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Interleaved TMS-fMRI in the Investigation of Multisensory Integration

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Abstract

To provide a more reliable percept of our environment, the brain combines information from multiple senses. Cross-modal deactivations are one of the possible types of multisensory influences that can be observed within the human brain. By suppressing potentially distracting neural activity in non-matching sensory areas these deactivations may mediate an increase in the salience of a sensory event. However, the exact mechanisms underlying cross-modal deactivations are still not known. The intraparietal sulcus (IPS) is a multisensory region that has been shown to modulate activity in sensory cortices. Here, we applied TMS to the right IPS during fMRI with the aim at determining the role of this region in the cross-modal deactivations during visual, auditory and audiovisual stimuli presentation. As a prior requirement to the interleaved TMS-fMRI experiment, we designed an experimental paradigm and stimuli that elicited robust activations and deactivations even in the presence of possible TMS confounding effects. Although the results obtained so far are still inconclusive with respect to the neural mechanism of cross-modal deactivations, they have provide important insights to guide future experiments.

Keywords: Multisensory Integration, Cross-modal Deactivations, right Intraparietal Sulcus, Interleaved TMS-fMRI

Resumo

De forma a providenciar uma percepção mais fidedigna do ambiente que o rodeia, o cérebro combina informação proveniente de múltiplos sentidos. Desactivações de modalidade cruzada constituem um dos vários tipos de influências multisensoriais observáveis no cérebro humano. Ao suprimir actividade neural potencialmente perturbadora em áreas sensoriais que não correspondem ao estímulo que está a ser apresentado, estas desactivações podem mediar um aumento na proeminência de um evento sensorial. No entanto, até ao momento não se conhecem quais os mecanismos subjacentes a este tipo de desactivações. O sulco intraparietal é uma região multisensorial capaz de modular a actividade nos córtexes sensoriais. Com o objectivo de determinar o papel desempenhado por esta região nas desactivações de modalidade cruzada durante a apresentação de estímulos visuais, auditivos e audiovisuais, estimulou-se o sulco intraparietal direito com TMS durante fMRI. Como requisito prévio da experiência de TMS-fMRI, elaborou-se também um paradigma experimental e estímulos capazes de provocar activações e desactivações robustas. Embora inconclusivos no que diz respeito aos mecanismos neuronais das desactivações de modalidade cruzada, os resultados obtidos até agora oferecem informação importante para orientar futuras experiências.

Palavras-chave: Integração Multisensorial, Desactivações de modalidade cruzada, Sulco Intraparietal, “Interleaved” TMS-fMRI

Contents

List of Abbreviations

A	Auditory Stimulation
AV	Audiovisual Stimulation
BOLD	Blood- Oxygenation-Level-Dependent
EPI	Echo-Planar Imaging
FEF	Frontal Eye Fields
FLASH	Fast Low-Angle Shot
fMRI	Functional Magnetic Resonance Imaging
FOV	Field-Of-View
FWHM	Full Width Half Maximum
GE	Gradient Echo
IPS	Intraparietal Sulcus
LCD	Liquid Crystal Display
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MR	Magnetic Resonance
TE	Time of Echo
TI	Time of
TMS	Transcranial Magnetic Stimulation
TR	Time of Repetition
RF	Radiofrequency
rips	right Intraparietal Sulcus
ROI	Region Of Interest
rTMS	repetitive Trancranial Magnetic Stimulation
SD	Standard Deviation
SPM	Statistical Parametric Map
STS	Superior Temporal Sulcus
V	Visual Stimulation

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1. Introduction

1.1. Multisensory Integration

Combination of information from different senses is indispensable for an appropriate response in everyday situations. For instance, in the theater or in the movies we perceive visual and auditory information in parallel and this information is processed in our brain to create a coherent and unified percept.

Behaviorally, integration of different sensory modalities has been shown to have several advantages, since it can lower reaction times (Gielen et al., 1983) or improve detection of degraded stimuli (Driver and Spence, 1998, McDonald et al., 2000). Furthermore, the combination of different sensory inputs can result in alterations of the quality of the sensory percept or illusions (McGurk and McDonald, 1976).

Traditionally, it is assumed that at the neural level multisensory integration occurs mostly in higher association cortices and specialized subcortical structures. This assumption is supported by several studies, revealing sensory integration in areas such as the superior temporal sulcus (STS), the frontal cortex and the intraparietal sulcus (IPS). Recent studies propose, though, that integration of information provided by different sensory modalities is not restricted to higher order association areas. Indeed, accumulating evidence suggests that areas that have been hitherto considered as unimodal can in fact be modulated by stimulation of several senses (Macaluso and Driver, 2005; Schroeder and Foxe, 2005; Ghanzafar and Schroeder 2006).

Various types of multisensory influences can be observed within the human brain. First, input from one sensory modality can modulate the response to input from a different sensory modality. Second, even when presented alone, input from one sensory modality may induce co-activations or deactivations in cortices primarily involved in processing stimuli from a different sensory modality. In fact, at the level of the fMRI BOLD response, presentation of unimodal auditory, visual or somatosensory stimuli activates the corresponding sensory cortex, while it induces a decrease in activity in non-matching sensory cortices. By suppressing potentially distracting neural activity in non-matching sensory areas these deactivations may mediate an increase in the salience of a sensory event. In addition to this input-driven cross-modal deactivations between sensory cortices (Laurienti et al., 2002), a similar response profile can be induced by attentional modulation. In the latter case, subjects are presented with bimodal stimuli (Shomstein & Yantis, 2004; Johnson & Zatorre, 2006) and transiently shift their attentional focus between different sensory modalities. These similar response profiles raise the question whether attentional modulation may be a common mechanism both for deactivation induced by unisensory input and explicit attentional shift during bisensory stimulation (Johnson & Zatorre, 2005). However, not all studies find attention modulates activity of sensory areas (Rees et al., 2000). Furthermore, attentional modulation in itself is not an explanatory concept and it may be more useful to understand the underlying mechanisms formally in terms of top down or bottom up effects. Thus, some previous functional imaging studies have argued that activity in the sensory-specific cortex may be modulated via back-projecting pathways from multisensory areas that serve to interconnect the different sensory cortices (Calvert et al., 2000). In contrast, other studies reported that interactions between unimodal cortices occur very early in time, making it less plausible that information passes through higher association cortices before going to the sensory cortices (Foxe et al., 2000; Molholm et al., 2002; Murray et al., 2005). Interleaved transcranial magnetic stimulation (TMS) and functional magnetic resonance (fMRI) might be a useful tool to understand the mechanisms underlying cross-modal deactivations.

1.2. Combined TMS-fMRI

1.2.1. Functional Magnetic Resonance Imaging

A great deal of work has been done using animal models to understand how individual neurons integrate multisensory cues. Although these studies have provided a substantial amount of information, it is necessary to extend this knowledge to studies that identify the neural substrates involved in multisensory perceptual processes in the human brain. One way to achieve this goal is to use non-invasive functional brain imaging technologies, or more specifically functional magnetic resonance imaging (fMRI).

The magnetic resonance imaging (MRI) signal arises from the interaction of an applied magnetic field with the hydrogen nuclei, or protons, that are found primarily in the water present in the body. Every nucleus possesses an angular momentum, also referred to as spin. Because protons are charged particles, their rotation produces a magnetic moment. Since the hydrogen proton has a spin of ½, its energy level diagram consists only of two allowed energy levels, which represent the two possible orientations that the magnetic moment may assume in an externally applied static magnetic field B_0: parallel or antiparallel. Prior to the application of B_0, each magnetic moment points in a random direction, generating a global net magnetization that is infinitesimally small. The presence of B_0 tends to align the magnetic moments parallel to it, which causes the protons to precess around the axis of the magnetic field with a frequency proportional to B_0, called the Larmor frequency. In order to obtain a signal, transitions between the protons in the parallel and the anti-parallel level must be induced. Therefore, a second oscillating magnetic field B_1 is introduced perpendicular to B_0 to excite the protons away from their resting state, thus creating a precessing transverse magnetization. This second magnetic field is created by a radiofrequency (RF) coil (RF pulse) and oscillates at the Larmor precession frequency. After the application of a RF pulse, the relaxation back to the original low energy state can be described as changes in two dimensions, longitudinal re-growth and transverse relaxation, which are exponential processes.

Most MRI studies acquire a series of slices through the anatomical area of interest. To select a slice, a slice selective excitation pulse is applied. This pulse will perturb from their equilibrium position along the static field direction only those spins at specific points on a plane, and leaves unperturbed spins outside the selected plane. In practice this is done by applying a radiofrequency excitation pulse in the presence of a magnetic field gradient orientated in the direction perpendicular to the desired plane. After selecting the slice, one has to encode the other two dimensions to produce a two-dimensional image. This is achieved by applying phase-encoding and frequency-encoding (also known as readout) gradients. In this regard, the concept of k-space proves to be very useful. K-space is a graphic matrix of digitized MR imaging data that represents the image prior to Fourier transform analysis. All points in k-space contain data from all locations within an MR image. The image is then the Fourier transform of k-space (Webb, 2003).

fMRI is based on blood-oxygen-level-dependent (BOLD) signal and its relationship with neural activity. It takes advantage of the fact that neuronal activation is accompanied by an increased blood flow, which leads to a washout of deoxyhemoglobin in venous compartments. Since deoxyhemoglobin is paramagnetic, it attenuates the signal intensity of gradient-echo MR images. Hence, with less quantity of deoxyhemoglobin in the blood, the local magnetic signal is slightly increased giving rise to the BOLD signal (Logothetis et al., 2004). Although it offers non-invasive maps of neural activation in the brain, the BOLD signal only provides an indirect measure of brain activity. Therefore, fMRI can only show correlations but cannot establish causal links between brain activity and behavior.

1.2.2. Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a technique for noninvasive stimulation of the human brain. TMS is based on the principles of electromagnetic induction. By applying a brief pulse of current through a stimulation coil held on the subject's scalp, a time-varying magnetic field perpendicular to the current is produced. In turn, this magnetic field induces an electrical current in the brain. If the right stimulation parameters are used, the current induced will depolarize cortical neurons and generate action potentials (Cowey, 2005).

The TMS equipment consists of a pulse generation unit, the stimulator, and an electromagnetic stimulation coil. The most widely used types of coil are the circular coil and the figure-of-eight coil. Figure-of-eight coils can be approximately viewed as two round coils mounted side to side, with the current rotating in opposite directions in the two coils. The focality of TMS is maximized with the use of this second type of coils. While with circular coils the maximum electric field induced in the brain lies in a ring under the coil, the electric field with figure-of-eight coils is strongest under the centre of the coil. Focality also depends on the size of the coil, smaller coils producing stronger and more focal fields (Siebner, 2009). Another important feature of TMS is that the induced magnetic field is inversely proportional to the square of the distance between the coil and the cortex, meaning that only superficial areas are prone to direct stimulation, whereas deeper areas might only be excited indirectly by propagated activity from the region beneath the coil (Bestmann et al., 2008).

