not result from eye movements, twitches, and startle effects, a subset of 6 subjects participated in an additional TMS experiment that was performed outside the scanner with identical paradigm and parameters. The TMS stimulation protocol was also identical, except for the TMS intensities that were newly defined as 30% (low TMS) and 60% (high TMS) of total output to account for the absence of the high-current filter that was used in the fMRI experiment to prevent MR images from being affected by RF noise (Moisa et al. 2009). For each subject, data in 2 runs were acquired for each TMS location.

Horizontal and vertical eye movements were recorded using a ViewPoint Eyetracker system (Arrington Research Inc., Scottsdale, AZ, USA) (220 Hz sampling rate). Eye position data were automatically corrected for blinks. For each subject, the mean distance (degrees) from the fixation cross, the number of saccades (defined by eye velocity threshold > 30°/s), and the number of blinks were quantified.
The 3 indices were independently entered into a 3-way repeated measures analysis of variance (RM-ANOVA) with the factors TMS stimulation location (right IPS and vertex), TMS intensity (no TMS, low TMS, and high TMS), and sensory modality (A, V, and Fix). None of the 3 RM-ANOVA revealed any significant main effects or interactions demonstrating that differences in eye movements are unlikely to account for the observed activation profile in our fMRI data.

### Discussion

This concurrent TMS-fMRI study investigated the effect of IPS-TMS on the (de)activations in the visual and auditory cortices under 3 sensory contexts: auditory, no, and visual stimulation. Our results demonstrate that IPS-TMS generally increased activations in the auditory cortex irrespective of the sensory stimulation context. Comparing IPS-TMS and Vertex-TMS suggests that this increase in activation level in the auditory cortices results from both coactivations induced by TMS clicks and top-down effects from IPS. In contrast, IPS-TMS influenced activations in the visual cortex in a state-dependent fashion: IPS-TMS suppressed activation in the cuneus under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation. Since TMS to the vertex as a control site exerted a comparable suppression in the visual cortex under auditory and no stimulation but amplified the response to visual stimulation.
Figure 3. (A) (center top) Increased activations for high relative to no intensity IPS-TMS pooled (i.e., summed) across sensory stimulation contexts are rendered on a template of the whole brain. Height threshold of $P < 0.01$ uncorrected, no extent threshold, for illustrational purposes only. (center bottom) Parameter estimates (mean ± standard error of the mean, RFX model) for no TMS (light gray), low intensity TMS (medium gray), and high intensity TMS (dark gray) in the cuneus at the given coordinates (i.e., activation peaks) are shown separately for auditory (left), fixation (middle), and visual (right) contexts. The bar graphs represent the size of the effect in nondimensional units (corresponding to % whole-brain mean). (left bottom) Nonspecific TMS effects were identified by inclusively masking the effects of high > no IPS-TMS with 1) high > no Vertex-TMS intensity and 2) high > low Vertex-TMS intensity at $P < 0.01$ (yellow) and $P < 0.05$ (red) uncorrected. (right bottom) Specific “true” TMS effects were identified by inclusively masking the effects of high > no IPS-TMS with 1) high > low IPS-TMS intensity and 2) high IPS-TMS > high Vertex-TMS intensity at $P < 0.01$ uncorrected. Effects are displayed on axial slices of cytoarchitectonic maps (Eickhoff et al. 2005). (B) TMS effects that depend on sensory stimulation context. (left) Interactions between TMS intensity and sensory stimulation ($[V > A]_{high\text{-}\text{IPS-TMS}} > [V > A]_{low\text{-}\text{IPS-TMS}}$) are displayed on sagittal and axial slices of cytoarchitectonic maps (Eickhoff et al. 2005). (B) TMS effects that depend on sensory stimulation context. (left) Interactions between TMS intensity and sensory stimulation ($[V > A]_{high\text{-}\text{IPS-TMS}} > [V > A]_{low\text{-}\text{IPS-TMS}}$) are displayed on sagittal and axial slices of cytoarchitectonic maps (Eickhoff et al. 2005). Height threshold: $P < 0.01$, uncorrected (for illustrational purposes only). Extent threshold > 0 voxels. The effects are inclusively masked with 1) the interaction between visual versus auditory stimulation and high versus low IPS-TMS intensity ($[V > A]_{high\text{-}\text{IPS-TMS}} > [V > A]_{low\text{-}\text{IPS-TMS}}$) and 2) the interaction between visual versus auditory stimulation and high IPS-TMS versus high Vertex-TMS ($[V > A]_{high\text{-}\text{IPS-TMS}} > [V > A]_{high\text{-}\text{Vertex-TMS}}$) at $P < 0.01$ uncorrected. (right) Parameter estimates (mean ± standard error of the mean, RFX model) for auditory (left), fixation (middle), and visual (right) contexts.
These results suggest that IPS-TMS may not only increase activations in the auditory cortex via nonspecific auditory confounds but possibly also via top-down effects from IPS. Since the real and pseudo-TMS clicks strongly perturbed the auditory cortex even in the fixation or visual stimulation conditions, it is not surprising that the auditory deactivations were attenuated, and the TMS effects were not state dependent.

