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Corresponding Author:	Galit Yovel Tel Aviv University Tel-Aviv, N/A ISRAEL
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	gality@post.tau.ac.il
Corresponding Author's Institution:	Tel Aviv University
Corresponding Author's Secondary Institution:	
First Author:	Boaz Sadeh
First Author Secondary Information:	
Other Authors:	Boaz Sadeh
Order of Authors Secondary Information:	
Abstract:	<p>Transcranial Magnetic Stimulation (TMS) is an effective method for establishing a causal link between a cortical area and cognitive/neurophysiological effects. Specifically, by creating a transient interference with the normal activity of a target region and measuring changes in an electrophysiological signal, we can establish a causal link between the stimulated brain area or network and the electrophysiological signal that we record. If target brain areas are functionally defined with prior fMRI scan, TMS could be used to link the fMRI activations with evoked potentials recorded. However, conducting such experiments presents significant technical challenges given the high amplitude artifacts introduced into the EEG signal by the magnetic pulse, and the difficulty to successfully target areas that were functionally defined by fMRI. Here we describe a methodology for combining these three common tools: TMS, EEG and fMRI. We explain how to guide the stimulator's coil to the desired target area using anatomical or functional MRI data, how to record EEG during concurrent TMS, how to design an ERP study suitable for EEG-TMS combination and how to extract reliable ERP from the recorded data. We will provide representative results from a previously published study, in which fMRI-guided TMS was used concurrently with EEG to show that the face-selective N1 and the body-selective N1 component of the ERP are associated with distinct neural networks in extrastriate cortex. This method allows us to combine the high spatial resolution of fMRI with the high temporal resolution of TMS and EEG and therefore obtain a comprehensive understanding of the neural basis of various cognitive processes.</p>
Author Comments:	<p>Dear Allison,</p> <p>1. Please note that we used the JoVE enl file for EndNote reference style, but the reference list looks somewhat different than in the "instructions for authors". Specifically, most references include "PII" or "DOI" in parentheses, followed by the full</p>

	identifier number.
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Question	Response

Dear Allison

Thank you for allowing us to resubmit a revised version of our manuscript “Extracting visual evoked potentials from EEG data recorded during fMRI-guided Transcranial Magnetic Stimulation”. Revisions are marked in red font throughout the text. We also attach three photos to assist the scriptwriters, not to be included in the publication.

We hope that you will find the manuscript suitable for publication in the *Journal of Visualized Experiments*.

Best,
Boaz Sadeh & Galit Yovel

Extracting visual evoked potentials from EEG data recorded during fMRI-guided Transcranial Magnetic Stimulation

Boaz Sadeh¹ and Galit Yovel^{1,2}

¹School of Psychological Sciences; ²Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, Israel

Short Abstract

This paper describes a method for collecting and analyzing electroencephalography (EEG) data during concurrent transcranial magnetic stimulation (TMS) guided by activations revealed with functional magnetic resonance imaging (fMRI). A method for TMS artifact removal and extraction of event related potentials is described as well as considerations in paradigm design and experimental setup.

Long Abstract

Transcranial Magnetic Stimulation (TMS) is an effective method for establishing a causal link between a cortical area and cognitive/neurophysiological effects. Specifically, by creating a transient interference with the normal activity of a target region and measuring changes in an electrophysiological signal, we can establish a causal link between the stimulated brain area or network and the electrophysiological signal that we record. If target brain areas are functionally defined with prior fMRI scan, TMS could be used to link the fMRI activations with evoked potentials recorded. However, conducting such experiments presents significant technical challenges given the high amplitude artifacts introduced into the EEG signal by the magnetic pulse, and the difficulty to successfully target areas that were functionally defined by fMRI. Here we describe a methodology for combining these three common tools: TMS, EEG and fMRI. We explain how to guide the stimulator's coil to the desired target area using anatomical or functional MRI data, how to record EEG during concurrent TMS, how to design an ERP study suitable for EEG-TMS combination and how to extract reliable ERP from the recorded data. We will provide representative results from a previously published study, in which fMRI-guided TMS was used concurrently with EEG to show that the face-selective N1 and the body-selective N1 component of the ERP are associated with distinct neural networks in extrastriate cortex. This method allows us to combine the high spatial resolution of fMRI with the high temporal resolution of TMS and EEG and therefore obtain a comprehensive understanding of the neural basis of various cognitive processes.

Introduction

Transcranial Magnetic Stimulation (TMS) generates momentary interference to the normal neural activity in target areas of the brain. By creating this transitory neural interference and measuring a behavioral or physiological change, we can draw a causal link between the target area and the measured experimental effect (for a review see ^{1,2}). Such an experimental effect may be, for example, a performance on a cognitive task or a change in electrophysiological (EEG) activity. Indeed, in recent years researchers have started using TMS in combination with EEG to directly relate cortical areas with event-related potentials (ERP) or oscillatory activity patterns (e.g. ²⁻⁷). In this methodological paper we will describe a particular and useful framework for combining TMS and EEG: fMRI-guided TMS during an ERP experiment. First, we will detail how to apply TMS to areas predefined by fMRI, while recording EEG data. We will then describe an experimental design that allows extraction of reliable ERP. The goal of such an experiment is to causally link brain areas revealed with functional MRI to ERP components of interest. Lastly, we will give a specific example of a study relating face and body selective ERPs with face and body selective areas that are revealed with fMRI.

What is the benefit of linking EEG signals with fMRI activations? EEG and fMRI are commonly used tools to measure cortical responses to visual input. For example, category-selectivity in the visual pathway was assessed for different visual object categories such as faces, body parts, and written words, both by means of ERP extracted from EEG data ^{8,9}, and functional MRI ¹⁰⁻¹². The signals measured by these two common research tools are, however, of fundamentally different nature. EEG carries information about neural electrical activity with great temporal precision, but very low spatial resolution and may reflect a mixture of many separate underlying sources. The fMRI provides an indirect measure of neuronal activity relying on the slow hemodynamic changes occurring during stimulus presentation or/and task execution, but presents this activity with a higher spatial resolution. Establishing a correlation between the two measures can thus be of great interest, but is limited in that it does not imply a causal link between the scalp-recorded electrophysiological response and the areas revealed with functional MRI. Even when measured simultaneously (e.g. ¹³⁻¹⁵), a directional causal relation between EEG and activity in functionally defined cortical areas cannot be determined. TMS is a tool that can assist achieving the establishment of such a causal relationship.