TMS can be applied as single pulses or as train of pulses (rTMS). The effects of single TMS pulses are short-lived and several seconds are needed before the next pulse can be delivered. On the other hand, rTMS can be used to modulate the excitability of the stimulated area even beyond the duration of the TMS application, and can depend on the prior history of activation before rTMS is applied (Bestmann et al., 2008). When TMS is used in humans, specific safety issues need to be taken into account, in particular when trains of TMS are applied. The main risk of TMS is that it can induce epileptic seizures, in particular if rTMS is applied at high frequency and intensity to the cortex. In order to minimize the risk of inducing a seizure, a safety guideline (Wassermann, 1998) restricting the use of rTMS to specified stimulation frequencies and parameter combinations has been published, and when followed, no cases of epileptic seizures have been reported so far. Investigation of possible irreversible long-term effects also revealed that no, or only mild effects that lasted up to 1 h post-rTMS. (Sack & Linden, 2003).

Unlike fMRI, TMS can be used to establish causal structure-function relationships: when temporarily disturbing a brain region results in degraded task performance, this region is indeed causally contributing to the observed behavior. This is known as the virtual lesion approach. Alternatively, TMS can be used to induce activity by adding extra “noisy” activity to ongoing processing (Siebner et al., 2009).

1.2.3. Interleaved TMS-fMRI

Despite its widespread use, the exact mechanisms by which TMS exerts its effects on cortical circuits are not yet fully understood. TMS may not only directly activate local neurons and intracortical connections but also affect processing in remote brain regions either via neural interregional connections or due to compensatory mechanisms (Bestmann et al., 2008). Such remote effects are usually not revealed by standard TMS studies and inferences are normally restricted to the targeted site of stimulation. The combination of TMS and fMRI allows a direct monitorization of the effects of TMS on brain activity, not only at the site of stimulation but also across the entire brain. For instance, when TMS is used to induce activity and the resulting BOLD activity is measured, direct proof of the connectivity between the stimulated area and co-activated areas is obtained.

There are, nevertheless, some technical aspects involved in the combination of both techniques. One of these aspects involves the safety of the subjects and the research personnel. In the static magnetic field, the TMS coil experiences additional forces that might cause the coil to move or break violently, causing injury. The use of figure-of-eight coils of two wire loops with counter-rotating currents can solve this problem, because the torques in the wire loops are in opposite direction and, in theory, this eliminates motion (Bohning et al., 2003).

In addition, combining TMS with fMRI can provoke EPI image distortions. The mere presence of TMS can lead to signal dropouts up to 2 cm underneath the coil (Bestmann et al., 2003). Since the distance between the TMS coil placed over the skull and the intended cortical stimulation area is in general greater, this does not normally constitute a problem in human studies. More importantly, image distortions can also arise when TMS pulses are applied during data acquisition**.** When a TMS pulse is applied during RF excitation, the steady-state longitudinal magnetization is affected, which can change the signal intensity of the subsequently acquired volumes and possibly resulting in false-positive activations (Bestmann et al., 2003). On the other hand, TMS pulses during readout distort the images but do not have any carry-over effects. One possible technical solution is applying TMS pulses while deliberately sacrificing those images that are acquired during the administration of the pulse and substituting them by its temporal neighbors during post-processing. This approach benefits from the fact that BOLD responses exhibit a delayed maximum effect after about 4-6 seconds and therefore no relevant physiological information is afflicted (Bestmann et al., 2003). Instead, one can introduce temporal gaps between two slice acquisitions. It has been shown that MR images can be distorted for up to 100 ms after the application of a TMS pulse (Shastri et al., 1999, Bestmann et al., 2003). Interleaving data acquisition with gaps with at least 100 ms constitutes therefore another alternative. This approach is nevertheless limited to rTMS at low frequencies on the order of 1-2 HZ. Further technical problems include the fact that the RF noise created by the TMS stimulator is picked up by the MR scanner. This can be solved by placing the stimulator outside the scanner room and by using a filter to remove the noise. Moreover, the accurate placement of the coil over the intended stimulation location inside the scanner is a difficult task.

Recently, TMS was combined with fMRI in an online fashion to directly image the changes in neural activation caused by TMS. In particular, this methodical combination was used to test hypothesis about functional interactions between fronto-parietal and visual cortical areas. Ruff et al. (2006) demonstrated how short bursts of TMS over the frontal eye fields (FEF) can modulate neural activity in early visual cortex. More recently, the authors also showed how stimulating the right human IPS elicits a pattern of activity changes in the visual cortex that strongly depends on current visual context (Ruff et al., 2008). Similarly, Sack et al. (2007) applied TMS to the parietal cortices during fMRI to study the contribution of left and right IPS to visuospatial processing during visuospatial judgements. Their results suggest that right parietal TMS influenced neural activity both in the stimulated region and in remote ipsilateral middle frontal gyrus, and also contributed to reduction in spatial cognition performance. Such findings indicate that focal TMS applied to a particular brain region has both local and remote neural effects in the brain and that these effects are state-dependent, that is, depend upon current neuronal excitability. Combining TMS with fMRI has therefore already shown to be a promising tool to refine how interactions between components of extended networks may support cognition, perception and behavior.

To our knowledge interleaved TMS-fMRI has not been used yet for investigating multisensory integration. Given its characteristics, this technique is potentially useful when investigating multisensory integration. Yet, there are some additional problems that need to be taken into account. When a TMS pulse is applied, a clicking sound is produced and muscle twitches or potential startle effects can be induced. If in a multisensory integration experiment auditory or tactile stimuli are presented, the auditory and somatosensory TMS co-activations can interfere with the actual stimulation and have a confounding effect. In addition, the muscle twitches also limit the range of possible stimulation areas. Applying TMS pulses to certain areas of the brain causes discomfort for the subjects, which automatically excludes these areas as possible stimulation sites. These TMS non-specific effects depend not only on the stimulation site, but also upon the intensity and frequency used (Bestmann et al., 2008). The co-activations need therefore to be controlled and the choice of stimuli to be used has to take into account these co-activations. In other words, the experiment needs to be carefully designed. The use of factorial designs may help, by testing for interactions between different stimulation parameters (e.g. stimulation intensity) and task condition (e.g. absence or presence of visual or auditory stimulation). Furthermore, the insertion of appropriate control sites can also be used as a way to exclude potentially confounding effects. This can only work if the non-specific TMS effects remain constant, while the TMS-evoked functional changes on brain activity depend on the specific function and connectivity of the stimulated area (Bestmann et al., 2008). An additional problem is the lack of space inside the MR coil, which means that particular areas are not accessible and cannot be stimulated. Therefore, some potential areas of interest for multisensory integration are excluded for the application of TMS pulses.

1.3. Purpose of the current work

This project aimed to shed light on the question to which extent the multisensory effects in the auditory and visual cortices that are observed at the level of the BOLD response are mediated via feedback from higher-level areas rather via direct connections to visual areas or subcortical mechanisms.

Previous studies have shown that cross-modal modulations in the sensory cortices can occur in the form of deactivations (Laurienti et al, 2002; Johnson & Zatorre, 2005). The exact mechanisms by which these deactivations occur are still not known. The IPS is a multisensory region that is capable of modulating activity in sensory cortices, in particular in the visual cortex (Ruff et al., 2008). Simultaneous TMS-fMRI allows the investigation of inter-regional interactions in the human brain and their possible functional consequences for perception and cognition. By applying TMS to the right IPS (rIPS) during fMRI we hoped to determine the role of this region in the cross-modal deactivations during visual, auditory and audiovisual stimuli presentation.

The project was carried out at the Max Planck Institute for Biological Cybernetics, Tübingen, more specifically in the Cognitive Neuroimaging Group, and was jointly supervised by Dr. Uta Noppeney and Dr. Axel Thielscher.

2. Methods

2.1. General Considerations

Before starting with the actual TMS-fMRI experiment, there are several methodological aspects that have to be taken into account. For instance, the experimental setup has to be tested to ensure that the different components are well synchronized with each other and that their combination doesn't induce artifacts on the images to be acquired or on the functioning of each of the single components. Moreover, since auditory stimuli are to be presented, auditory co-activations induced by TMS have to be minimized as potential confounds. On the other hand, it is necessary to construct a set of stimuli that are able to reproduce the cross-modal deactivations reported by other studies (Laurienti et al., 2002; Johnson & Zatorre, 2006). Consequently, the present work comprised three different steps. The first step consisted in establishing and testing our experimental setup, while the second step concentrated on reproducing the deactivations using fMRI, given this experimental setup. After these two steps had been achieved, it was possible to proceed with the third step, in which TMS pulses were applied to the IPS. These steps, especially the first two, were performed in parallel, which makes it sometimes difficult to describe each one of them without referring to the others. Therefore, the different sections describing each step individually will be linked via cross-references.

The sequence and setup used for data acquisition, as well as the way the stimuli were presented and the fMRI data were preprocessed were a common feature to all of the three steps, being therefore described next.

2.1.1. Stimulus presentation

Visual and auditory stimuli were presented using Cogent (John Romaya, Vision Lab, UCL; http://www.vislab.ucl.ac.uk/), running under Matlab 6.5.1 (Mathworks Inc., Natick, MA, USA) on a Windows PC.

When trying to reproduce the deactivations, visual stimuli were back-projected onto a Plexiglas screen using a LCD projector (JVC Ltd., Yokohama, Japan) visible to the subject through a mirror mounted on the MR-head coil. In the interleaved TMS-fMRI experiment, visual stimuli were presented through special eye goggles (VisuaStimDigital, Resonance Technology Inc.), as the presence of the TMS coil inside the scanner interfered with the visibility of the screen through the mirror.

In all situations, auditory stimuli were presented in monophonically at approximately -16 dB, using MR-compatible electrodynamic headphones (MR Confon GmbH, Magdeburg, Germany).

In the second and third steps described, subjects had to perform a target detection task, which was done using a MR-compatible custom-built button device connected to the stimulus computer.

