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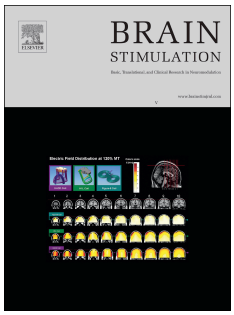

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Comparison of cortical EEG responses to realistic sham versus real TMS of human motor cortex

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Abstract

Background: The analysis of cortical responses to transcranial magnetic stimulation (TMS) recorded by electroencephalography (EEG) has been successfully applied to study human cortical physiology. However, in addition to the (desired) activation of cortical neurons and fibers, TMS also causes (undesired) indirect brain responses through auditory and somatosensory stimulation, which may contribute significantly to the overall EEG signal and mask the effects of intervention on direct cortical responses.

Objectives: To test differences in EEG responses to real TMS at intensities above and below resting motor threshold (RMT) and a realistic sham stimulation.

Methods: 12 healthy subjects participated in one session in which single-pulse TMS was applied to the left motor cortex in 3 different blocks, 150 pulses per block: 110%RMT, 90%RMT and realistic sham stimulation. Cortical responses were collected by a 64 electrode EEG system. TMS evoked potentials (TEPs) and TMS induced oscillations were analyzed.

Methods: 12 healthy subjects participated in one session in which single-pulse TMS was applied to the left motor cortex in 3 different blocks, 150 pulses per block: 110%RMT, 90%RMT and realistic sham stimulation. Cortical responses were collected by a 64-channel EEG system. TMS evoked potentials (TEPs) and TMS induced oscillations were analyzed.

Results: TEPs from all conditions differed significantly, with TEPs from 110%RMT showing overall highest amplitudes and realistic sham lowest amplitudes. Sham stimulation had only minor effects on induced cortical oscillations compared to pre-stimulus baseline, TMS at 90%RMT resulted in a significant increase (50-200 ms)

followed by a decrease (200-500 ms) in power of alpha and beta oscillations; TMS at 110% RMT led to an additional increase in beta power at late latencies (650-800 ms).

Conclusions: Real TMS of motor cortex results in cortical responses significantly different from realistic sham. These differences very likely reflect to a significant extent direct activation of neurons, rather than sensory evoked activity.

Highlights

- Supra- and subthreshold TMS of motor cortex was compared to realistic sham
- Responses were measured with EEG as evoked potentials and induced oscillations
- Responses to real TMS vs. realistic sham presented significant differences
- Sensory evoked potentials have only limited impact on motor cortex TMS-EEG responses
- TMS-EEG responses reflect in part direct activation of the brain by TMS

Introduction

The combination of electroencephalography (EEG) with transcranial magnetic stimulation (TMS) has enabled important advances in investigating cortical physiology through analysis of electrophysiological responses recorded from the brain [1, 2]. Conventionally, motor cortex was targeted by TMS due to the availability of motor evoked potentials (MEPs) recorded through electromyography (EMG) from hand muscles as an indirect measure of corticospinal excitability [3]. TMS evoked EEG potentials (TEPs) serve as a more direct measure of cortical excitability and connectivity that enables analysis of spatiotemporal cortical response profiles, e.g., before and after brain stimulation or pharmacological interventions [4, 5]. For TMS target sites other than motor cortex, TEPs gain additional importance, as there are no other straightforward electrophysiological outcome measures available [1].

The technical challenges regarding the design of EEG amplifiers for compatibility with TMS have largely been solved, and it is now possible to analyze neural responses a few milliseconds after the TMS pulse [2, 6]. Nevertheless, interpretation of the EEG response to TMS regarding its origin remains difficult, since this response is a combination of the experimentally desired (i.e., TMS evoked) activation of the brain, and experimentally undesired responses, such as indirect brain activation due to somatosensory and auditory inputs, that are inevitably caused by excitation of trigeminal nerve endings in the scalp and the TMS click sound, as well as non-neural signals, such as scalp muscle activation. The click sound generated by the coil during each TMS pulse results in a prominent auditory evoked potential (AEP), which contaminates the EEG signal of interest [7, 8], but can be partially mitigated using masking noise [9]. Similarly, somatosensory input due to vibration of the stimulating coil during the TMS pulse, as well as direct activation of peripheral sensory nerves in

the scalp, result in somatosensory evoked potentials (SEP) in the EEG signal [10]. The use of a spacer between the coil and the scalp has been proposed to reduce sensory activation [11, 12]. Despite these efforts, there is still concern that remaining signals from these auditory and somatosensory inputs still act as significant confounders in the analysis of TMS-EEG [11].

Consequently, incorporation of a sham stimulation condition has been advocated in TMS-EEG research, to ensure that observed effects cannot be attributed to experimentally undesired responses. However, it is not trivial to design a proper TMS sham condition that does not produce effective direct cortical stimulation but is otherwise equivalent to real TMS in all its indirect effects [13]. Realistic sham procedures that incorporate both an auditory click, as well as a weak electrical stimulation to reproduce the skin sensation and scalp muscle activation near the TMS target, have been proposed as a possible solution, although so far only few studies have adopted this approach [13, 14]. Another issue, specifically concerning TMS of motor cortex, is the re-afferent feedback from the evoked muscle twitch with suprathreshold TMS intensities, which contributes to TEPs [10, 15] and to the spectral pattern of TMS induced cortical oscillations [16-18], adding to the contamination of the direct cortical response signal.

In this study, we aim to disentangle these phenomena by comparing EEG responses generated by real TMS of the motor cortex vs. a realistic sham stimulation. Specifically, we compared EEG responses to TMS with an intensity above motor threshold (eliciting MEP), TMS with an intensity below motor threshold (without eliciting MEP), and realistic sham stimulation. We expected to find differential EEG responses between these conditions, which would allow identification of brain

responses caused by direct activation by TMS rather than indirect activation by somatosensory or auditory inputs or re-afferent feedback.