A simultaneous EEG-TMS study is methodologically challenging, mostly due to the high voltage artifact introduced to the EEG signal by the magnetic stimulation (see Fig. 1, for a review see ¹⁶). This artifact consists of a transient short living pulse-related disturbance, often followed by a slower secondary (or residual) artifact that may last a few hundreds of milliseconds after the pulse is delivered (Fig. 2A), thus overriding most ERP components of interest. This secondary artifact may include mechanical sources such as currents induced by the magnetic pulse into the wiring and the slow decay of these currents in the skin, and physiological sources such as muscular activity over the scalp and auditory or somatosensory evoked potentials elicited by the operation of the coil ¹⁷⁻²⁰. Although the mechanical sources of interference probably produce larger amplitude artifacts than the physiological ones, these different artifacts cannot be separated, and the existence of any of them in the signal can confound the results. One

possible solution is the application of repetitive TMS pulses prior to EEG recording ("offline TMS"), as opposed to simultaneous EEG-TMS. The inhibitory effect of such a protocol on cortical activity persists for several minutes (and up to half an hour) after the stimulation, and EEG can be measured during this effective time window and compared with baseline, pre-TMS, EEG data. Repetitive stimulation, however, is by definition lacking the high temporal resolution that online TMS may offer, where pulses can be administered at a precise timing relative to trial onset at the millisecond resolution. The effect of repetitive stimulation may also propagate via cortical connections across a wider area than desired and therefore significantly reduce the spatial resolution as well.

To take advantage of both the spatial and temporal resolution that TMS can provide, a simultaneous EEG-TMS combination can be applied. However, this requires methods for removal of artifacts generated by the magnetic stimulation on the EEG signal. Very few offline mathematical solutions for TMS artifact removal have been proposed^{16,21,22}, although no method is agreed upon, and no one method may be optimal for all experimental designs. A "clipping" system, consisting of a sample-and-hold circuitry, was also developed to momentarily stop EEG acquisition during TMS pulse delivery²⁰. This technique not only requires specialized hardware, but may not completely remove the residual TMS artifact. In this paper we will describe an adaptation of an EEG-TMS methodology developed by Thut and colleagues¹⁹, particularly suitable for ERP studies. This technique allows reliable extraction of ERP while eliminating all the residual noise components caused by the TMS pulse (see Fig. 2). We will further provide general guidance towards a successful EEG-TMS experimental setup.

Another challenge in TMS studies addressed in this methodological paper is finding the best coil position and angle for an accurate targeting of the desired cortical area. We will describe the use of a stereotactic navigation system to coregister the subject's head with the pre-acquired functional MRI images. Although the navigation system can be used to localize anatomically-defined brain structures, an fMRI-guided targeting is particularly useful since for many functions and experimental effects the precise location of activation cannot be inferred from anatomical markers alone. For such functional regions of interest (ROI), the definition of an area is made for each participant individually.

To illustrate all of the above, we will provide an example of a study we conducted previously, in which EEG was recorded concurrently with TMS guided by fMRI activations⁷. In this study, a double dissociation was made between face-selective and body-selective ERPs: although face and body ERPs peak around the same latency and electrode sites, targeting individually defined face-selective and body-selective areas in the lateral occipital lobe enabled us to dissociate the neural networks underlying each ERP response. Finally, we will try to give more general advice for optimizing EEG recording during TMS application.

Protocol

The experiment is conducted in two separate sessions. During the first session a functional MRI experiment (e.g. a functional localizer) is carried out in order to define the desired TMS target areas on an individual subject basis. The fMRI results are then fed into a stereotactic navigation system for an accurate TMS targeting. The second session is held following the analysis of the fMRI data, during which EEG is recorded concurrently with TMS. The protocol described here was approved by the ethics committee of the Tel-Aviv Sourasky Medical Center.

In the example given in this paper, data were analyzed with Matlab version 7.7 (R2008b). The Statistical Parametric Mapping (SPM 5) software for Matlab and MarsBar toolbox for SPM²³ were used for fMRI data processing.

1) fMRI session and fMRI data analysis

1.1) Run a functional MRI task using an echo planar imaging (EPI) sequence to determine the desired foci of activation to be targeted with TMS. For a better dissociation between adjacent areas, such as the OFA and EBA in the example below, high-resolution scanning is recommended. Voxels of 3mm³ or smaller, obtained with an MRI head-coil of 8 channels or more, are sufficient for delineating adjacent regions.

1.2) Run a T1-weighted structural scan to obtain neuroanatomical data. Make sure that the face of the participant is entirely included in the field of view of this scan, since external markers on the image (e.g. the tip of the nose) will be later used to coregister the subject's head with its scan.

1.3) After data collection, use the MarsBar toolbox for SPM to define the desired brain regions of interest based on contrasts between the experimental conditions. Use the contrasts face>objects to define the Occipital Face Area (OFA), and bodies>objects to define the Extrastirrate Body Area (EBA). To further ensure that the two cortical targets are functionally distinct, use "conjunction" (in MarsBar) to mask from each ROI any voxels responding to the other experimental condition (exclude face-voxels from the EBA, and body-voxels from the OFA).

1.4) Corregister the structural T1 images with the functional scans, using SPM.

1.5) Copy the files of the structural scan, as well as the relevant functional contrast files, to a portable drive in order to upload to the navigation system.

2) Preparing a paradigm for the EEG-TMS experiment that will allow ERP extraction

Described in the section below is a method for collecting EEG data during TMS application in a way that allows extraction of reliable and replicable ERPs¹⁹. The advantage of this technique is that it easily handles the secondary, long lasting, TMS artifact, and is robust enough even to

allow restoration of data at electrodes located right under the TMS coil, where the artifact is of the highest voltage and longest duration.

2.1) Organization of the paradigm

2.1.1) Run the different TMS conditions (the different target brain areas, as well as a no-TMS condition) in separate blocks.

2.1.2) Within each block present the participant with all stimulus conditions (e.g. faces, objects, scenes and so forth) randomly in an event-related design.

2.1.3) For better quality of ERP and TMS-noise template (below) make sure to have at least 50 trials per condition.