2.1.2. Data Acquisition

A 3T SIEMENS MAGNETOM TIM Trio System (Siemens, Erlangen, Germany) was used to acquire both, high-resolution structural images (176 sagittal slices, TR = 2300 ms, TE = 2.98 ms, TI = 1100 ms, flip angle = 9°, FOV = 240 x 256 mm, image matrix = 240 x 256, voxel size = 1 x 1 x 1 mm^3) and T2*-weighted axial echoplanar images (EPI) with blood oxygenation level dependent (BOLD) contrast. The EPI sequence used was adapted for interleaved TMS-fMRI experiments. Gaps of 110 ms are introduced after every 425 ms in the gradient echo (GE) EPI sequence, i.e. after each sixth slice (GE-EPI, Cartesian k-space sampling, TR = 3210 ms, TE = 40 ms, flip angle = 90°, FOV = 192 x 192 mm, image matrix 64 x 64, 36 slices acquired sequentially in ascending direction, 3 x 3 x 2.6 mm^3 voxels, slice thickness 2.6 mm, interslice gap 0.4 mm). Each pause is introduced to allow the delivery of one TMS pulse 10 ms after each sixth slice without interference with image quality (Bestmann et al., 2003).

There were four sessions with a total of 241 volume images per session. The first 3 volumes were discarded to allow for T1-equilibration effects.

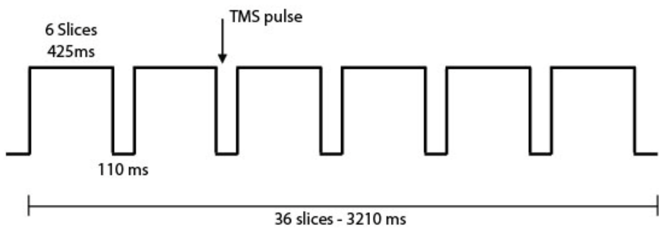


Figure 1 – Schematic representation of the EPI sequence used in the experiment.

2.1.3. Pre-Processing

The functional MRI data were preprocessed and analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) (Friston et al., 1995).

In order to account for movement artifacts, scans from each subject were realigned using the first as a reference, spatially normalized into MNI standard space using the mean-realigned image as the source image, and were resampled to 3x3x3 mm^3 voxels. The template image used for normalization is based on average data provided by the Montreal Neurological Institute. In order to enhance the signal-to-noise ratio and to enable intersubject functional anatomical comparison, images were also spatially smoothed by convolution with a Gaussian kernel of 8 mm full width at half maximum (FWHM). The timeseries in each voxel was high-pass filtered at 1/128 Hz.

2.2. First Step – Establishing the Experimental Setup

An essential aspect when performing an experiment is to make sure that the experimental setup is running appropriately and is adjusted to the goals of research. In this experiment there are three main aspects that have to be taken into consideration: synchronization of stimuli presentation and TMS, occurrence of possible artifacts due to the interaction of the different components used in the setup, and auditory co-activations induced by TMS.

2.2.1. Synchronization of Stimuli Presentation and TMS

The synchronization between the presentation of the stimuli and the application of the TMS pulses is essential for concurrent TMS-fMRI experiments.

A script programmed by Axel Thielscher running on a Windows PC remotely controls the TMS stimulator box. In order to control the intensity of the stimulator, an analog-to-digital converter connects the PC controlling the stimulator to the stimulator itself and the intensity set in the script is thereby transmitted to the stimulator. To ensure synchronization of the TMS pulses with the visual and auditory stimuli, the script for visual and auditory stimulation was also programmed to run on the triggers from the scanner. Based on the MR triggers, start indices for each stimulation block are determined and introduced both in the visual and auditory stimulation script and in the script that controls the TMS stimulator. This enables to control which stimulation intensity should be applied to each block.

2.2.2. Checking for Image Artifacts and Stimuli Presentation Distortions

As previously said, auditory stimuli are presented using electrodynamic headphones, while visual stimuli are presented either by using special goggles or by projecting stimuli onto a screen that is viewed by the subject with the help of a mirror. TMS pulses are applied by placing an appropriate TMS coil inside the scanner, which is connected to the TMS stimulator placed outside the scanner room. Even though the individual components are not thought to induce any EPI image artifacts or distortions of the visual or auditory stimuli presentation, it is essential to ensure that their combined use does not induce any artifacts either.

Distortions of visual and auditory stimuli presentation during the application of a TMS pulse were evaluated in a subjective fashion by an experimenter that placed himself inside the scanner having headphones and goggles on, and TMS pulses were applied approximately over the desired location.

Additionally, it is necessary to assure that all components combined do not induce image artifacts. In fact, it is possible that, when a component is applied alone, the noise it creates is not strong enough to induce artifacts. Yet, when it is applied in combination with other components, this noise can be extremely amplified, thereby inducing image artifacts that are not acceptable. In order to account for this possibility, phantoms were scanned with and without the delivery of TMS pulses. Apart from analyzing the phantom data to check if unwanted activations emerged, EPI sequences with zero angle RF excitation pulse were carried out to determine the noise level of the data.

2.2.3. Accounting for TMS auditory co-activations

When a TMS pulse is applied, a sound is produced that induces auditory activations. This may interfere with the actual auditory stimulation and lead to false interpretations of the results. To account for this problem, two strategies were considered.

In the replacement strategy, pseudo TMS-clicks were introduced only when TMS was not to be applied and were off in periods where TMS stimulation was present. In order for this strategy to work, an exact recording of the actual TMS-sound is necessary, so that it is not possible to differentiate between pseudo TMS-clicks and the actual TMS-sound. To record the TMS-sound, an MR compatible microphone was used and several TMS pulses were recorded with the TMS coil placed inside the scanner. Unfortunately, when the recordings were reproduced through the electrodynamic headphones, pseudo TMS-clicks and the actual TMS-sound were judged perceptually different by the experimenter placed inside the scanner.

Therefore, a masking strategy was considered, which is based on introducing pseudo TMS-clicks throughout the whole experiment, including baseline periods. In this case, the synchronization between pseudo TMS-clicks and the actual TMS-sound is essential, since both sounds must be perceived as being simultaneous when TMS pulses are applied.

Apart from this synchronization, which was successfully accomplished, it was necessary to regulate the intensity of the pseudo TMS-clicks, so that they masked the actual TMS-clicks. Behaviorally, this was achieved by placing the experimenters inside the scanner and running a Matlab script that was written so as to reproduce a pseudo TMS-click and deliver a TMS pulse sequentially. By pressing the appropriate button from the button device connected to the PC running the script, experimenters could adjust the intensity of the pseudo TMS-clicks until they considered it to be appropriate for the masking procedure.

Nonetheless, a verification that the masking strategy works out in terms of the activations that were induced was still required. In order to do so, data from two subjects were acquired while subjects were presented with the fixation cross as visual input and the pseudo TMS-clicks as an auditory input. Additionally, there were periods where TMS was applied and periods in which only the pseudo TMS-clicks were present. The comparison of periods with TMS and without TMS should not yield any differences as far as auditory activations were concerned. The performing of this comparison allowed for the evaluation of the success of the masking strategy. Moreover, in a later stage of the experiment, one subject was scanned with the TMS coil positioned over the vertex and two centimeters apart from the skull, while she was presented with the final set of stimuli chosen for the experiment (see *2.3.2. Stimuli and Tasks*). Since this coil position is thought not to induce any specific TMS effects, any potential activations have to result from non-specific TMS effects, such as auditory activations elicited by the TMS clicks. During the data acquisition, periods of high intensity TMS alternated with periods of low intensity TMS (see *2.4.3. TMS Stimulation)*. Comparing the effect of low intensity TMS with high intensity TMS under auditory stimulation (i.e. presentation of TMS pseudo clicks as masks) enables us to evaluate the effectiveness of the auditory masking stimulus. If the TMS clicks at both intensities were effectively masked by the synchronous TMS pseudo-clicks, then the comparison of different intensities of TMS pulses when using an effective TMS coil position should not reveal any differential activations.

2.3. Second Step - Reproducing the Deactivations

In the second step, we designed an experimental paradigm and stimuli that elicited robust activations and deactivations that have previously been reported in the literature. The main challenge here was to design effective auditory and visual stimuli that replicated these activation results despite confounding and masking effects of the pseudo TMS clicks. In addition, task context was manipulated to evaluate the effects of selective and divided attention on auditory and visual deactivations.

2.3.1. Participants

Four right-handed male participants (aged 26-29 years, mean age: 28, SD: 1.25) with no history of neurological or psychiatric illness took part in the fMRI experience. Participants had normal or corrected-to-normal visual acuity and reported normal hearing. All participants gave full consent prior to participation and the study was approved by the local ethics committee.

2.3.2. Stimuli and Tasks

Several stimuli sets were evaluated and optimized to show robust cross-modal deactivations in all subjects.

Based on the study of Laurienti and colleagues (Laurienti et al., 2002) that reported cross-modal deactivations in both auditory and visual cortices, the first set of stimuli being tested was a flickering checkerboard at a frequency of 4 Hz as the visual stimulus and white noise as the auditory stimulus. This kind of auditory stimulus was not salient enough when compared with the pseudo TMS-clicks and was therefore replaced with a continuous looming auditory stimulus. This new set of stimuli (checkerboard + looming auditory stimulus) revealed that a more salient visual stimulus was also necessary. Therefore, the visual stimulus was changed to a looming white ring presented in a black background. In addition, in order not to be influenced by TMS, the auditory stimulus was also changed to have duration of 425 ms and 110 ms gaps (sampling rate: 44100 Hz, see Figure 2).

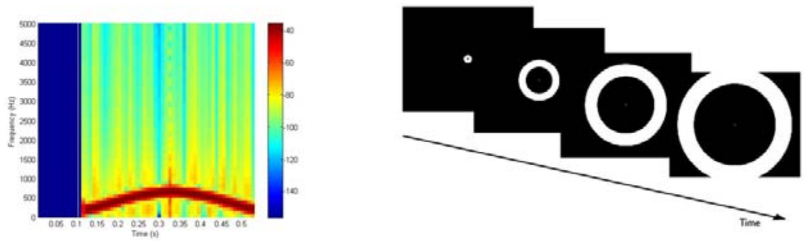


Figure 2 – Spectrogram of the auditory looming stimulus (left) and time line of the looming ring visual stimulus (right).

A target detection task was used to prevent subjects from stop paying attention to the stimuli. In each session, a target was presented in 6 out of a total of 26 blocks and subjects were asked to press a button every time a target appeared. Blocks in which targets appear and the exact moment targets appear within blocks were pseudo-randomized and thereby subjects did not know when a target was to be expected. Targets consisted of red crosses presented for 300 ms at the center of the screen and beeps with a frequency of 700 Hz and duration of 300 ms. Two versions of the target detection task were tested: In the "focused attention" task, the target modality matched the modality of the block in which they appeared in. Therefore, apart from the unimodal visual and auditory targets appearing in the visual and auditory blocks, respectively, there was an audiovisual target for the audiovisual block that consisted in the simultaneous presentation of both unimodal targets. No targets appeared in the baseline blocks. In the "divided attention" task, unimodal targets were randomly distributed through all types of blocks.