Methods

Subjects

The sample for the present study was drawn from a previous experiment (Desideri et al, under review). Subjects included 12 healthy right-handed individuals (4 males, age range 22-51 years, mean age ± s.d. 27.5 ± 7.7 years, Edinburgh Handedness inventory laterality score 75 ± 23). Experiments were conducted in accordance with the Declaration of Helsinki and within the current TMS safety guidelines of the International Federation of Clinical Neurophysiology [19]. All subjects provided written informed consent prior to participation, and the study was approved by the ethics committee of the medical faculty of the University of Tübingen (protocol 716/2014BO2).

Experimental design

The experiment involved a single session, with the application of single-pulse TMS to the hand area of the left primary motor cortex. Stimulation was divided into 3 separate blocks: 1. Real TMS with stimulation intensity of 110%RMT; 2. Real TMS with intensity of 90%RMT; and 3. Realistic SHAM stimulation. The order of the blocks was randomized across subjects, who were blinded to the nature of the stimulation applied in each block. To test the quality of blinding, subjects filled a questionnaire at the end of the experimental session, designed to report the order in which each condition (110%RMT, 90%RMT or SHAM) was applied according to their impression.

Experimental set-up & procedure

Participants were seated in a comfortable armchair with their hands relaxed and were required to watch a fixation cross 1 m in front of them. The experiment involved a figure-of-

eight TMS coil (PMD70-pCool, 70 mm winding diameter, Research 100, MAG & More, Germany), which delivered single pulses with a biphasic waveform (single cosine, 160 µs period). The coil orientation was 45° with respect to the midline, resulting in the major component of the electric field induced in the brain underneath the coil pointing from lateral-posterior to medial-anterior. MEPs were recorded from the right abductor pollicis brevis muscle in a bipolar belly-tendon montage through surface electromyography (EMG, 5 kHz sampling rate, 0.16 Hz -1.25 kHz bandpass filter) using adhesive hydrogel electrodes (Kendall, Covidien). The motor "hotspot" was defined as the coil position and orientation eliciting, at a slightly suprathreshold stimulation intensity, maximum MEP amplitudes [20]. The RMT was determined as the minimum stimulation intensity that produced MEPs > 50 µV in the target muscle in more than 50% of the trials [20]. Coil position angulation and orientation were kept constant relative to the participant's head using a stereoscopic neuronavigation system based on a standard Montreal Neurological Institute (MNI) brain anatomy (Localite GmbH, Sankt Augustin, Germany).

EEG signal was recorded from 64 channels arranged in the International 10-20 montage [21] in a TMS compatible Ag/AgCl sintered ring electrode cap (EasyCap GmbH, Germany). Data were acquired in DC mode (5 kHz sampling rate, 1.25 kHz low-pass anti-aliasing filter). The impedance at the interface between skin and all EEG electrodes was <5 kΩ throughout the experiment. A 24-bit 80-channel biosignal amplifier was used for EEG and EMG recordings (NeurOne Tesla with Analog Real-time Out Option, Bittium Biosignals Ltd., Finland). To minimize TMS-evoked auditory potentials, white noise was applied to the subjects through earphones, with attached plugs that attenuate external noise [6, 9]. The loudness of the white noise was individually adjusted to optimally mask the TMS click.

In the SHAM block, the original coil was disconnected from the TMS stimulator, while still positioned over the subject's scalp on the "hotspot" target. A second identical coil was then connected to the TMS stimulator, which was used to produce the typical TMS click at a stimulation intensity of 90%RMT. The second coil was positioned next to the first coil in the air and held by a fixation arm, but kept at a distance of 20 cm away from the scalp, which

models showed to produce only a negligible electric field in the cortex, thus avoiding undesired neuronal stimulation [22]. To simulate the scalp sensation associated with TMS, electrical stimulation of the scalp with 200 µs pulse duration, 200 V compliance voltage and 2.50 mA output current was delivered through two round electrodes (diameter 1 cm) integrated in the EEG cap, covered in conductive gel, with the cathode placed between Cz and CP1, corresponding to the position of the electrode CCP1h in the high-density 5% EEG montage, and the anode placed between FC5 and C3, corresponding to the position of the electrode FCC5h, and connected to a constant current high voltage electrical stimulator (Constant current stimulator DS7A, Digitimer Ltd, UK).

In the original experiment (Desideri et al., under review), TMS triggers in each block were generated randomly at the positive peak, negative peak or at random phase of the ongoing sensorimotor µ-oscillation (for details, see [23]). For the purposes of the present study, only the trials with TMS triggered at random phase were used, to avoid possible confounding factors on the EEG response from stimulation at specified brain states [24] (Desideri et al., under review). TMS triggers were applied with a jittered minimum inter-trial interval of 2 s.

Data analysis

EEG and EMG data processing and analysis were performed using customized analysis scripts on MATLAB R2017b and the Fieldtrip open source MATLAB toolbox [25]. The continuously recorded EEG signal was segmented with respect to the trigger markers in the data. The epochs were defined from 500 ms before the marker to 1 s after the marker. Additionally, trials from the 90%RMT dataset which elicited any MEP with a peak-to-peak amplitude of >25 µV within 20 - 40 ms after TMS were excluded, to ensure that the data in the 90%RMT condition exclusive contained trials without MEPs.