2.2) Set the timing of the TMS pulse/pulses to the desired latency after image onset. This is done via writing to a parallel port, from which a cable goes to the TMS stimulator. This function is available in most software for psychological experiments, such as Psychtoolbox (versions 2 or 3) for Matlab²⁴ or E-Prime (see table 1). Jitter the inter stimulus interval (ISI) to reduce stimulus (and pulse) predictability (e.g. add a random value between 0 to 500 ms at each ISI).

2.3) Prepare an additional blank-screen condition:

2.3.1) Prepare trials during which TMS will be applied at the same intensity, but with no stimulus presentation on the screen. These blank-screen TMS trials will be served to calculate a TMS artifact template in the absence of visual stimulation.

2.3.2) Set the number of repetitions of the blank trials to be identical to the number of repetitions of any one of the experimental conditions within the block.

2.3.3) For an accurate representation of the shape of the TMS residual artifact, randomize the blank trials throughout the whole block rather than presenting them all at the beginning or at the end.

3) Setting up the EEG and the neuronavigation system, and conducting the experiment

Accurate TMS targeting of individually defined ROIs is possible with the use of a stereotactic navigation system, comprised of an infrared camera, infrared sensors mounted on the participant's head, and a specialized software.

3.1) Screen participants based on TMS safety criteria. Exclude from participation subjects with a self or family history of epilepsy, subjects with other neurological conditions or with frequent migraines, and subjects on psychoactive drugs. Although not typically screened for, subjects with suspicion for autonomic disorders such as vasovagal syncope (mostly manifested as a tendency to faint easily) may also be excluded. Instruct participants to avoid alcoholic drinks

starting the prior evening, and caffeinated drinks at least two hours before the experiment. For further instructions and discussion of safety see ²⁵, and the Magstim safety review (http://joedevlin.psychol.ucl.ac.uk/tms/docs/magstim_safety.pdf).

3.2) Prepare the neuronavigation system:

3.2.1) Before the session starts, feed the structural scan files into the navigation system's software.

3.2.2) Overlay the functional MRI results (the contrasts) on the structural images.

3.2.3) Using the Neuronavigation software, mark the desired targets on the images, as well as the external anatomical markers that will serve for coregistration: the tip of the nose, the deepest part of the nose bridge often referred to as nasion, and the tragus of each ear.

3.3) Mount the EEG cap on the participant's head and connect the electrodes:

3.3.1) Try to keep electrode impedance not higher than 5kΩ.

3.3.2) To avoid TMS-related heating of the electrodes, use as little gel as possible. To achieve good impedance with little amount of gel perform a thorough skin preparation. Optionally, ask participants to wash their hair before coming to the experiment.

3.3.3) Make sure that the electrode wires do not cross each other and are oriented away from the coil's location. Avoid loops in the wires.

3.3.4) Use a high sampling rate for a better representation of the noise artifact. It is recommended to use 1kHz or above, as most previous studies using this method have done ^{7,26-28}.

3.3.5) Place the reference and ground electrodes as far from the coil as possible. In this example, areas in the occipital cortex were targeted using a nose reference and an Fz ground ⁷. For other examples see ^{3,4,27,29,30}. Note that data can be re-referenced offline to a new reference as needed, such as the common average.

Note: For a review about optimization of TMS-EEG setup, see ³¹.

3.4) Coregister the subject's head with the scan, as follows:

3.4.1) Mount the infrared detectors on the participant's head.

3.4.2) Coregister the head location with the navigation system using the predefined markers (tip of the nose, etc. See Fig. 3). It is advised to repeat the coregistration between blocks to ensure accurate coil placement at all stages.

3.5) Locate the target areas:

3.5.1) Have the subject seated with the chin resting on a chinrest at the desired distance from the screen.

3.5.2) Make sure the participants are comfortable at their chair, as they are asked to refrain from movements during the experimental blocks (important for an accurate noise-template measurement).

3.5.3) Choose a TMS target from the navigation system (Fig. 3).

3.5.4) Using the pointer tool (see table 1), let the navigation system guide the user to the optimal coil location and mark it with a small sticker on the electrode cap. It is important to hold the pointer perpendicular to the head. Repeat this stage prior to each block. Note that it is not recommended to use online navigation during the block (navigating the coil itself while holding it) since any coil movement should be avoided for a best measurement of the TMS artifact template. It was found that predefining and marking the TMS location is the optimal way to achieve stable stimulation.

3.5.5) Guide the coil's precise center to the marker, while being held by a holder. Make sure it is tangential to the head.

3.6) Set the TMS intensity to the desired value. Administer one test pulse for the participant's approval.

3.7) Run the experimental block.

3.8) No-TMS condition: if a specialized sham TMS coil is not available, place the TMS coil next to the subject's head and tilt it in 90°. Run the block as usual, including the blank trials.

The reader may also refer to the JoVE video paper by Andon and Zatorre³² for further demonstration of the navigation system.

4) Analyzing the EEG data and computing ERP

4.1) Remove the immediate pulse artifact, as follows:

4.1.1) If a clipping device is not available (see above), the first step in the EEG data processing would be to cut out the immediate TMS pulse artifact itself from the data. Note that this step can be skipped if filters are not desired. But if filters are applied, the sharp-edge shape of the artifact will create distortions in the data. A narrow time-window of 10 to 15ms after pulse onset should suffice, but be sure to verify this by visual inspection of the data.

4.1.2) Connect the two cut ends created after pulse removal. Two main methods for accomplishing this have been suggested in previous reports: 1. simply join together the two ends remaining after pulse removal (see ²⁶ and Fig. 1); 2. interpolate a line between the two cut ends by generating equally spaced values between them ⁷. The equation used for this interpolation is as follows: for each missing data point y at sample x , compute $y = y_0 + ((y_1 - y_0) * (x - x_0)) / (x_1 - x_0)$ where x_0 and y_0 are the coordinates for the last data point before the cut segment, and x_1 and y_1 are the coordinates for the first data point after the cut segment. Both techniques assure that filters can be applied on the data without producing ripples due to sharp voltage steps, as demonstrated in Fig. 1. Refer to the work by Reichenbach and colleagues ²⁷ for a more complex 3rd order polynomial interpolation.

4.2) Apply the subtraction method:

4.2.1) For each experimental block, including a no-TMS control condition if present, calculate an averaged ERP to the blank screen trials by time-locking them to trial start (as if an image had been presented).