To determine which task was more effective in producing robust deactivations, a total of four sessions were acquired, two for the focused attention task and two for the divided attention task. The order in which the sessions were presented was counterbalanced within the subjects. Subjects were not aware of the difference between both tasks.

2.3.3. Experimental design

The 2x2x2 experimental design manipulated (1) visual stimulation (presence vs. absent), (2) auditory stimulation (presence vs. absent) and (3) task (divided attention vs. focused attention).

Focused attention / Divided attention

	Visual present	Visual absent
Auditory present	AV	A
Auditory absent	V	+

Figure 3 – Experimental design. 2x2x2 factorial design with the factors (1) visual stimulation (presence vs. absent), (2) auditory stimulation (presence vs. absent) and (3) task (divided attention vs. focused attention).

Stimuli were presented in a block design, in which pseudo-randomly ordered blocks of 20 seconds alternate with fixation baseline periods of 10 seconds. Apart from visual, auditory and audiovisual blocks, a long fixation block, in which only a fixation cross was displayed, was introduced in order to serve as a later control for the TMS experiment. In both focused and divided attention conditions, subjects had to detect visual (circle), auditory (beep) or audiovisual (circle+beep) targets that were randomly interspersed amongst the main stimuli. In the divided attention condition, targets from all sensory modalities were presented irrespective of the sensory modality of the block. In the focused attention condition, only targets from the sensory modality of the non-target stimuli within a particular block were presented.

2.3.4. fMRI Data Analysis

After preprocessing the data (*see 2.1.3. Pre-Processing*) SPM5 was also used for further analysis of the data. The fMRI experiment was modeled in a blocked design fashion with regressors entered into the design matrix after convolving each block with a canonical hemodynamic response function. In addition to modeling the 8 conditions in our 2 x 2 x 2 factorial design, the first statistical model included both target blocks and target onsets. Nuisance covariates included the realignment parameters to account for residual motion artifacts. All subjects were analyzed within a common fixed effects analysis. Condition-specific effects for each subject were estimated according to the general linear model. This involved creating the following contrast images for each subject:

(1) *Activations during visual stimulation under focused attention task:* V_{foc} (+)
(2) *Deactivations during visual stimulation under focused attention task:* V_{foc} (-)
(3) *Activations during auditory stimulation under focused attention task:* A_{foc} (+)
(4) *Deactivations during auditory stimulation under focused attention task:* A_{foc} (-)
(5) *Activations during visual stimulation under divided attention task:* V_{div} (+)
(6) *Deactivations during visual stimulation under divided attention task:* V_{div} (-)
(7) *Activations during auditory stimulation under divided attention task:* A_{div} (+)
(8) *Deactivations during auditory stimulation under divided attention task:* A_{div} (-)

In each case, these contrasts were used in a global null conjunction analysis of the 4 subjects.

2.4. Third Step – Stimulating the IPS

The actual interleaved TMS-fMRI experiment started with this step. Using the set of stimuli and the experimental setup attained in previous steps, the right IPS was stimulated with low and high intensity TMS in order to assess its role in the cross-modal deactivations in the visual and auditory cortices. Before stimulating, the site of stimulation has to be determined for each of the single subjects and low and high intensities have to be established. Furthermore, an a-posteriori confirmation of the stimulation site was performed. Here, in addition to how the fMRI data was analyzed, a description of how all these points were achieved will be given.

2.4.1. Participants

Three right-handed participants (2 female and 1 male, aged 25-28 years, mean age: 26, SD: 1.53) with no history of neurological or psychiatric illness took part in the experience. Participants had normal or corrected-to-normal visual acuity and reported normal hearing. All participants gave full consent prior to participation and the study was approved by the local ethics committee.

2.4.2. Experimental Design

A 2x2x2 factorial design was used for the TMS experiment, manipulating (1) visual stimulation (presence vs. absent), (2) auditory stimulation (presence vs. absent) and (3) stimulation intensity (low vs. high).

A looming ring and a looming sound were used as visual and auditory stimulation, respectively. The focused attention task was used as the target detection task (*see 2.3.2. Stimuli and Tasks*).

2.4.3. TMS Coil Positioning & Post-Hoc Verification

The location of TMS was based on the Talairach coordinates (x = 38, y = -44, z = 46) in the IPS reported by Bremmer et al. (Bremmer et al., 2001). This study used moving visual, auditory and tactile stimuli with the aim at identifying the neural mechanisms underlying human polymodal motion information processing. Their results showed increased activity in the IPS evoked by all three stimulus modalities.

To determine where to place the coil for each one of the single subjects, these coordinates have to be transformed into individual space. In order to do so, structural images of each subject, acquired previously (see *2.1.1. Data Acquisition*), are transformed into MNI space using the segmentation and normalize options from SPM5, giving both the transformation and the inverse transformation from the individual space into MNI space. The coordinates (x = 38, y = -44, z = 46), which were transformed from Talairach into MNI space, were marked on these normalized images using MRicroN. This mark was then transformed back into individual space by applying the inverse transformation to it, allowing us to obtain the desired coordinates in individual space. After having the coordinates for each individual subject, the nearest coil position on the scalp was calculated using a Matlab script written by Axel Thielscher. This position together with the high-resolution structural images, were then loaded and saved into a neuronavigation system (BrainView, Frauenhofer IPA, Stuttgart, Germany). Prior to the interleaved fMRI-TMS experiment, subjects were registered with the neuronavigation system to mark the desired stimulation position on the subject’s scalp.

Although subjects’ head were fixed with cushions inside the RF coil after the correct positioning of the TMS coil was achieved, it is still possible that subjects move during the experiment and thereby possibly change the relative position of the TMS-coil and the scalp, which consequently may change the stimulation location. The acquisition of a fast structural image (fast low-angle shot [FLASH], 100 slices, 128x128 matrix, voxel size = 2x2x3 mm, TR = 452 ms, TE = 2.46 ms) between each EPI sequence allowed for a-posteriori verification of the exact stimulation location after each one of the four sessions.

These FLASH images were first checked for coregistration using SPM5 in order to verify is there were no significant head displacements present from one session to the other. If no significant head movements were detected, the last FLASH image was used to reconstruct the coil position. In order to do that a custom-written Matlab script was used to extract the coil from the FLASH image, allowing for a visualization of the actual coil position relative to the subject's head in BrainVoyager.

2.4.4. TMS Stimulation

High intensity was determined as being 120% of the subject's motor threshold inside the scanner, which was assessed beforehand (motor threshold ranged from 55% – 61% total output, mean: 57%). The motor threshold was used as a reference for the stimulation intensities in order to follow the safety protocol for TMS experiments, which is defined relative to the motor threshold. Low intensity was set at 60% motor threshold. Being below motor threshold intensity, low intensity blocks are thought not to induce any activity in the area being stimulated and thus may be used as a control condition. These blocks were chosen over no-TMS blocks, because, by using low intensity blocks, auditory and somatosensory co-activations were still evoked, and the difference between the experimental conditions in these blocks and in high intensity blocks was minimized. Whether low intensity or high intensity was applied to a certain block, was controlled by the same script that controls the TMS stimulator via an analog-to-digital converter (see *Synchronization of Stimuli Presentation and TMS*).

In each block, repetitive pulses of TMS (rTMS) were applied at a frequency of 1.9 Hz in gaps between acquisitions of 6 subsequent slices, thus avoiding image artifacts due to TMS pulses (see *Data Acquisition*). Biphasic magnetic stimuli were delivered by a MagPro X100 stimulator (MagVenture, Denmark) with an MR-compatible figure of eight TMS coil (MRi-B88). The stimulator was placed outside the scanner room so as to eliminate interference of RF noise generated by the device. A custom-build mechanical coil-holding device with 6 degrees of freedom was used to place the coil inside the scanner and to positioning it tangentially over the marked location on the subject's scalp.

2.4.5. fMRI Data Analysis

After preprocessing the data (*see 2.1.3. Pre-Processing*) SPM5 was also used for further analysis of the data. The fMRI experiment was modeled in a blocked design fashion with regressors entered into the design matrix after convolving each block with a canonical hemodynamic response function. In addition to modeling the 8 conditions in our 2 x 2 x 2 factorial design, the first-level statistical model included both target blocks and target onsets. Nuisance covariates included the realignment parameters to account for residual motion artifacts. All subjects were analyzed within a common fixed effects analysis. Condition-specific effects under low intensity TMS were estimated for each subject according to the general linear model. This involved creating the following contrast images for each subject:

(1) *Activations during visual stimulation under focused attention task:* V_{low} (+)
(2) *Deactivations during visual stimulation under focused attention task:* V_{low} (-)
(3) *Activations during auditory stimulation under focused attention task:* A_{low} (+)
(4) *Deactivations during auditory stimulation under focused attention task:* A_{low} (-)

In addition, in order to make inferences about the effect of TMS under each of the four conditions (auditory, visual, audiovisual and fixation), the following contrasts were created:

(1) $A_{low} < A_{high}$ and $A_{low} > A_{high}$
(2) $V_{low} < V_{high}$ and $V_{low} > V_{high}$
(3) $AV_{low} < AV_{high}$ and $AV_{low} > AV_{high}$
(4) $Fix_{low} < Fix_{high}$ and $Fix_{low} > Fix_{high}$
(5) $(A_{high} > Al_{ow}) > (V_{high} > V_{low})$

Again, all these contrast images were used to perform a global null conjunction analysis of the subjects.

3. Results

In this chapter, the results obtained throughout all the steps of the experiment are presented. Initially, the results with respect to the assessment of the setup will be shown, in particular the ones that concern the testing for TMS-induced co-activations. Secondly, the results on the reproducibility of the deactivations in visual and auditory areas (as seen without TMS) during low-intensity TMS will be described. Finally, the results obtained when stimulating the right IPS will be characterized.

3.1. Establishing the Experimental Setup

Images displayed in the goggles remained unchanged and no auditory alterations were reported during TMS delivery. Additionally, analysis of the phantom data revealed no EPI image artifacts near the headphones, goggles and TMS coil.