EEG data preprocessing: For the 110%RMT and 90%RMT datasets, data from 1 ms before to 15 ms after the marker, where high amplitude TMS artifacts occur, were removed and cubic interpolated. For the SHAM dataset, data from 1 ms before to 40 ms after the marker needed to be removed and interpolated, as the electrical stimulation and scalp muscle

activation produced longer lasting artifacts. EEG data were then visually inspected. Epochs containing major artifacts were removed as well as channels that showed prominent noise in most of the epochs. Independent component analysis (ICA) based on FastICA algorithm with a symmetric approach and the “gauss” contrast function for finding the weight matrix [26] was applied. These specifications have been recommended for the processing of TMS-EEG data [27]. Data were submitted to a two-step ICA procedure, in which ICA components were visually inspected and removed based on their topography, single-trial time-course, average time-course and power spectrum [28]. In the first step, only components representing high amplitude TMS-related artifacts were removed. Then, data were filtered with a 1-80 Hz bandpass filter (zero-phase Butterworth, 3rd order) and a 49-51 Hz notch filter (zero-phase Butterworth, 3rd order) and down-sampled to 1000 Hz. Afterwards, ICA was again applied to the data, and components representing eye blinks and movements, persistent muscle activity or smaller amplitude TMS-related artifacts were removed. Finally, channels discarded during the visual inspection of the data were spline-interpolated using signal of the neighbor channels and data were re-referenced to the average reference signal [29].

TMS-EEG evoked potentials (TEPs): For the TEP analysis, the EEG trial epochs of a given block were lowpass filtered (45 Hz, zero-phase Butterworth, 3rd order) and averaged. We included the following 5 TEPs (with post-TMS time windows of interest) into further statistical analyses, as they correspond to those most reproducible according to the literature [5, 30]: P25 (20-30ms), N45 (35-60ms), P70 (60-80ms), N100 (85-140ms), P180 (150-230ms).

TMS-EEG oscillatory response: Aside from the TEPs, TMS induces oscillations which are not necessarily time-locked to the TMS pulse, i.e., changes in spontaneous oscillatory activity [16, 17]. To obtain the induced response, first, we isolated the induced activity in the time-domain by a channel-wise subtraction of the evoked response from each single trial [18, 31] for the epochs retained after data cleaning and after re-afferent feedback compensation (see below). Subsequently, we calculated the time-frequency representations (TFRs) convolving single trials with complex Morlet wavelets [32]. We have analyzed the frequency range from 6 - 45 Hz in steps of 1 Hz and the center of the wavelet was shifted in steps of 10 ms in the

time window -500 ms – 1000 ms relative to the TMS pulse. The length of the wavelet linearly increased from 2 cycles at 6 Hz to 9 cycles at 45 Hz. The result of the wavelet transformation is a complex time series for each frequency in the examined frequency range. We then obtained the TFRs of power taking the squared absolute values of the complex time series. This was followed by the individual trial normalization for each frequency, based on a z-transformation that used the trial's respective mean and standard deviation for the power of each frequency from the full trial length. This normalization procedure transforms all power data to the same scale, allowing comparison across participants, trials and electrodes [18, 33]. This full-length single-trial z-transformation calls for a pre-stimulus baseline correction, i.e., subtraction of mean value (over time) of the baseline period (from 300 ms - 100 ms before TMS), to ensure that the average pre-stimulus values do not differ from zero and that z-values can be interpreted as a modulation of the pre-stimulus oscillatory activity. Finally, for each subject and each experimental condition (110%RMT, 90%RMT, SHAM) we averaged the TFRs across trials.

Statistical analysis

All statistical analyses were performed on the MATLAB platform (R2017b, The Mathworks, USA). Responses in the blinding questionnaire were compared to the actual blocks using chi-square test of independence.

EEG data were analyzed, using all channels, by means of non-parametric cluster-based permutation statistics to control for the family-wise error rate [34]. Clusters were defined as ≥2 neighboring electrodes with a p-value <0.05. Monte Carlo p-values were subsequently calculated by means of a two-tailed test (i.e., significance level $p<0.025$), using 1000 iterations for TEPs, and 2000 iterations for induced oscillations.

Significant differences between TEPs in the 3 experimental conditions (110%RMT, 90%RMT, SHAM) were evaluated by means of four analyses of variance (ANOVAs), one for each TEP of interest (N45, P70, N100, P180). The amplitude of the signal was averaged across the respective time windows of interest, and channels were permuted in the cluster

based analysis. TEPs that presented clusters with $p<0.05$ in the ANOVA were further analyzed in post hoc pairwise comparisons, performed by t-tests using the same cluster based methods approach. For the P25 TEP, only the 110%RMT and 90%RMT conditions were compared because within this early period data analysis in the SHAM condition was compromised by the stimulus artifact. We disregarded a comparative analysis between 110%RMT and SHAM, as it would not be possible to attribute any of the observed differences between these two conditions to the TMS brain activation or to differences in somatosensory activation.

The same statistical procedures were repeated in an additional analysis, following normalization of the signal's amplitude. This involved subtraction of the signal's amplitude of each trial by the average of the whole trial's amplitude and dividing the result by the standard deviation of the whole trial's amplitude, obtaining a z-score. By normalizing the amplitudes across interventions, results obtained from the statistical cluster-based analyses would reflect primarily differences in the signal's spatial distribution between conditions.

Induced Oscillations were also analyzed with a cluster-based ANOVA to compare the 3 experimental conditions (110%RMT, 90%RMT, SHAM). Here, both the space (channels) and time dimensions were permuted in the cluster-based method, within a period 40 – 800 ms after the TMS pulse. This method was preferred instead of a predetermined set of time windows, given the absence of a consensus for time windows of interest to be used in the TMS induced oscillation analysis. Also, the present cluster-based statistics approach is appropriate for exploratory analyses, as it minimizes false-positives involved in testing multiple time-points [34]. Four ANOVAs were performed, one for each of the 4 frequency bands of interest: alpha (8-12 Hz), beta-1 (13-19 Hz), beta-2 (20-29 Hz), and gamma (30-45 Hz). Time-frequency points that presented clusters with $p<0.05$ in the ANOVA were proceeded to the pairwise post hoc comparison with cluster-based t-tests.