4.2.2) Subtract this averaged template from each trial of all other stimulus conditions. If several blocks were run for the same stimulation site, do this separately for each block, as the template will be slightly different between the blocks.

4.3) All other pre-processing and processing steps are carried out as in any other ERP experiment.

Representative Results

A concurrent EEG-TMS investigation was used to reveal whether the ERP responses to faces and bodies recorded over the occipito-temporal scalp are dissociated. When visual stimuli are presented, a prominent N1 component is recorded at posterior-lateral electrode sites. In particular, the N1 component is typically larger for faces and bodies than to other stimulus categories^{8,33}. By assessing the effect of stimulation on face and body-selective brain areas defined with fMRI on their respective face and body N1 component, we attempted to reveal whether the face and body N1 responses reflect (at least partially) non-overlapping sources, or rather the same network activity with quantitatively different activation levels.

We applied a double-pulse stimulation at 60 ms and 100 ms after image onset (see for example^{34,35}), to the face-selective and body-selective areas in the lateral occipital cortex - the Occipital-Face Area (OFA) and the Extrastriate Body Area (EBA) (Fig. 4A, see section 1.3 above for definition of the relevant fMRI contrasts). The two areas were stimulated in separate blocks, while subjects viewed images of faces and headless bodies. Results show that stimulation to the OFA enhance the N1 amplitude to faces but not to bodies, whereas stimulation to the EBA enhance the N1 to bodies but not to faces. Fig. 2B depicts the face N1 before and after TMS residual artifact subtraction, and Fig. 4B shows the specific effect of TMS on the N1 component as a function of stimulated area.

These findings show how fMRI-guided TMS during concurrent EEG recording can be applied to assess whether two (or more) neural networks are dissociated, as well as to establish a causal link between a functionally defined brain area and an electrophysiological signal.

Figure Captions

Figure 1. Data processing. Raw and processed data of a representative subject, at the lateral-occipital electrode PO8. **A.** Raw EEG data including two trials, each containing two TMS pulses separated by 40 ms (red arrows); **B.** Zoom into the data after pulse removal. The two pulses at each trial are removed from the data by cutting a window around the double-pulse (2 ms before first pulse to 16 ms after second pulse). The cut edges are then connected by interpolation (red arrows) as explained in 4.1.2; **C.** The interpolated segment allows filtering without creating edge artifacts. In this figure, a 40 Hz low-pass filtered ERP (red) is plotted against its non-filtered version (grey); **D.** As an alternative to interpolation, the free ends that are remained after pulse removal can be joined together (see for example²⁶, and point 4.1.2 in the text). Here, both methods are compared and show highly similar waveforms (blue and red traces mostly overlap), after low-pass filtering at 40 Hz. Red trace: linear interpolation method; blue trace: no interpolation (connected edges are taken apart for plotting purpose only, to keep consistency of time axis).

Figure 2. TMS artifacts and the subtraction technique. **A.** Left - ERP time-locked to the presentation of an image of a face, with a double-pulse TMS at 60 ms and 100 ms after image

onset. Each line represents an electrode. Note that for some electrodes the immediate TMS artifact is followed by a longer residual artifact. Right – Approximate coil location is symbolized by the two red circles, and a few electrodes are labeled for orientation; **B.** Artifact-subtraction procedure. The immediate pulse artifact is removed (concealed), a template of the residual noise is measured based on “TMS only” trials and subtracted from full trials. Adapted with permission from ⁷.

Figure 3. Stereotactic Navigation System. Top: Setting landmarks for coregistration. In order to coregister the structural scan of the head with the actual head position during the experiment, anatomical landmarks are marked on the image as shown by arrows. Then, the locations in space of the same landmarks on the subject’s head are provided to the system with the aid of a specialized tracker that is detected by the camera. **Bottom:** Functional brain areas can be precisely targeted. Activations are overlaid on the anatomical image, and desired areas are marked and saved. During the session the experimenter can load a pre-defined area to target with TMS.

Figure 4. Representative results. Double-pulse TMS was applied either to the right OFA or to the right EBA, at 60 ms and 100 ms after the onset of a face or a headless-body image. A dissociation between the face-N1 and the body-N1 responses was made. **A.** The two target areas in a representative subject; **B.** Left - double dissociation between the face and the body networks. TMS to the OFA enhanced the N1 response to faces, but not to bodies, relatively to TMS to the EBA. The opposite pattern is shown for headless-body stimuli. Right – N1 peak amplitude for faces and bodies, following OFA stimulation, EBA stimulation, and without TMS stimulation. Error bars denote the SEM. This figures was adapted with permission from ⁷.

Discussion

Having the unique ability to momentarily disrupt normal neuronal activity in selected cortical areas, in precise time points and with a relatively good spatial accuracy, TMS allows to causally link a stimulated brain area with a behavioral or a neurophysiological measure. In this paper we described a method for measuring EEG during concurrent TMS application, targeting functionally defined cortical areas^a, and applying an analysis that enables a reliable measuring of ERP responses. We gave an example from the literature in which TMS was used in combination with EEG and fMRI to ask whether given fMRI-defined brain areas (i.e. OFA and EBA) are causally associated with ERP responses to their preferred stimuli (i.e. faces and bodies).

The subtraction technique described, which was validated ¹⁹ and applied in several studies ^{7,26,27}, has several noteworthy advantages: it allows elimination of the residual long-lasting TMS artifact covering the time-window of most significant ERP components; it equally eliminates the artifact components from muscular, mechanical (electrical interference to the electrodes) and non-desired cortical (e.g. auditory) origins; and it is robust and reliable even in electrodes lying

^a The same technique can also be applied for anatomically-defined areas

413 directly under or in proximity to the coil. Note that line noise can also be pronounced in these
414 electrodes, in addition to the enhanced amplitude TMS pulse artifact, since the coil might be
415 touching or lying in close proximity to the electrode or the wires. The technique demonstrated
416 here enables the extraction of ERPs at these electrode sites as well. This is of primary
417 importance since very often the evoked responses of interest originate in or near the
418 stimulated cortical area. Furthermore, recovering signals from the entire scalp is necessary in
419 cases where source reconstruction algorithms are desired.

420
421 The combination of research tools such as TMS, EEG and fMRI, each putting forward different
422 aspects of neural activity and attacking similar questions from different angles, is a promising
423 move forward in the research of human cognition and brain function. It can be expected that
424 TMS will be increasingly used in combination with EEG to causally associate cognitive or
425 behavioral functions to electrical activity, and to further explore currently developing fields
426 such as synchronization, brain oscillations and connectivity, in high temporal and spatial
427 resolution.