The results presented in the following show the effectiveness of our masking strategy used to control for nonspecific TMS effects (auditory co-activations) and are based on the data from one subject, scanned with the TMS coil placed two centimeters apart from the vertex. Figure 4 depicts the parameter estimates in a spherical region of interest (ROI) in the auditory cortex with a radius of 6 mm. The centre of the ROI was defined as the voxel exhibiting peak activation when analyzing the auditory conditions with low and high intensities together. The error bars reflect the standard deviation within the ROI. Under auditory stimulation, there is a small increase in activation with high intensity TMS. The same pattern occurs under audiovisual stimulation. During fixation blocks, the auditory cortex seems to be deactivated and this deactivation seems to be slightly larger under high intensity TMS stimulation.

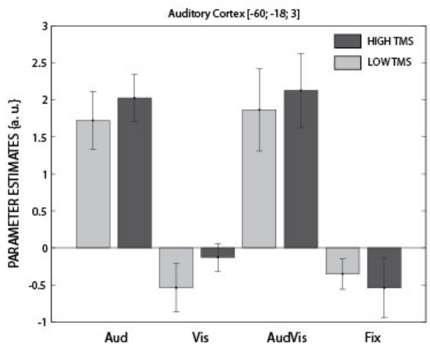


Figure 4 – Parameter estimates for auditory (AUD), visual (VIS), audiovisual (AUDVIS) and fixation (FIX) conditions in a spherical region of interest in the auditory cortex (p< 0.01 uncorrected). The bar graphs represent the size of the effect in non-dimensional units.

3.2. Reproducing the Deactivations

The results shown in the following were derived from a global null conjunction analysis of four subjects thresholded at p<0.01 (uncorrected) at the voxel level and at 50 voxels at the cluster level. The results encompass only the testing of the last set of stimuli described in the methods session. Results for the divided and focused attention tasks are presented separately. For each task, in order to identify brain regions that show deactivations under visual and auditory stimulation, we defined the contrasts V(-) and A(-), respectively. An overview of the pattern of activations under the divided attention task can be seen in Figure 5.

Auditory Stimulation   Visual Stimulation

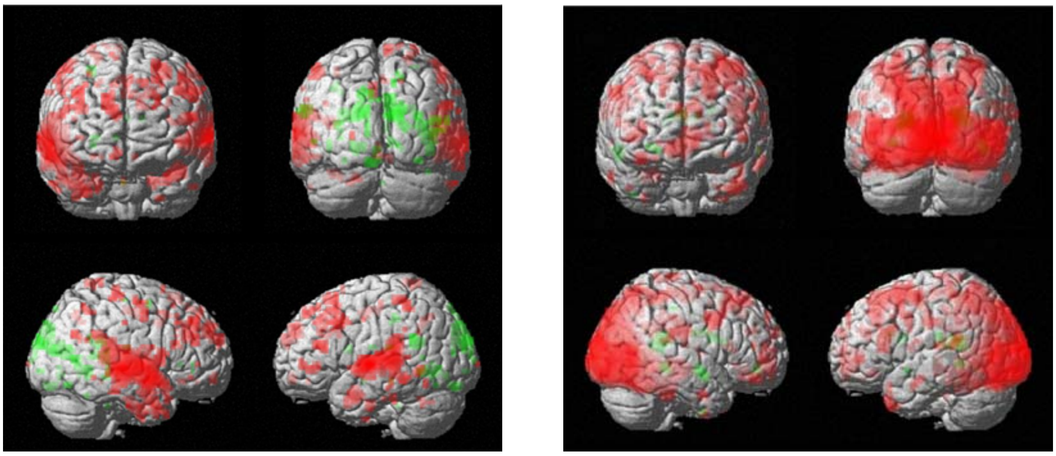
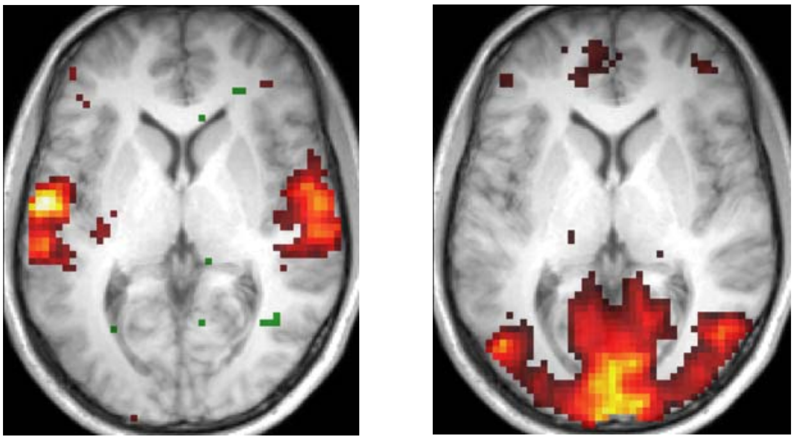


Figure 5 – Overview of activations (red) and deactivations (green) during auditory (left) and visual (right) stimulation under the divided attention task.

Under the divided attention task, both visual and auditory stimulation induced very strong activations in the corresponding cortices. In addition, visual stimulation also induced activation in the left superior frontal gyrus and cingulate gyrus (Figure 6). Nevertheless, only negligible deactivations are visible in the auditory cortex. The visual cortex demonstrated some deactivation due to auditory stimulation, but this deactivation was not significant (<50 voxels at the cluster level) (Table 1).

Table 1. Deactivations under the divided attention task.

	MNI Coordinates (mm)			*Z Score*
Brain Regions	X	y	Z	
Visual Cortex				
Right Superior Occipital Gyrus	18	-96	24	3.69
Right Cuneus Gyrus	9	-87	30	2.95



Figures 6 – Overlaid on a mean structural image of the subjects are the activations (red) and deactivations (green) relative to baseline during auditory (left) and visual stimulation (right) under the divided attention task. Images were obtained by the global null conjunction analysis of four subjects ($p < 0.01$ uncorrected). This axial image, located at Z=4, was chosen because it includes both auditory cortex and early visual areas.

During the focused attention task, the deactivations induced in the visual and auditory cortices were much more reliable and spatially more extended (Figure 7).The deactivations in the visual cortex resulting from auditory stimulation also comprised early visual areas, particularly around in the calcarine and lingual sulci and extended to the cuneus (Figure 8 (left), Table 2). Similarly, visual stimulation induced modulatory effects in terms of deactivations in areas of the auditory cortex, such as the superior and medial temporal gyrus, Heschl's gyrus (Figure 8 (right), Table 2). These modulatory effects in the non-matching cortices were present not only in the group analysis, but in three out of four of the individual subjects.

Auditory Stimulation　　Visual Stimulation

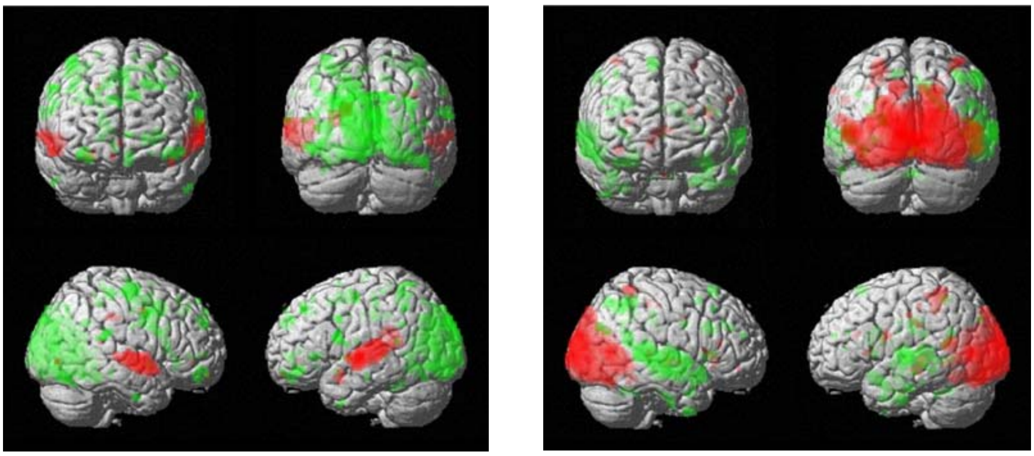
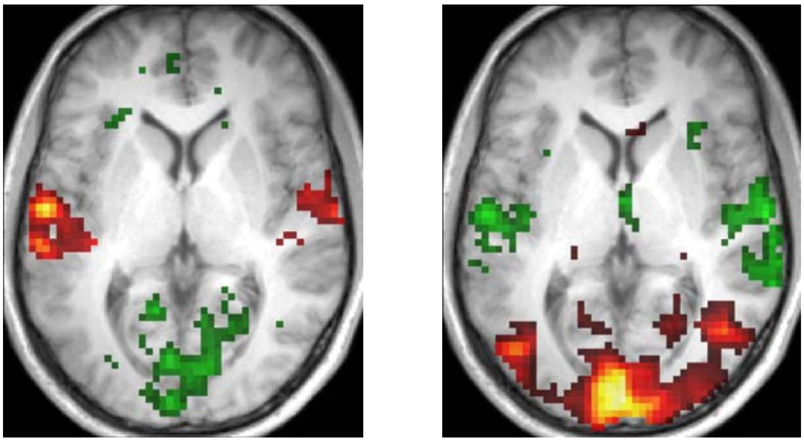


Figure 7 – Overview of activations (red) and deactivations (green) during auditory (left) and visual (right) stimulation under the focused attention task.



Figures 8 – Overlaid on a mean structural image of the subjects are the activations (red) and deactivations (green) relative to baseline during auditory (left) and visual stimulation (right) under the focused attention task. Images were obtained by the global null conjunction analysis of four subjects ($p < 0.01$ uncorrected). This axial image, located at Z=4, was chosen because it includes both auditory cortex and early visual areas.

Table 2. Deactivations under the focused attention task.

	MNI Coordinates (mm)			*Z Score*
Brain Regions	x	y	Z	
Visual Cortex				
Right occipital cortex				
Calcarine Gyrus	9	-84	15	4.58
Lingual Gyrus	9	-63	6	3.51
Superior Occipital Gyrus	18	-93	33	3.44
Left occipital cortex				
Cuneus Gyrus	0	-78	21	4.83
Lingual Gyrus	-6	-81	0	4.34
Calcarine Gyrus	-9	-78	15	4.25
Inferior Occipital Gyrus	-45	-75	-9	3.57
Auditory Cortex				
Right temporal cortex				
Superior Temporal Gyrus	63	-15	3	4.98
Middle Temporal Gyrus	57	-9	-12	4.55
Left temporal cortex				
Middle Temporal Gyrus	-60	-15	0	5.05
Heschel Gyrus	-42	-15	6	3.95
Superior Temporal Gyrus	-45	-21	0	3.84

The parameter estimate plots from spherical ROIs (6 mm radius) in the auditory and visual cortices also illustrate the difference between the deactivations induced with both tasks (Figure 9). The centers of the ROIs were defined as the voxels exhibiting peak activation during auditory (for the ROI in the auditory cortex) and visual stimulation (for the ROI in the visual cortex) for each task. The error bars reflect the standard deviation within the ROI. The plots confirm that the deactivations induced in the cross-modal cortices during the focused attention task (Figure 9 (B)) are stronger than the ones induced during the divided attention task (Figure 9 (A)).