Results

Blinding

The analysis of the blinding questionnaire suggests that the subjects were able to distinguish between the conditions applied, $X^2 = 40$ (df=4, N=12); p<0.001 (**Table 1**). This was because all subjects could correctly identify the 110%RMT condition associated with muscle twitches. Comparing solely the 90%RMT and SHAM conditions, no statistical relation between the conditions and the subjects' responses was observed, as the null hypothesis could not be excluded: $X^2 = 2.66$ (df=1, N=12), p=0.102. This suggests that subjects could not reliably distinguish realistic sham TMS from sub-threshold real TMS.

Session applied	Responses to the blinding questionnaires			TOTAL number of sessions
	110%RMT	90%RMT	SHAM	
110%RMT	12 (100%)	0 (0%)	0 (0%)	12
90%RMT	0 (0%)	8 (66.6%)	4 (33.3%)	12
SHAM	0 (0%)	4 (33.3%)	8 (66.6%)	12

Table 1: Contingency table of the number of subjects' responses to the blinding questionnaire versus the actual session the subjects received.

TMS Evoked Potentials (TEPs)

The average percentage (±1 s.d.) of excluded trials during data processing was 3.9±2.4% (110%RMT), 3.3±2.1% (90%RMT) and 2.8±1.7% (SHAM). The average number of components excluded in the first-step ICA were, respectively: 7.4±2.1, 7.0

±2.4 and 4.7±1.6, and the average number of components excluded in the second-step ICA were, respectively: 23.9±8.9, 24.5±6.4, and 26.5±4.7.

Stimulation (110%RMT, 90%RMT and SHAM) over the left motor cortex resulted in a series of deflections of the EEG signal, that differed among each other already at visual inspection (**Figure 1**). Cluster-based ANOVA showed that the signals from all TEPs were statistically different, both for the sensor-level absolute amplitudes (in μV, **Figure 1**, top panels) and the z-transformed normalized amplitudes (**Figure 1**, bottom panels).

Pairwise comparisons showed that 110%RMT trials presented a significantly higher amplitude of TEPs (P25, N45, P70 N100 and P180) compared to 90%RMT. The difference was expressed mostly in channels located in proximity of the stimulation site (**Figure 2,** upper panels). The 90%RMT trials presented a significantly higher amplitude of N45, N100 and P180 but not P70 when compared to SHAM. All differences were in clusters centered around the vertex (**Figure 2,** upper panels). Following the normalization of signal amplitude, differences between conditions 110%RMT and 90%RMT remained significant (**Figure 2,** lower panels). In contrast, the difference in N100 between 90%RMT and SHAM was no longer significant, while the differences in N45 and P180 remained, and a new significant difference was observed in P70 (**Figure 2,** lower panels).

TMS Induced Oscillations

Stimulation (110%RMT, 90%RMT and SHAM) over the left motor cortex resulted in a series of changes in the power of ongoing oscillatory activity (**Figure 3**). A comparison between all interventions revealed a statistical difference in the oscillatory frequencies corresponding to the alpha (α), low-beta (β1) and high-beta (β2) bands. The differences occurred in 3 separate post-TMS pulse periods: an early

response with increased power (around 50-200 ms), followed by a depression (around 250-500 ms), and a late response with increased power (after 650 ms), with respect to the baseline period.

A pairwise comparison indicated higher increase in power of cortical oscillations in the frequency bands α, β1 and β2, around 50-200 ms, in the condition 90%RMT, compared to SHAM, in a cluster of channels comprising the stimulated area and the contralateral hemisphere, followed by a larger decrease in power of the oscillations in the frequency bands α and β1, around 250-500 ms, in a cluster of channels comprising mostly the stimulated area (**Figure 4**). The pairwise comparison between 110%RMT and SHAM indicated a similar pattern of differences. A larger increase in power of the oscillations in the frequency bands β1 and β2 was observed around 650-800 ms in the 110%RMT condition compared to both 90%RMT and SHAM (**Figure 4**).

Discussion

The objective in this study was to compare EEG responses generated by TMS of the motor cortex at supra- and sub-threshold intensities and by realistic sham stimulation. We found that TMS evoked and induced EEG responses present distinct patterns when generated by single-pulse TMS above RMT, below RMT or a realistic sham stimulation.

Motor cortex TMS at 90%RMT effectively activates the brain, as has been demonstrated by inhibition of ongoing motor activity [35], generation of intracortical inhibition and facilitation in paired-pulse TMS protocols [36], or elicitation of corticospinal volleys in epidural spinal recordings [37]. Therefore, the 90%RMT and SHAM conditions should differ only with regard to effective (but subthreshold for generation of MEPs) cortical stimulation by TMS, while indirect sources of brain activation by auditory input caused by the TMS click and somatosensory inputs caused by excitation of scalp nerve endings should be similar. Nevertheless, the N45, N100 and P180 TEP amplitudes were significantly larger in the 90%RMT than SHAM condition. TEPs evoked by the 90%RMT condition followed the pattern described in previous reports of motor cortex stimulation below RMT [15, 38], and these TEPs remained even after subtracting the responses caused by the realistic SHAM (**Figure 2**). It is very likely that this difference between TMS 90%RMT and sham is mostly caused by direct cortical activation by the TMS pulse. The analysis of the signal after amplitude normalization suggested also a significant difference in the spatial distribution of TEPs between 90%RMT and SHAM conditions, except for the N100. The realistic sham stimulation evoked cortical responses with a negative peak at around 100 ms after stimulation, followed by a positive peak at around 200 ms (**Figure 1**), as expected from sensory and auditory evoked cortical activity generated

by TMS [7, 10, 39]. Given the presence of auditory click and scalp sensation in all conditions in the present study, it is expected that their cortical responses would share this feature. It is possible that the spatial difference of the N100 between 110%RMT and 90%RMT was due to the re-afferent input from the motor evoked potential in the 110%RMT condition, skewing the voltage distribution of the cortical evoked potential towards the sensorimotor cortex of the stimulated hemisphere.