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436 437 **Disclosure**

438 The authors declare that they have no competing financial interests.
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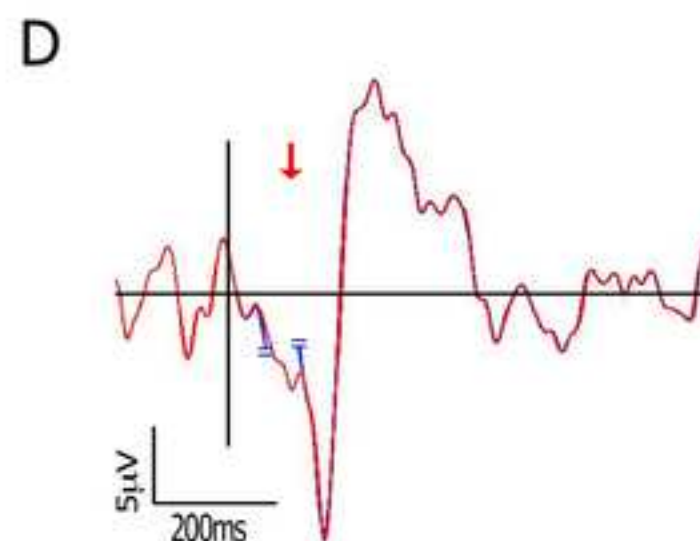
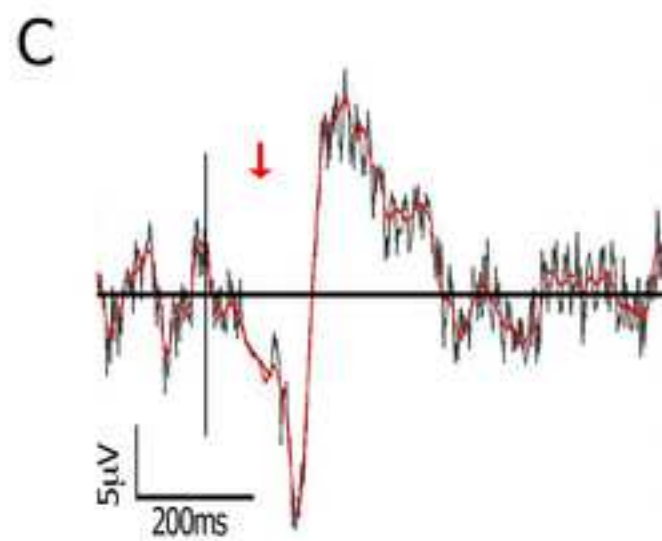
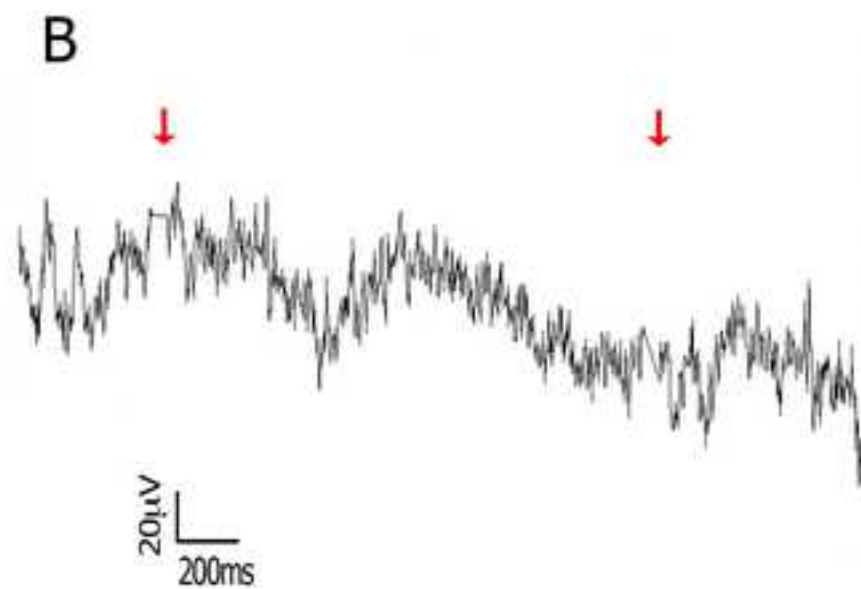