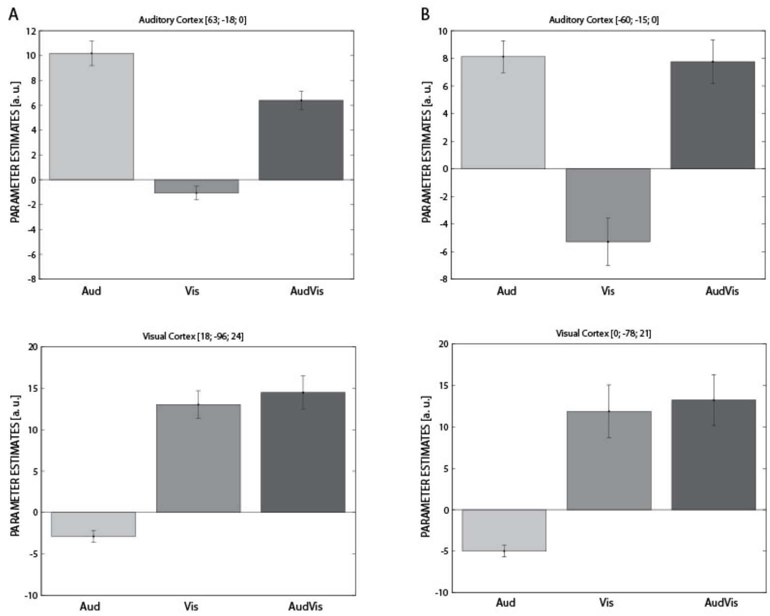


Figure 9 – Parameter estimates during auditory (AUD), visual (VIS), audiovisual (AUDVIS) blocks in the auditory and visual cortices during the divided attention task (A) and during the focused attention task (B) ($p< 0.01$ uncorrected). The bar graphs represent the size of the effect in non-dimensional units.

3.3. Stimulating the IPS

This section includes the results obtained by analyzing the data collected in the interleaved TMS-fMRI experiment. First, the reconstructed stimulation location for each of the single subjects will be shown, followed by the imaging results obtained for the conditions with low intensity TMS. Finally, the effects of high vs. low intensity TMS stimulation will be outlined.

3.3.1. Reconstructing of the Stimulation Location

Placing the coil over the exact location on the skull is a difficult task. Thus, it is important to ensure post-hoc that the area stimulated during the experiment was accurate. In Figure 10, one can see the reconstruction of the stimulation site for each of the three subjects. As one can see, in all of the three subjects, the coil seems to be approximately over the desired location.

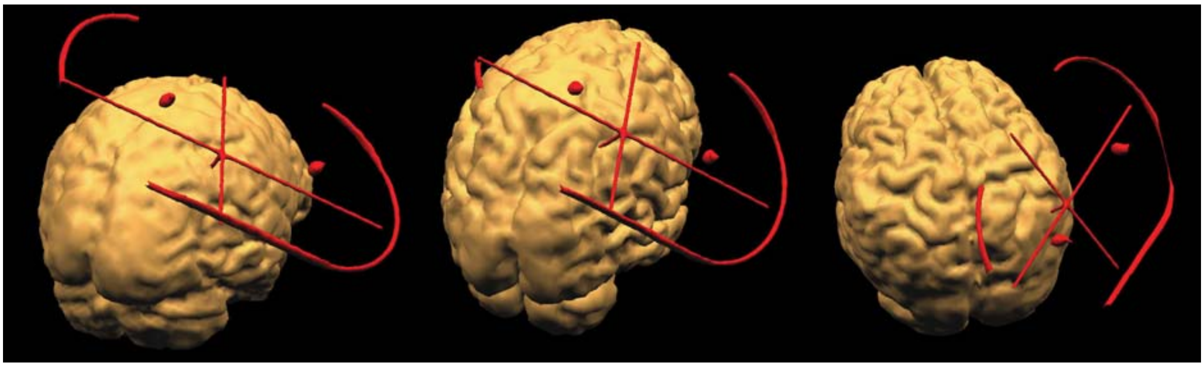


Figure 10 – (*from left to right*) Reconstruction of the stimulation site for AJ, NZ and MG

Comparing the intended stimulation coordinates with the ones that were actually achieved (Table 3) reveals the amount of inaccuracy of our placement strategy. On average, the actual stimulation location was ~10 mm away from the intended location and the stimulation region in all subjects was clearly the IPS. While this amount of inaccuracy is acceptable for a pilot study as presented here, it should be slightly improved in future scans.

Table 3. Intended and attained individual coordinates.

SUBJECTS	*Intended Coordinates (mm)*			*Reached Coordinates (mm)*			*Deviation (mm)*
	x	y	z	x	y	Z	
AJ	29.6	-50.3	37.7	32.6	-52.1	31.7	7.1
NZ	32	-57	42	21.9	-53.9	40.6	10.65
MG	35.4	-50.3	41.5	39.7	-59.3	33.5	12.78

3.3.2. Identification of the sensory system during low intensity TMS

In order to determine if the deactivation pattern was still present during the application of low intensity TMS, auditory and visual conditions under low intensity TMS were compared to baseline.

In Figure 11, one can observe the results obtained with a global null conjunction analysis. One of the subjects did not show deactivations in the auditory cortex due to visual stimulation. Given the small total number of subjects, he was left out of this particular conjunction analysis.

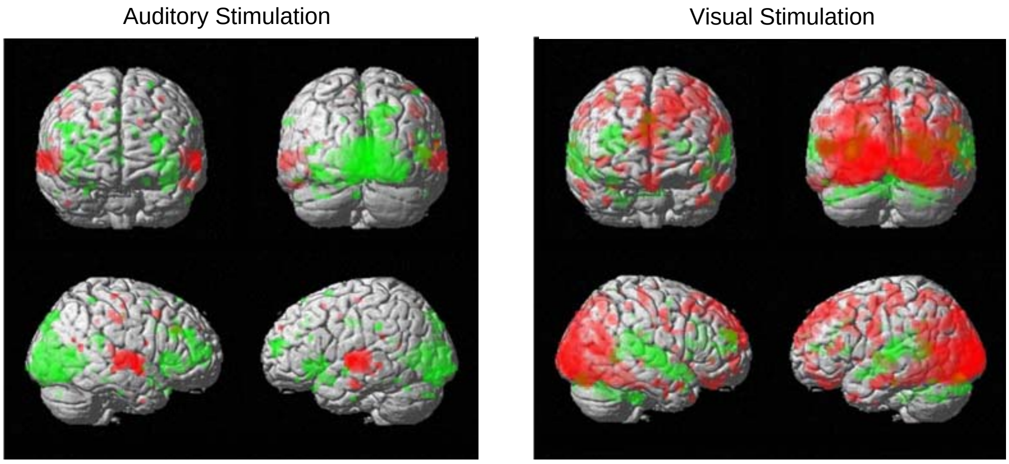


Figure 11 – Overview of activations (red) and deactivations (green) during auditory (left) and visual (right) stimulation under low intensity TMS.

As in the case where no TMS was applied, auditory and visual cortices showed significant activations in their corresponding sensory modalities. Additionally, the deactivations are also comparable with the ones obtained without TMS (Figure 12). In fact, the areas deactivated are essentially the same in both occasions (Table 4).

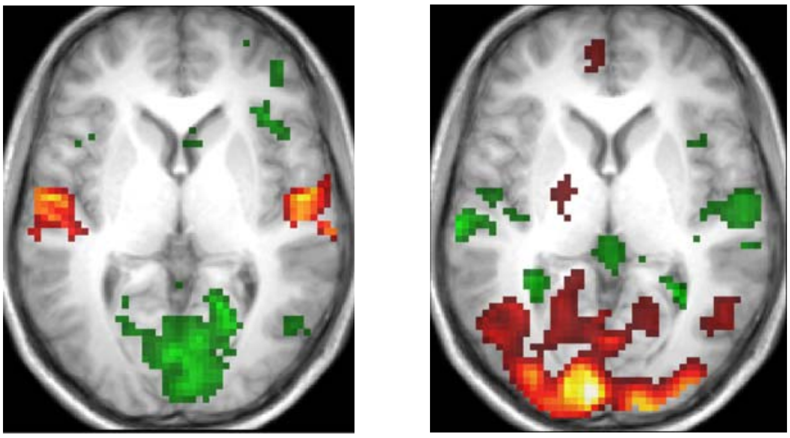


Figure 12 – Overlaid on a mean structural image of the subjects are the activations (red) and deactivations (green) relative to baseline during auditory (left) and visual stimulation (right) under low intensity TMS ($p < 0.01$ uncorrected). This axial image, located at Z=4, was chosen because it includes both auditory cortex and early visual areas.

Table 4. Deactivations under low intensity TMS.

	MNI Coordinates (mm)			*Z Score*
Brain Regions	x	Y	Z	
Visual Cortex				
Right occipital cortex				
Calcarine Gyrus	6	-81	12	4.87
Lingual Gyrus	18	-57	3	4.83
Fusiform Gyrus	33	-75	-12	4.78
Inferior Occipital Gyrus	36	-75	-6	3.89
Cuneus Gyrus	21	-81	45	3.44
Left occipital cortex				
Calcarine Gyrus	-3	-96	-6	5.06
Lingual Gyrus	-3	-78	3	4.62
Auditory Cortex				
Right temporal cortex				
Superior Temporal Gyrus	60	-30	6	4.95
Middle Temporal Gyrus	48	0	-15	4.22
Left temporal cortex				
Superior Temporal Gyrus	-66	-21	6	6.30
Heschel Gyrus	-45	-12	6	2.83

3.3.3. Effects of TMS

To facilitate the determination of the effects induced by applying TMS to the right IPS, a direct contrast between high and low intensity TMS was performed for each of the four different stimulation conditions.

Comparing low intensity to high intensity TMS under auditory stimulation ($A_{low} > A_{high}$), did not reveal any significant differential activations. In contrast, comparison of high vs. low intensity TMS ($A_{low} < A_{high}$) revealed significantly less deactivations in both lower and higher order visual cortex, as well as activations in the right auditory cortex and in frontal brain regions (Figure 13). Among the visual areas that were more activated during low intensity TMS are the fusiform and lingual gyrus and the calcarine sulcus. Activations in the V5+/MT area are also visible. This activations suggest that the deactivations in the visual cortex due to visual stimulation are reduced with high intensity TMS. In the auditory cortex, activations were restricted to the right middle and superior temporal gyri (Table 5).