Moreover, sham stimulation had only minor effects over induced oscillations, especially when compared to the effects of 110%RMT and 90%RMT stimulation (**Figure 3**). Specifically, the 90%RMT resulted in increased power of oscillations in the alpha and beta frequencies in an early period, followed by decreased power of alpha and beta-1 frequencies in a later period, as described in previous studies [17, 18]. These observations provide further evidence that these patterns originated by direct cortical stimulation by the TMS pulse, rather than by auditory or somatosensory evoked activity.

Significant differences were also found comparing suprathreshold TMS (110%RMT) with subthreshold TMS (90%RMT). Stimuli applied at intensities above RMT by definition elicit a motor response, which in turn leads to a re-afferent somatosensory evoked potential [40]. Motor re-afference from MEPs has been shown to interfere with the signal from TEPs, from approximately 40 ms after TMS pulse on, corresponding to the cumulative latencies of the MEP and somatosensory evoked potentials [6, 10]. When stimulating the motor cortex at 100%RMT, one previous study found an increased amplitude of TEPs at latencies around 60 ms in the temporoparietal region in trials that elicited MEPs compared to those that did not, suggesting that this difference is probably caused by the re-afferent feedback from MEPs [38]. A similar result was also observed in our study (**Figure 2**). However,

intensity of motor cortex stimulation per se has also been correlated to TEP amplitudes, regardless of the presence of MEPs [15]. It is likely that stimuli with higher intensities are able to depolarize neurons in a larger and deeper cortical area, thus leading to higher TEP amplitudes. Also, a study using functional magnetic resonance imaging and suprathreshold TMS suggested that the activation in motor cortical areas due to the re-afference potential does only explain 10-20% of the activation while 80-90% are attributable to direct brain activation by suprathreshold TMS [41]. Activation of motor output neurons by 110%RMT TMS, including connection of these neurons to the contralateral motor cortex through interhemispheric connections, might have been responsible for higher amplitudes found in the P25 around the contralateral motor cortex with 110%RMT TMS compared to 90%RMT (**Figure 2**) [42]. Moreover, due to its short latency, it is unlikely that the amplitude of this TEP was influenced by re-afference or any other sensory evoked activity [6].

Changes in cortical oscillations following TMS have also been previously explored, with increase in power of alpha and beta frequency bands in the period 50 – 200 ms after TMS [10, 16], with larger changes with increasing TMS intensities, and no change following sham stimulation [16]. Later studies identified a decrease in power in these frequency bands in a later period 200 – 500 ms after TMS pulse [17, 18]. The latency of this alpha and beta power decrease (event related desynchronization, ERD) may suggest a correspondence to sensory evoked activity [43], such as the motor re-afference [40]. In this line, it was demonstrated in one previous study that the decrease in power of the ERD (200 – 350 ms after the TMS pulse, alpha and beta frequency bands) was larger in ~110%RMT trials that elicited high amplitude MEPs, compared to trials with low amplitude MEPs, supporting that re-afference signals from the muscle twitch contributed to the ERD [17]. In contrast, we observed

no significant difference in alpha/beta ERD between the 110%RMT and 90%RMT condition, but ERD was absent in the SHAM condition (**Figure 4**). Another possibility is that alpha/beta ERD over sensorimotor cortices elicited by TMS may simply reflect overall cortical activation, which would include cortico-cortical and cortico-subcortical circuits directly activated by TMS and, to a lesser extent, the re-afferent feedback from the MEPs [18]. Accordingly, one study demonstrated that patients with severe disorders of consciousness, unlike healthy controls, failed to present TMS induced alpha and beta desynchronization [44], likely representing a consequence of the breakdown of cortico-cortical neuronal processing in this condition [45, 46]. Induced oscillations in the 110%RMT condition presented a significantly larger power increase in the beta band (event related synchronization/ERS) in a late time window (650 – 800 ms) compared to 90%RMT, suggesting that this phenomenon might correspond specifically to the motor re-afference. Late beta ERS (after approximately 1 s) has been shown to correlate to somatosensory re-afference, as both intentional finger movements and peripheral nerve stimulation without motor response were able to generate beta ERS [40, 47] Also, post-movement beta rebound in latencies beyond 500 ms was found to be increased following executed movements, compared to movement planning, suggesting a role of re-afference in this phenomenon [48].

The present study has some limitations. As mentioned, the signal at latencies up to 40 ms in the sham condition was lost due to artifacts. Analysis of these data could have added to the understanding of the contribution of downstreaming activity from the motor cortex to the early TEPs. Latencies beyond 40 ms in conditions using suprathreshold TMS are always subject to interference from re-afferent signals, thus limiting the comparison of the effects between different TMS intensities. A future study might overcome this limitation by pairing TMS with peripheral stimulation in all intensities, and subtracting the evoked potential from peripheral stimulation from the

TEPs; or by blocking peripheral nerve conduction with local anesthetic nerve block [49]. It would also be of interest to investigate the comparison between different TMS intensities and realistic sham in other cortical areas. A recent preliminary report suggests that effective TMS evoked potentials in other brain regions, namely the frontal and parietal cortex, share many similar features with the responses from sham stimulation [50]. Future studies would be valuable to further confirm these observations to provide guidance for a more accurate extraction of signals that reflect direct cortical activation using TMS-EEG. In summary, our data demonstrate that real TMS of motor cortex results in EEG responses that reflect to a significant extent activation of the brain by the TMS pulse rather than by indirect sources of auditory, somatosensory or re-afferent inputs. Our findings are in close agreement with one previous study that demonstrated that TEPs are genuine cortical responses because they were detectable only when preserved cortical tissue was stimulated in patients with traumatic or ischemic brain lesions, in the presence of otherwise intact nerves in the scalp and cranial muscles [51].