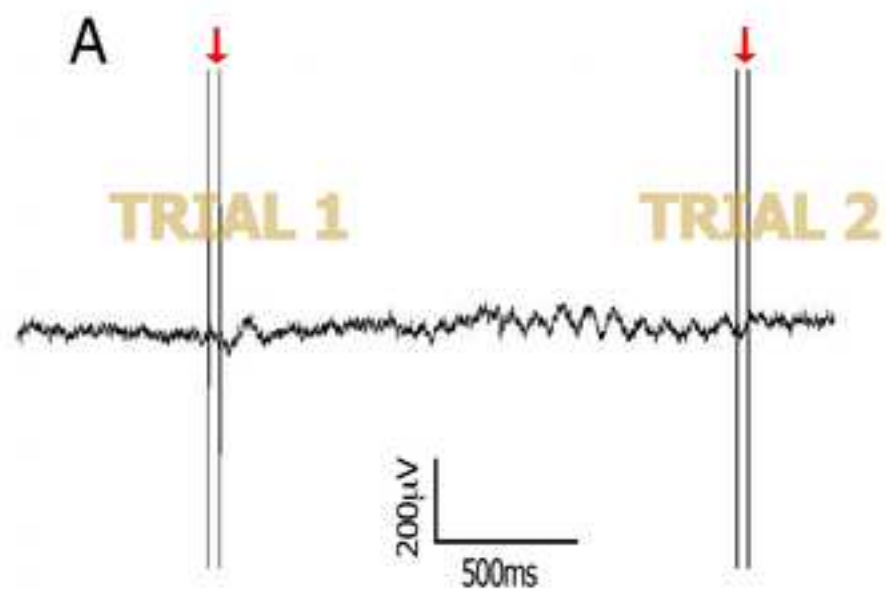
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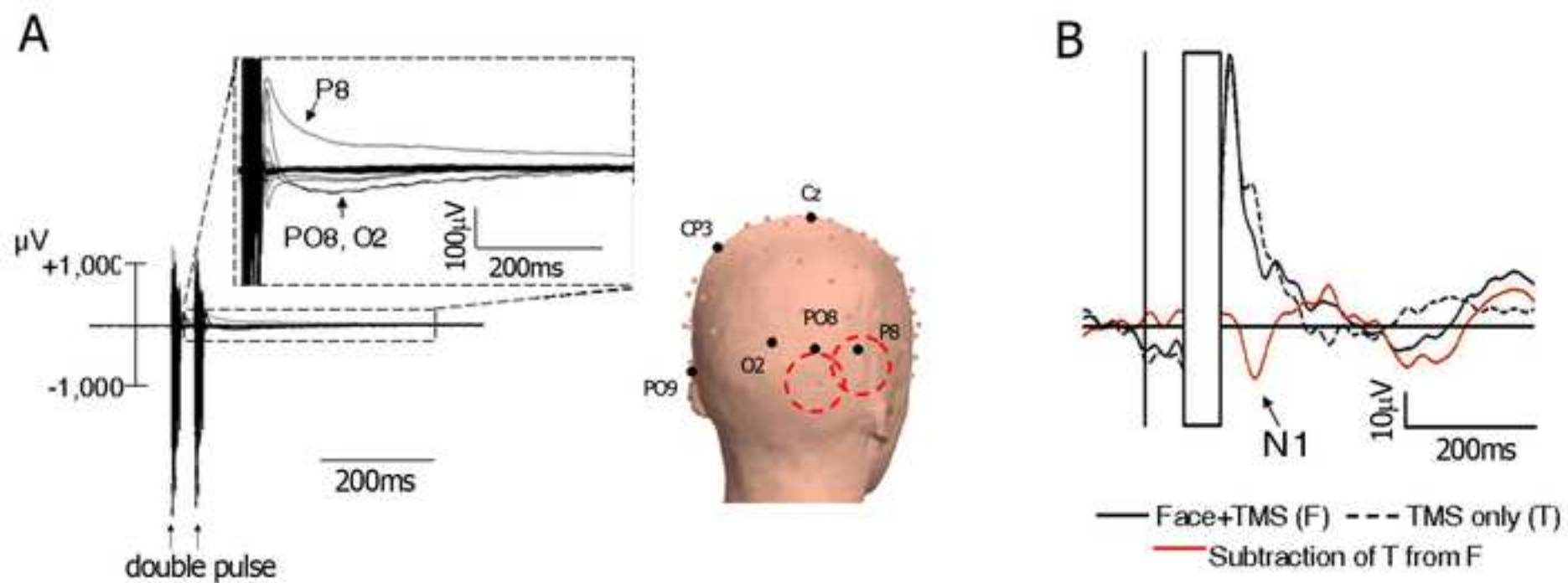
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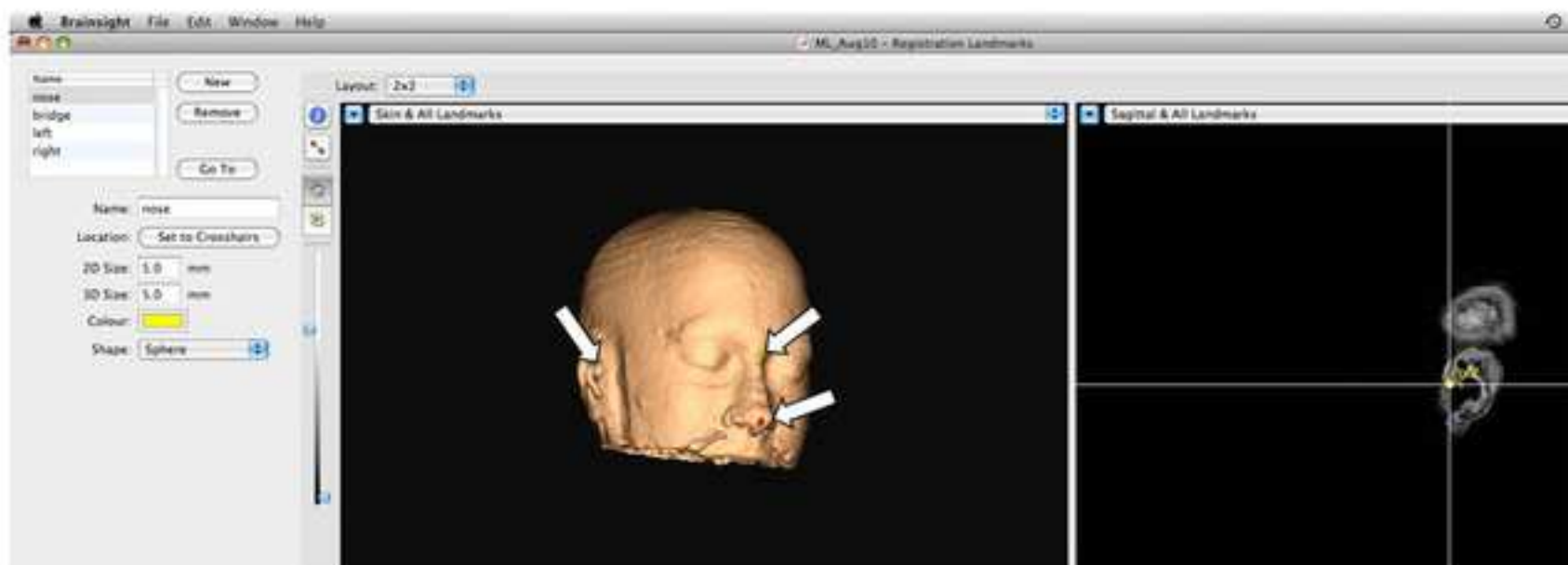
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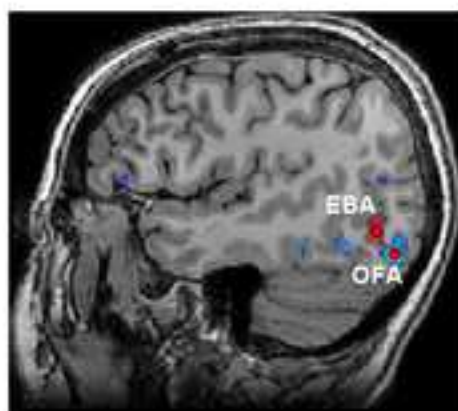