In contrast, in the visual cortex IPS-TMS increased the auditory-induced deactivations as well as the visual-induced activations. IPS-TMS similarly induced deactivations in the visual cortex in the absence of any stimulation. At first sight, these state-dependent TMS effects seem to be in accordance with our hypothesis. Yet, the response profile we observed in the visual cortex may not necessarily reflect true state-dependent IPS-TMS effects, but as we will argue below be generated by a mixture of true and nonspecific TMS side effects similarly to the TMS effects in the auditory cortices. Taking the multisensory nature of the neocortex serious (Ghazanfar and Schroeder 2006), activations and deactivations in the visual cortices can in principle be mediated via audiovisual interactions as a consequence of the TMS side effects on activations in the auditory cortex. In line with this conjecture, a recent study (Werner and Noppeney 2011) demonstrated that auditory input suppressed activations in the visual cortex but amplified the BOLD response to concurrent visual inputs. Since high relative to low intensity clicks increased activations in the auditory cortex, it is conceivable that the auditory cortex in turn induces deactivations in the visual cortex in the absence of visual stimulation but amplifies the response to concurrent visual stimulation. In other words, the auditory TMS clicks themselves can induce BOLD effects in the auditory cortex that exert different influence on the visual cortex depending on the sensory stimulation context. This multisensory perspective is important because it highlights that BOLD effects due to TMS side effects in the auditory cortex emerge 1) not only in the auditory cortex and 2) in a nonlinear fashion (i.e., they interact with the sensory stimulation context). Therefore, they can impede not only the interpretation of the main effect of TMS intensity but also interactions between TMS intensity and sensory stimulation context. In short, they cannot simply be eliminated or ignored when considering state-dependent TMS effects in unsensory processing (as has been argued in previous studies). Instead, even state-dependent TMS effects need to be carefully considered in the context of stimulation conditions to other control sites (e.g., vertex).

Indeed, TMS to the vertex as a control site induced a comparable deactivation in visual cortices under auditory and no stimulation suggesting that our IPS-TMS stimulation was not effective in modulating crossmodal deactivations. While an absence of an effect needs to be interpreted with caution, it may point to a role of recently advocated thalamic mechanisms in mediating crossmodal deactivations (Hackett et al. 1998; Schroeder et al. 2003; de la Mothe et al. 2006; Cappe et al. 2009). So possibly, competition between sensory signals in multiple modalities may already be arbitrated via gating mechanisms at the thalamic level. Obviously, this suggestion remains speculative and needs to be substantiated in future studies.

### Table 2: TMS-induced effects

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>MNI coordinates (mm)</th>
<th>Z score</th>
<th>Z score masking 1</th>
<th>Z score masking 2</th>
<th>$P_{\text{FWE}}$ value</th>
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<td>Left superior temporal gyrus</td>
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<td>$-32$</td>
<td>$20$</td>
<td>$&gt;8$</td>
<td>7.41</td>
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Note: $P$ values are corrected for multiple comparisons within the entire brain, the visual cortex *, or the auditory cortex #, see Materials and Methods. FWE, familywise error.

For no TMS (light gray), low intensity TMS (medium gray), and high intensity TMS (dark gray) in the cuneus at the given coordinates (i.e., activation peaks) are shown separately for auditory (left), fixation (middle), and visual (right) contexts. The bar graphs represent the size of the effect in non-dimensional units (corresponding to % whole-brain mean). For illustrational purposes, the stars indicate the significance of the tests comparing individual conditions at the activation peak (uncorrected; **significant at $P < 0.001$, *** significant at $P < 0.01$, * significant at $P < 0.05$).
Mechanisms of TMS effects in auditory and visual cortices. (A) TMS effects in auditory cortex. (Left) Auditory clicks produced by TMS pulses induce auditory coactivations in auditory cortices (nonspecific mechanism). (Right) IPS-TMS exerts top-down effects on the neural activity in auditory cortices (specific mechanism). (B) State-dependent TMS effects in visual cortex. (Left) IPS-TMS increases visual activations directly via multiplicative gain control. (Right) IPS-TMS modulates the effect of neural activity in auditory cortices (induced by TMS clicks) on activations in the visual cortex via crossmodal interactions. For a colored version of this figure, see Supplementary Figure S3.

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

Notes
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References