Conclusion

Realistic sham TMS of the motor cortex elicits evoked and induced EEG potentials that are of significantly lower amplitudes compared to real TMS. These findings reinforce the evidence that most cortical responses observed with TMS-EEG are mostly unrelated to sensory evoked potentials caused by scalp stimulation and/or auditory stimulation from the TMS pulse, provided proper masking noise and ear protection are used. Nevertheless, the presence of a non-zero signal caused by sensory evoked activity might act as a confounder. Therefore, the use of a sham-controlled design is advisable in TMS-EEG experiments to disentangle the signal

originated by direct cortical responses to TMS from auditory and somatosensory evoked activity, to ensure that the effects of experimental interventions are specifically attributed to the genuine cortical response to TMS.

Conflicts of interest

The authors declare that the research was conducted in the absence of any competing financial interest.

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Figure Legends

Figure 1. The top panel shows the EEG sensor amplitude using an average reference montage (μV); the bottom panel shows the normalized amplitudes (z-score). Left: Butterfly plot of the grand average across all subjects (n=12) and trials of each condition (110%RMT, 90%RMT and SHAM). The green curve is the signal recorded from electrode C3 underneath the stimulating coil over left motor cortex. Red dotted line indicates the TMS pulse. Shaded areas represent the latencies of typical TEPs observed after TMS of motor cortex (P25, N45, P70, N100 and P180). Right: Spatial distribution of voltage over the scalp averaged across the latency of each TEP. TEPs that presented statistical significance in the cluster-based ANOVA are marked with * ($p<0.001$), and statistical significance in the cluster based t-test are marked with † ($p<0.010$).

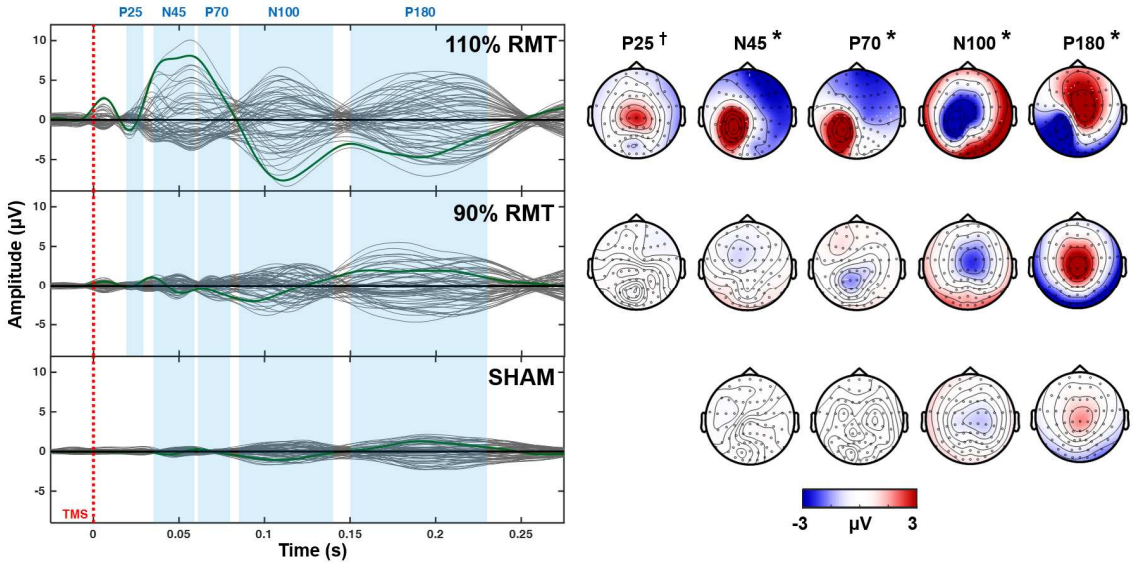
Figure 2. The upper panels show the EEG sensor amplitude using an average reference montage (μV); the bottom panels show the normalized amplitudes (z-score). Top: Butterfly plots of the difference between interventions. The green curve represents electrode C3. Red dotted lines indicate the TMS pulse. Cyan areas represent the latencies of typical TEPs observed after TMS of motor cortex (P25, N45, P70, N100 and P180) which presented statistical significance in the ANOVA ($p<0.001$). Mid: Topographical plots of the statistical differences (t-values) of TEP amplitudes indicated by the bold black line on the butterfly plots, channels highlighted (*) belong to clusters in which statistical significance was expressed. Red indicating more positive amplitude in the first condition, and blue indicating more negative amplitudes. P-values of the statistical tests are displayed next to the respective cluster. Bottom: Time courses of the average of the voltages from the EEG channels

628 that comprised the significant electrode clusters, depicted in the above topographical
629 plot (Pos Clust: Positive clusters; Neg Clust: Negative cluster), areas in cyan
630 correspond to latencies of typical TEPs described above, shadows around the
631 average curves correspond to ±1 SEM.

632

633 **Figure 3.** Left: Time-frequency plots of the induced oscillations from the average
634 across subjects and all EEG channels of each condition (110%RMT, 90%RMT and
635 SHAM). Black area around time=0 corresponds to the TMS artifact. Middle-Bottom:
636 Time-frequency plot of the average across all subjects, conditions and EEG
637 channels, dotted rectangles indicate the time-frequencies where the cluster-based
638 ANOVA detected a statistical difference between conditions (respective p-values to
639 the right of the topographical plots). Topographical plots indicate the distribution of
640 the standardized power (z-value) of the TMS induced oscillations from each
641 condition, within the time-frequencies where the cluster-based ANOVA detected a
642 statistical difference: Frequency indicated to the left of the plots (α, β1, β2), post-
643 trigger period and p-value of the ANOVA indicated to the right of the plots.