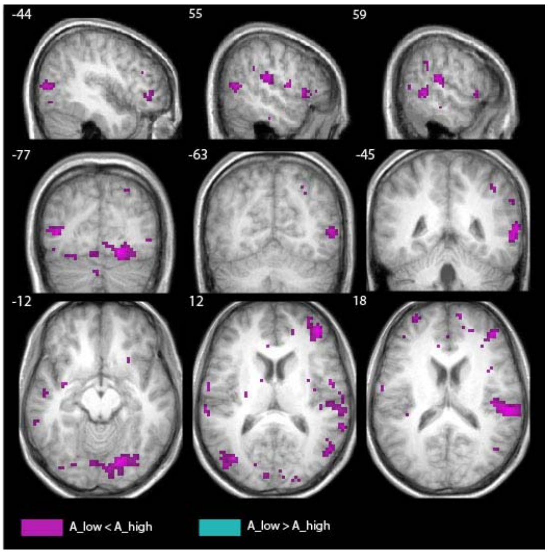


Figure 13 - Overlaid ion the mean structural image of the three subjects are the cortical areas that activated for $A_{low} > A_{high}$ (cyan) and $A_{low} < A_{high}$ (violet). Images were obtained by the global null conjunction analysis of three subjects (p<0.001 uncorrected).

Contrary to the results for auditory stimulation, the comparison between low and high intensity TMS during visual stimulation only revealed significant results for $V_{low} > V_{high}$ (Figure 14). Interestingly, apart from the activation of some visual areas (V5+/MT), only the motor and somatosensory cortices contra-lateral to the stimulation location were more activated for low vs. high intensity TMS stimulation.

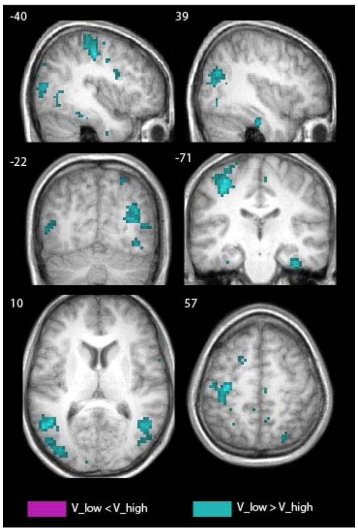


Figure 14 - Overlaid ion the mean structural image of the three subjects are the cortical areas that activated for $V_{low} > V_{high}$ (cyan) and $V_{low} < V_{high}$ (violet). Images were obtained by the global null conjunction analysis of three subjects (p<0.001 uncorrected).

Approximately the same motor and somatosensory areas were also more activated for low intensity TMS than for high intensity TMS during audiovisual stimulation (Figure 15). In addition, the reverse contrast

($AV_{low} < AV_{high}$) showed activations in right prefrontal regions and in the IPS, predominantly in the stimulated areas in the right IPS.

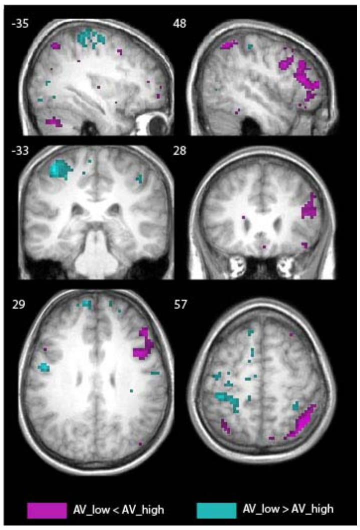


Figure 15 - Overlaid ion the mean structural image of the three subjects are the cortical areas that activated for $AV_{low} > AV_{high}$ (cyan) and $AV_{low} < AV_{high}$ (violet). Images were obtained by the global null conjunction analysis of three subjects (p<0.001 uncorrected).

Under the fixation condition only medial parietal and orbitalfrontal areas were activated for high intensity more than for low intensity TMS (Figure 16).

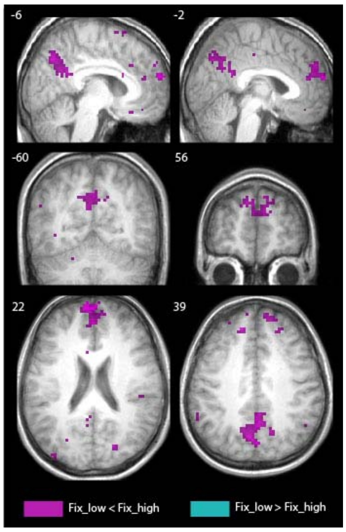


Figure 16 - Overlaid ion the mean structural image of the three subjects are the cortical areas that activated for $Fix_{low} > Fix_{high}$ (cyan) and $Fix_{low} < Fix_{high}$ (violet). Images were obtained by the global null conjunction analysis of three subjects (p<0.001 uncorrected).

The effects of TMS were further characterized in parameter estimates in regions of interest in the visual and auditory cortices, as well as in the TMS stimulated area (right IPS). The parameter estimates in the right IPS show an increase of activation for high intensity TMS for all conditions, exception for visual stimulation (Figure 17). One aspect to mention is the strong deactivation during low intensity TMS in the fixation blocks.

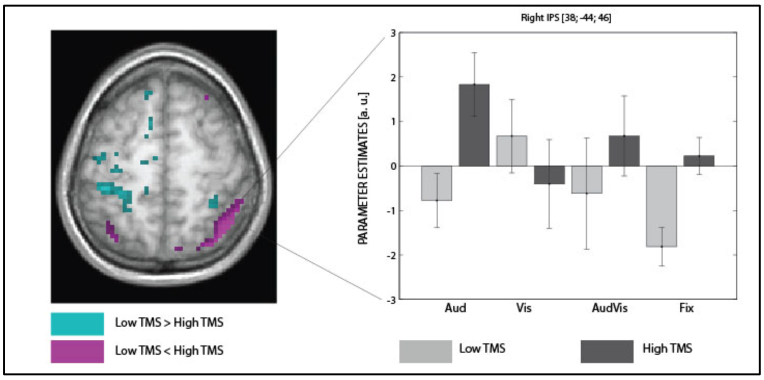


Figure 17 – Parameter estimates in the right IPS [38, -44, 46] (p<0.001 uncorrected).

The pattern of deactivations visible in the IPS seems to the propagated to the auditory cortex. Except under audiovisual stimulation, this is also true for the visual cortex (Figure 18-20). In visual areas, in particular in the V5+/MT area (Figure 19), there is a decrease of activation with high TMS stimulation in response to the matching sensory stimulus. The same pattern is visible under audiovisual stimulation. On the other hand, in both lower and higher order visual cortices, activity increases when auditory stimulation is presented (Figure 18-19).

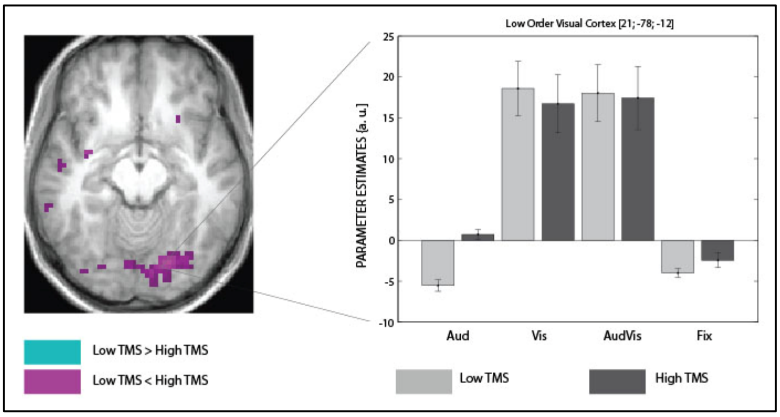


Figure 18 – Parameter estimates in lower order visual cortex [21, -78, -12] (p<0.001 uncorrected).

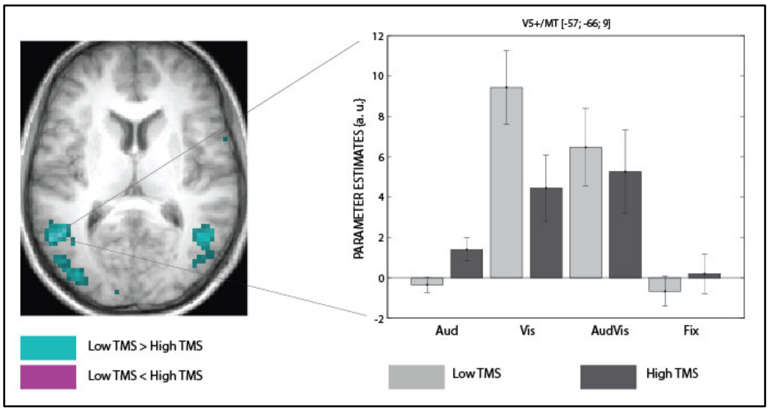


Figure 19 – Parameter estimates in higher order visual cortex [-57, -66, 9] ($p<0.001$ uncorrected).

In contrast, in the auditory cortex, activity increases with high intensity stimulation when auditory stimulation is present, and deactivations due to visual stimulation also increase, although not significantly. In addition, there are clear deactivations under both low and high intensity TMS during the fixations periods.

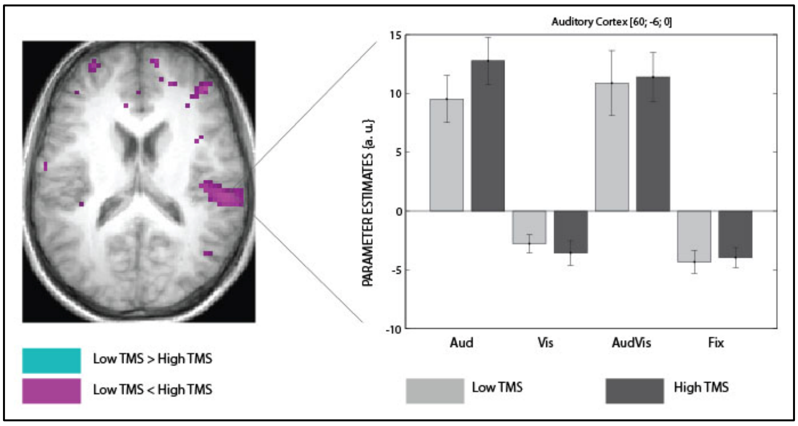


Figure 20 – Parameter estimates in auditory cortex [60, -6, 0] ($p<0.001$ uncorrected).