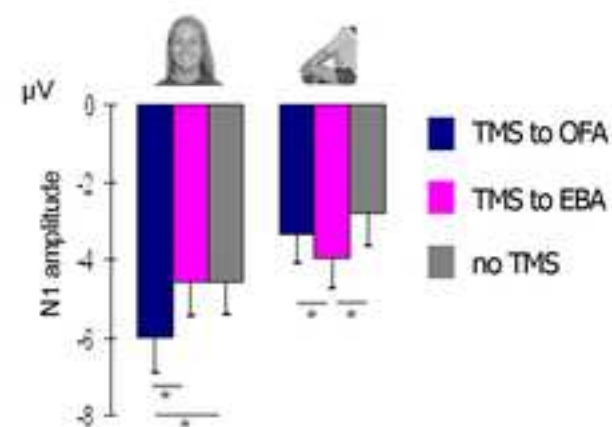
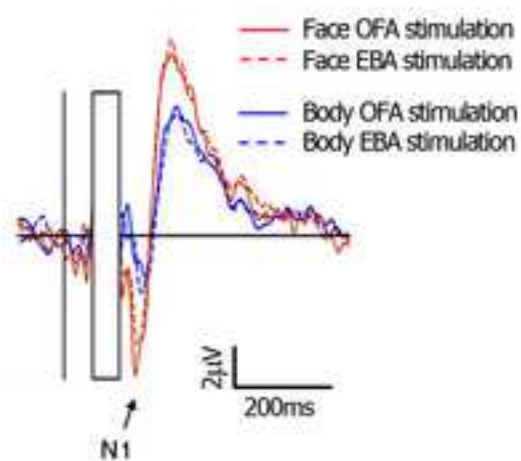
*Figure_3
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A



B



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
3.0T Signa MRI scanner	General Electric		
BrainAmp amplifier	Brain Products GmbH	BP-01300	
Electrode input box	Brain Products GmbH		Optional
PowerPack - battery for amplifier	Brain Products GmbH	BP-02615	
BrainCap - 32 flat electrodes on a flexible cap	Brain Products GmbH	BP-0300MR	Flat electrodes should be used to assure a shorter distance between coil and scalp. If larger (e.g. pin type) electrodes are used, remove the ones under the coil
TMS Super Rapid2 stimulator	Magstim		
50mm double coil	Magstim		
Coil holder			Any mechanical arm or tripod that can hold the coil, be adjusted to the right angle and location, and keep the coil steady during stimulation

Chinrest			
Polaris infrared camera	Rogue Research Inc		
Polaris trackers and pointer tool	Rogue Research Inc		
BrainSight workstation and software	Rogue Research Inc		
BrainVision Recorder software	Brain Products GmbH	BP-00010	
MATLAB software	The MathWorks Inc.		
SPM for Matlab	Wellcome Department of Imaging Neuroscience, London, UK		
MarsBar region of interest toolbox for SPM			
Psychtoolbox for MATLAB			This toolbox and the E-prime software (below) are examples for stimulus presentation software capable of delivering commands to the TMS stimulator and to the EEG recorder with reliable timing

E-Prime software

Psychology
Software Tools,
Inc.

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>> We have obtained permissions from the publisher for Fig. 4, and cite it accordingly.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This is a comprehensive paper explaining with certain details all the procedures which should be considered in an fMRI-EEG-TMS study. This kind of methodological papers are necessary in order to standardise the procedures of data acquisition and analyses across different laboratories.

Major Concerns:

N/A

Minor Concerns:

p. 4. 90. It is written: "However, this requires methods for removal of artifacts generated by the magnetic stimulation on the EEG signal. Very few offline mathematical solutions for TMS artifact removal have been proposed 16-17". There is at least another paper that should be added here: "Offline removal of TMS-induced artifacts on human electroencephalography by Kalman filter. Morbidi F, Garulli A, Prattichizzo D, Rizzo C, Manganotti P, Rossi S. J Neurosci Methods. 2007 May 15;162(1-2):293-302.

>> We added the above citation (Morbidi et al., 2007). Several other citations were added in the revised form of the manuscript most are listed throughout this reply (see also changes in red fonts in the main text).

p.6. 205 "3.2.3) Use a high sampling rate for better representation of the noise artefact". Here you should write an example of high sampling rate (i.e. 1000, 5000 Hz).

>> We now specify a minimal recommended sampling rate (1kHz) on section 3.3.4. Please note that in addition, and following the comments of all three reviewers, we have made a general attempt to flesh out the manuscript with more details, instructions, examples, and values where possible (see protocol).

p.9. 311 and in the main body of text is written: "C. The interpolated segment allows filtering without creating edge artifacts. In this figure, a 40Hz low-pass filtered ERP (red) is plotted against its non-filtered version (grey); D. Alternatively to interpolation, the free ends remained after pulse removal can be joined together (see for example 25, and point 4.1.2 in the text)". The method used to avoid the TMS artefact in the reference 25 by Fuggetta, G., Pavone, E. F., Walsh, V., Kiss, M. & Eimer, M is very intuitive. However in the current version of the paper is not clear how to create the interpolated segment. It is very important especially for a methodological paper to offer the possibility to replicate the same methods employed by the authors. It is important to add a paragraph in the text where you explain in practical terms how to perform the "interpolation" of missing EEG data between 2ms before first TMS pulse to 16ms after second TMS pulse.

>> Following this comment and other comments below, we now explain in precise and straightforward terms the options for accomplishing this step (section 4.1.2).

We thank the reviewer for the comments.

Additional Comments to Authors:
N/A

Reviewer #2:

Manuscript Summary:

The paper "Extracting visual evoked potentials from EEG data recorded during fMRI-guided Transcranial Magnetic Stimulation" shares a technical achievement in combining 3 neuroscience tools with the research community. It allows other researchers to use TMS while recording EEG without risk of data loss or distortion of the EEG data by the TMS pulse or by filtering. It offers a straight forward method (making a response template based on blank screen trials), easy to operate and compute, which solves the artifact issue as well as a variety of side issues such as the noise effect of the TMS on the ERP. I therefore find the paper to be useful to other researchers and well worth publishing.

Major Concerns:

N/A

Minor Concerns:

1. The first reference to figure 1 in the text appears in line 214, after the reference to Fig. 2 (p.72). Please change figure numbers accordingly. Also note the missing period in line 214, ("Fig 1").