644 **Figure 4.** Time-frequency plots of the difference of the induced oscillations between
645 conditions, from the averages across all subjects (n=12). Dotted rectangles indicate
646 the time-frequencies where the pairwise cluster-based t-tests detected a significant
647 difference between interventions (p-values indicated next to respective topographical
648 plots). Topographical plots indicate significant differences from the pairwise cluster
649 based t-tests, with clusters of channels indicated by (*). The frequency bands are
650 indicated to the left of the plots (α, β1, β2), p-value of the t-tests are indicated to the
651 right of the plots.


P25
N45
P70
N100
P180
110% RMT
90% RMT
SHAM
TMS
Amplitude (μV)
Time (s)
P25 †
N45 *
P70 *
N100 *
P180 *
-3
μV
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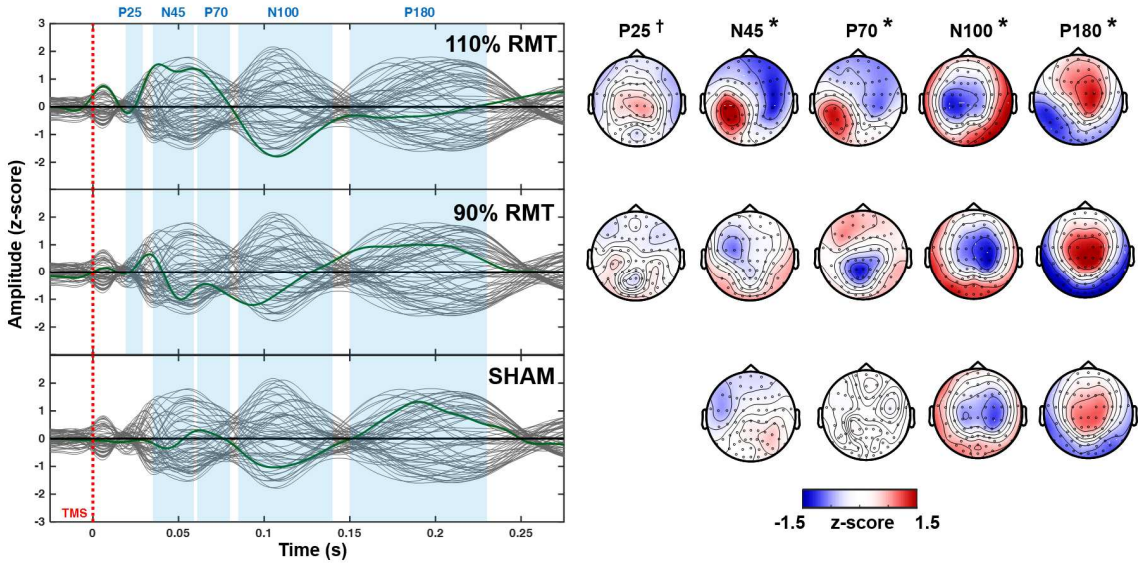
P25
N45
P70
N100
P180
110% RMT
90% RMT
SHAM
TMS
Amplitude (z-score)
Time (s)
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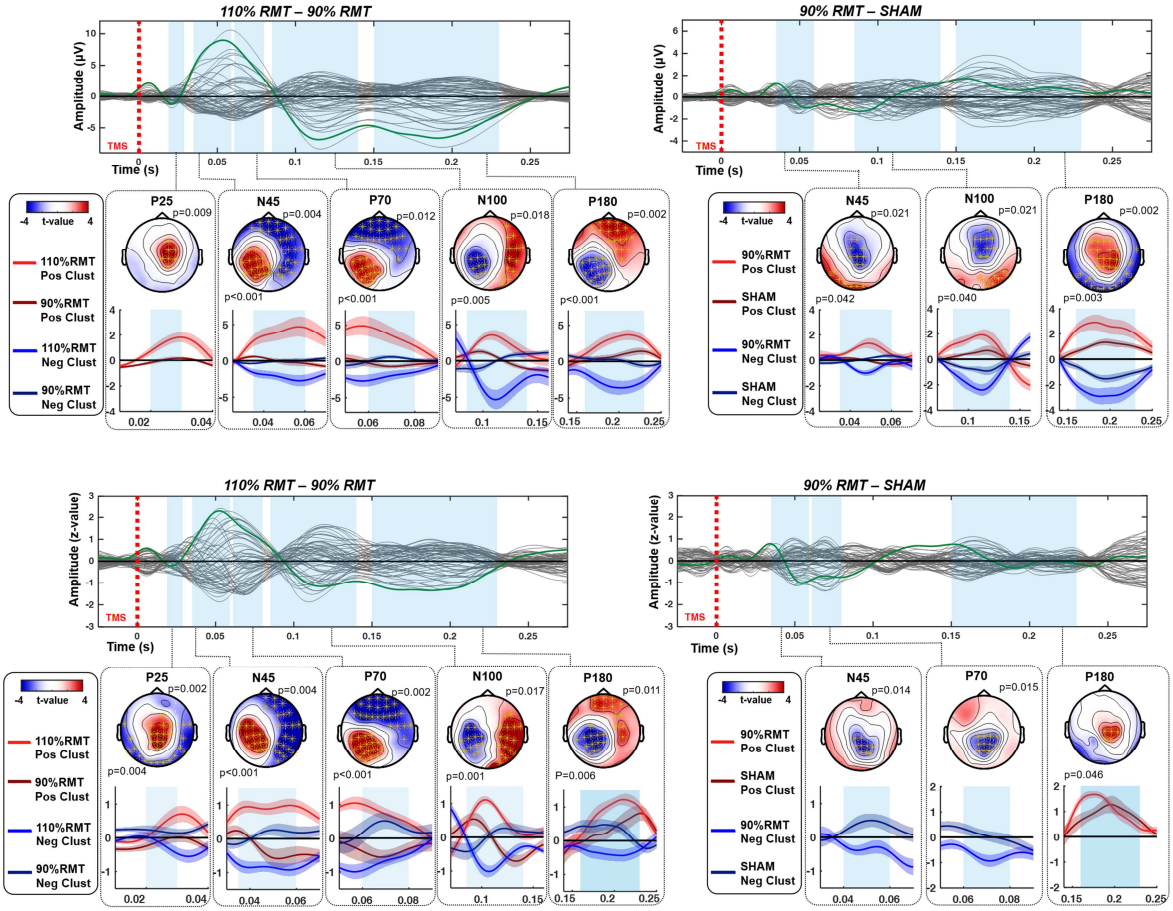
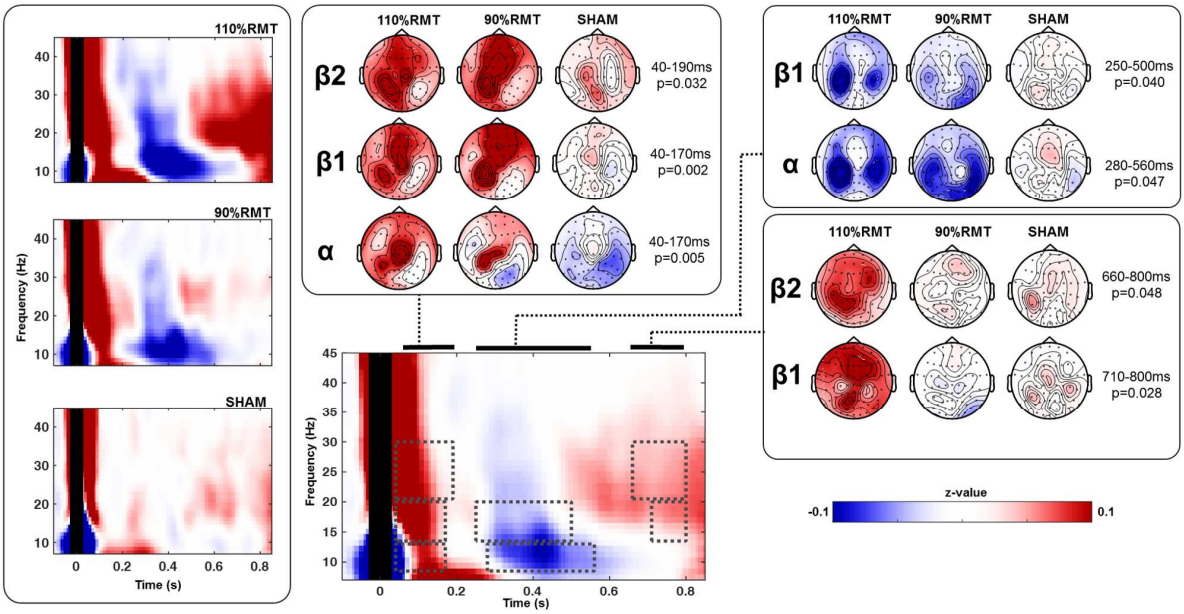
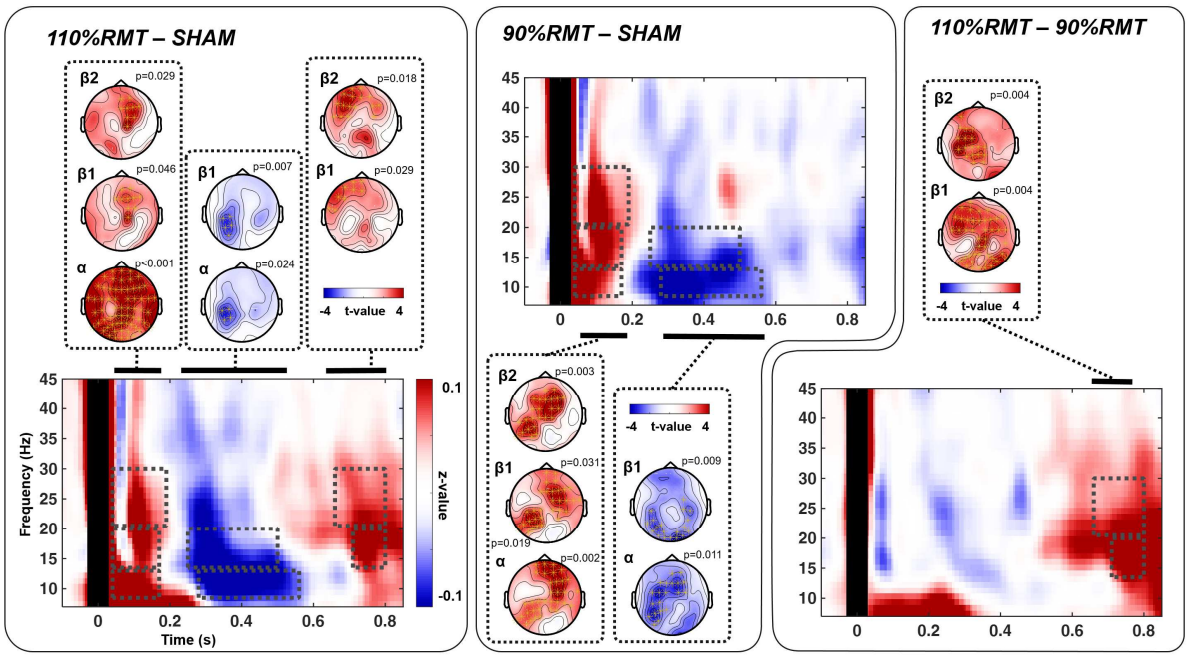
N45 *
P70 *
N100 *
P180 *
-1.5
z-score
1.5







Highlights

- Supra- and subthreshold TMS of motor cortex was compared to realistic sham
- Responses were measured with EEG as evoked potentials and induced oscillations
- Responses to real TMS vs. realistic sham presented significant differences
- Sensory evoked potentials have only limited impact on motor cortex TMS-EEG responses
- TMS-EEG responses reflect in part direct activation of the brain by TMS