In order to investigate the common pattern of the parameter estimates in all the regions of interest, we tested for the interaction (A_{high} > A_{low}) > (V_{high} > V_{low}) (Figure 21). That is, we tested for an *increase* in activition for high vs. low intensity TMS during auditory stimulation blocks, combined with a *decrease* of activition with during visual stimulation. Apart from the motor and somatosensory areas, this interaction shows activations in both low and high order visual areas and it also shows some significant activation under the stimulated area (Table 5).

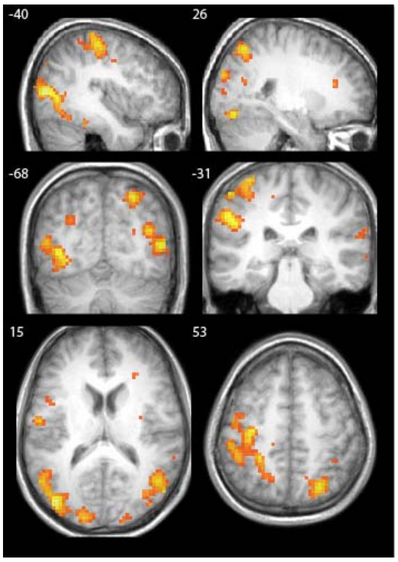


Figure 21 – Overlaid on the mean structural image of the three subjects are the cortical areas that activated for (A_{high} > Al_{ow}) > (V_{high} > V_{low}). Images were obtained by the global null conjunction analysis of three subjects ($p < 0.01$ uncorrected).

In summary, stimulation with low intensity TMS elicited the cross-modal deactivations in the visual and auditory cortices. In fact, the areas deactivated during low intensity TMS approximately coincide with the ones obtained in the focused attention task, where no TMS was applied.

When comparing low with high intensity TMS, a common activation profile was observed in the sensory cortices and right IPS as shown in the parameter estimates. The modulation of visual and auditory activations by TMS stimulation in the auditory and visual cortices imitates the activation profile observed in the IPS, suggesting an indirect effect of right IPS TMS on the sensory cortices. Whereas there is an increase in activation with high intensity TMS under the auditory, audiovisual and fixation conditions, a decrease in activation is evident under visual stimulation. Yet, despite the common qualitative activation profile, the regions differ in terms of their mean activations over all conditions. Furthermore, activation differences between conditions vary from a quantitative perspective. Indeed, the increase in activation under auditory stimulation in the IPS is much larger than the increases observed in the auditory and visual cortices. On the other hand, the decrease in activity during visual stimulation is larger in the high order visual cortex when compared to the decrease in the IPS during visual stimulation.

The pattern was further analyzed by testing for the interaction (A_{high} > Al_{ow}) > (V_{high} > V_{low}). Apart from the motor and somatosensory areas, the only areas that were activated were lower and higher order visual association areas and the right IPS. No significant activations in the auditory cortex were visible. Table 5 lists the main peaks of activation for some of the contrasts tested.

Table 5. Activated areas for the different contrasts tested.

	MNI Coordinates (mm)			*Z Score*
Brain Regions	X	Y	Z	
$Al_{ow} < A_{high}$				
Fusiform Gyrus	21	-78	-12	4.12
Middle Occipital Gyrus	-42	-75	9	3.96
Lingual Gyrus	9	-81	-9	3.35
Calcarine Sulcus	18	-90	3	3.05
Right Superior Temporal Gyrus	60	-27	18	4.08
Right Middle Temporal Gyrus	66	-45	9	3.76
Left Superior Temporal Gyrus	-66	-27	12	3.45
$Vl_{ow} < V_{high}$				
Left Middle Temporal Gyrus	-54	-57	12	4.46
Left Middle Occipital Gyrus	-42	-84	12	3.56
Right Middle Temporal Gyrus	39	-69	27	3.74
Right Middle Occipital Gyrus	45	-60	12	3.73
$(A_{high} > Al_{ow}) > (V_{high} > V_{low})$				
Left Middle Occipital Gyrus	-42	-84	12	4.99
Right Middle Temporal Gyrus	51	-63	9	4.50
Left Middle Temporal Gyrus	-57	-60	12	4.04
Precentral Gyrus	-33	-18	57	3.93
Fusiform Gyrus	24	-78	-12	3.90
Superior Parietal Gyrus	27	-69	54	3.65
Left Superior Occipital Gyrus	-18	-96	18	3.47

4. Conclusions and Future Work

This work comprised three different steps: First, we had to develop an experimental setup that minimizes the confounding non-specific TMS effects on activations elicited by audiovisual stimulation. Second, we designed an experimental paradigm that reliably elicits cross-modal deactivations. Third, we obtained initial pilot data during the interleaved TMS-fMRI experiment. The experimental setup was quite complex. Apart from the usual difficulties in all TMS-fMRI experiments, the audiovisual paradigm required special control of non-specific TMS effects inducing co-activations in the auditory cortex. To account for these co-activations a masking procedure was introduced: auditory clicks were presented as masks and were synchronized with both the auditory stimulation and the TMS-pulse application, increasing the complexity of the setup. This work reflects the importance of having a good experimental design that is appropriate for the questions that are being investigated and that accounts for all possible confounding effects.

Regarding the reproducibility of the cross-modal deactivations, the combination of the focused attention task and audiovisual looming stimuli proved to produce reliable deactivation in the non-matching sensory cortices. In contrast, no significant deactivations were observed when subjects were engaged in a divided attention task. These results are in accordance with some previous studies using endogenous attention (Johnson & Zatorre, 2006) and add to the amount of evidence of cross-modal influences between sensory cortices.

Although the results obtained while stimulating with TMS are still very preliminary, it revealed important findings that guide our future experiment.

Direct TMS effects are reflected in the profile of activations in the rIPS. In fact, under almost all conditions high intensity TMS induced an increase in activation in the rIPS. This increase with high intensity was expected, since in this study TMS was used to induce activity, rather than to disturb it. Consequently, this profile was also expected under the fixation condition. Nevertheless, during fixation, the strong deactivations relative to baseline during low intensity TMS were not anticipated. This could be explained by the lack of an absolute baseline level in fMRI experiments in combination with the use of very short baseline periods in our case (10 sec), which may lead to TMS carry-over effects from one block to the next. While activation increases were commonly observed for high intensity TMS under auditory and audiovisual stimulation, during visual stimulation, high intensity TMS had exactly the opposite effect. This response profile suggests that the rIPS was modulated by TMS and by the auditory and visual input in a complex manner. The difference in the pattern of activation under visual stimulation might be due to the fact that rIPS is predominantly a visual area so that the observed difference echoes the state-dependence of TMS-induced effects in this region. That is, the impact of a TMS pulse depends on the current level of activity at the time the TMS pulse is applied (Siebner et al., 2009).

The same pattern of activations manifested in the rIPS was also observable in both the visual and auditory cortices. Even though the patterns in the sensory cortices were not merely a copy of the one visible in the rIPS (but were also influenced by specific auditory and visual stimulation), this suggests that the effect of TMS on the rIPS seemed to be propagated to the sensory cortices to modulate their activity. In the visual cortex, the deactivations under auditory stimulation were attenuated during high intensity TMS, suggesting that the rIPS might indeed play a role in the cross-modal deactivations in the visual cortex. Furthermore, a dissociation between low and high order visual areas was apparent: while in low order visual areas high intensity TMS only induced very little decrease in activation during visual stimulation, suppression of visual activation was quite strong in high order areas, in particular in the V5+/MT area. The stronger suppression in visual areas associated with movement processing might be related to the fact that the stimuli presented in both modalities were moving stimuli.

In the auditory cortex high intensity TMS amplified activations during the presentation of the matching stimulation. Two potential mechanisms may account for this finding. First, the masking procedure for the TMS auditory co-activations may not have removed these non-specific effects completely, despite the promising results from the initial pilot that focused on non-specific TMS activations in the auditory cortex. This increase might, therefore, reflect a confound due to the extra auditory input. However, the increase in activation can also be a specific remote TMS effect indicating that TMS in the right IPS might have the inverse effect on the auditory cortex than the one it has in the visual cortex.

Even though these initial pilot studies have not yet produced conclusive results with respect to the neural mechanism of cross-modal deactivations, they have provide important insights to guide future experiments:

First, the strong deactivations in the IPS with low intensity TMS under the fixation condition probably result from the short fixation periods that do not permit appropriate decay of activation. Therefore, the insertion of longer (20 sec) baseline periods is necessary to attenuate TMS carry-over effects from activation into fixation periods.

Second, high and low intensity TMS had different effects in the IPS under auditory and visual stimulation. While high intensity TMS induced an activation increase during auditory stimulation, it induced a suppression of activation during visual stimulation. The introduction of no-TMS blocks will allow us to evaluate the effect of low intensity TMS and its interaction with sensory stimulation. Ideally, no-TMS blocks and low intensity TMS blocks should have the same effect. Including no-TMS blocks in the experimental design enables us to determine if the deactivations are due to a direct effect of low intensity TMS on the IPS.

Third, stimulation over the right IPS revealed so far a rIPS-induced effect on the visual and auditory cortices. This effect is not simply an additive propagation of the pattern of activations from the IPS to the sensory cortices, but is also modulated by the sensory input. In order to guarantee that these effects arise from stimulating the right IPS, the introduction of stimulation control sites is essential. Possible control sites are the vertex or the contra-lateral IPS. In future scans, it is also necessary to improve the positioning of the coil over the skull, in order to minimize the inaccuracy of the placement strategy.

Fourth, the definition of low intensity and high intensity may also have to be reconsidered. In order to follow the safety protocol defined for TMS experiments (Wassermann, 1998), low intensity and high intensity TMS were defined as being 60% and 120% of the individual motor threshold of the subjects. Nonetheless, these intensity values might not be appropriate for stimulation in the IPS. It has been shown that individual motor- and phosphene thresholds are not correlated (Stewart et al., 2001). Therefore, it is likely that the individual activation thresholds of the rIPS also differ from those of the motor cortices. Alternatively, one could define common high and low TMS intensities for all the subjects and still follow the safety protocol by only including subjects having motor thresholds that do not exceed a certain stimulation intensity.

Fifth, so far only three subjects participated in the TMS experiment. To allow for inferences at the population level, the optimized experimental design needs to be applied to a greater number of subjects. This will also allow us to dissociated robust effects from random variability. Finally, connectivity analyses using Dynamic Causal Modeling may also be used to go beyond inferences about regional activations and characterize how TMS alters the connectivity between brain areas.

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