>> Thank you for the remarks, we corrected both issues.

2. Line 48 and line 67: the reference indices are sequential and can be written as (e.g. 2-7) instead of (e.g 2,3,4-7).

>> The problem is now fixed (lines 49 and 69).

3. Line 65: I suggest describing the possible causal links in more detail. As it is written now a reader not in the field might understand that there might be a causal relation between the two measurements, implying that the fMRI signal evoked the ERP signal or vice versa. It should be therefore stated that the TMS is used to test causality in the sense that the same brain region which causes the fMRI signal to change also causes the ERP modulation. For example, the sentence "a directional relation between EEG and fMRI cannot be determined" can be restated similarly to line 350 discussing the affirmation of the causal relation ("fMRI defined brain areas...").

>> We thank the reviewer for bringing this up, causality is a term that should indeed be treated with caution. We revised the part in the introduction where the elements in the causal relation were not stated clearly. It is now stated that TMS helps to establish a causal link between functionally defined cortical areas and scalp-recorded ERPs (rather than between the 2 signals) (lines 67 – 71).

4. Line 163: PsychToolbox is referred to in the text and references but not in Table 1, without specifying that it is a Matlab toolbox. Matlab is included in Table 1 without reference in the text.

>> We now cite Matlab and Psychtoolbox (and also SPM and MarsBar) in the text and in the material table.

5. Line 205: please specify the lowest sampling rate you would consider or refer to a TMS-EEG work where this issue is discussed.

>> A recommended sampling rate (1kHz or above) is now given in 3.3.4. Please note that many other technical details were added in order to facilitate comprehension and replication, see changes in the corrected manuscript marked in red fonts.

6. Line 259: The term linear-interpolation should be made clearer. If the two end points were connected by a straight line then this can be put in these terms without using "linear interpolation" which might imply fitting a straight line to a curve. Alternatively, if this term was used to describe the substitution of missing data by a straight line in another work this work can be referred to.

>> A clear and more precise explanation of the interpolation now replaces the previous one (4.1.2). Please see also reply to reviewer #1 in the same topic. Indeed, our interpolation consists of connecting the two ends with a line, which eliminates filtered-related ripples created when sharp edges exist in the data (see for example Fig. 1).

Additional Comments to Authors:
N/A

Reviewer #3:

Manuscript Summary:

The authors outline the principles and methodology regarding their recent fmri-guided TMS-ERP paper. The method is important and the account is clear and this could be a valuable resource, but as it stands it requires more detail about how the data was collected and why it looks the way it does.

Major Concerns:

In general the descriptions tend towards the qualitative rather than the quantitative. For example, using a 'high' sampling rate is recommended, and that the reference electrode should be placed 'far' from the coil, and that 'High-resolution' fMRI scanning is recommended. Replication here needs more detail - what Hz, how many mm, what resolution.

>> We thank the reviewer for this comment. We revised several sections concerning data analysis and experimental procedure, including the ones mentioned by the reviewer hereby, to include more details and concrete information (see also comments and replies to reviewers 1 and 2). Where applicable, we now provide precise values. In some of these places we also give references to other works. These modifications can be found in the text in red fonts.

Safety information would also be important.

>> Some major safety points were added, such as pre-TMS screening and a couple of points related to EEG preparation. We also refer the reader to two documents about safety, for further reading (Rossi et al. (2009), and the Magstim safety review (http://joedevlin.psychol.ucl.ac.uk/tms/docs/magstim_safety.pdf). We think, however, that in the scope and goals of this work we cannot provide a complete review of TMS safety.

The data looks unusual: the raw EEG shown in Fig 2 especially in Fig 2 b and fig 2c contains unusual high-amplitude high-frequency noise. Is this due to the TMS? A cleaner section of data should be presented if available.

>> We had initially chosen particularly noisy channel data (now changed, please see below) to demonstrate that the reviewed technique is suitable even for electrode sites that are located right under the coil (which are often also the electrodes of interest), what may cause an increase of line noise amplitude in some cases. This is a main advantage of the technique, and we now added a note in the text regarding this (lines 367-373). We did, however, prepare a new figure as per the reviewer's request (now Fig. 1, after renumbering) with data of another subject at the same electrode site, which is less noisy.

Which of the several sources of the secondary artifact listed on pg 3 lns 76-78 are responsible for the TMS artifact shown e.g. at p8 in fig 3a which seems unusually large and long. What is the basis in the literature for such TMS artifacts?

>> Pulse-related artifacts and/or baseline shifts lasting for prolonged durations that can get up to a few hundreds of milliseconds, are discussed, for example, by Virtanen et al. (1999), Thut et al. (2005), Julkunen et al. (2008), and briefly in the consensus paper by Siebner and colleagues (Siebner et al., 2009). Such artifacts were also mentioned or inferred from the results in other works (such as Bender et al., 2005, Litvak et al., 2007, Iwahashi et al., 2009). Although we cannot conclude that these artifacts represent all the exact same sources of interference, their major cause is claimed to be currents induced by the pulse and their decay process (see for example Julkunen et al., 2008, Siebner et al., 2009), and may also depend on amplifier structure. But physiological components can also constitute parts of the overall recorded interference depending on the stimulation site (e.g. Thut et al., 2005). Even if smaller than the mechanical artifact, any other potential not directly related to the experimental manipulation, may confound the results. Please also note that the artifacts from physiological origins are also not equally distributed across electrode locations. For example, in the figures presented in the current work, auditory and somatosensory potentials are probably negligible if at all existent, but other electrodes may include them in a more pronounced manner. We now revised the introduction of the artifact to briefly explain these possible contributors, and added relevant references (lines 78-83).

We would like to add that one of the reasons many studies did not report such residual artifacts may be that they are very large only in electrodes placed in the direct vicinity of the coil (Virtanen et al., 1999), and these electrodes are often removed or excluded from processing (e.g. Komssi et al., 2004, Kahkonen et al., 2005). They may also differ across amplifiers. We now make clearer in our manuscript that a major advantage of the techniques discussed here is the ability to eliminate artifacts even in electrodes located directly below the coil (lines 367-373). This is of crucial importance since often the desired ERPs are produced in or near the cortical tissue being stimulated. Having full head coverage is also recommended if source estimation algorithms are applied.

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

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