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Disentangling EEG responses to TMS due to cortical and peripheral activations

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ABSTRACT

Background: the use of combined transcranial magnetic stimulation (TMS) and electroencephalography (EEG) for the functional evaluation of the cerebral cortex in health and disease is becoming increasingly common. However, there is still some ambiguity regarding the extent to which brain responses to auditory and somatosensory stimulation contribute to the TMS-evoked potential (TEP).
Objective/Hypothesis: to measure separately the contribution of auditory and somatosensory stimulation caused by TMS, and to assess their contribution to the TEP waveform, when stimulating the motor cortex (M1).
Methods: 19 healthy volunteers underwent 7 blocks of EEG recording. To assess the impact of auditory stimulation on the TEP waveform, we used a standard figure of eight coil, with or without masking with a continuous noise reproducing the specific time-varying frequencies of the TMS click, stimulating at 90% of resting motor threshold. To further characterise auditory responses due to the TMS click, we used either a standard or a sham figure of eight coil placed on a pasteboard cylinder that rested on the scalp, with or without masking. Lastly, we used electrical stimulation of the scalp to investigate the possible contribution of somatosensory activation.
Results: auditory stimulation induced a known pattern of responses in electrodes located around the vertex, which could be suppressed by appropriate noise masking. Electrical stimulation of the scalp alone only induced similar, non-specific scalp responses in the in the central electrodes. TMS, coupled with appropriate masking of sensory input, resulted in specific, lateralized responses at the stimulation site, lasting around 300 ms.
Conclusions: if careful control of confounding sources is applied, TMS over M1 can generate genuine, lateralized EEG activity. By contrast, sensory evoked responses, if present, are represented by non-specific, late (100–200 ms) components, located at the vertex, possibly due to saliency of the stimuli. Notably, the latter can confound the TEP if masking procedures are not properly used.

Introduction

A growing body of evidence suggests that EEG activity evoked by TMS reflects the summation of excitatory and inhibitory post-synaptic potentials arising from pyramidal and interneuronal activation [1,2]. However, there are several potential confounding factors. First, the TMS pulse produces a clear “click” sound each time it is discharged, thus eliciting an auditory-evoked potential (AEP) through air and bone conduction [3]. TMS also produces a tapping sensation on the scalp which might, at least in principle, evoke a somatosensory-evoked potential (SEP), defined as modality-specific activation of the contralateral primary somatosensory cortex following non-noxious stimulation of a body area

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[4,5]. Additionally, the magnetic field gives rise to artefacts due to activation of scalp muscles, especially when lateral areas are stimulated [6]. These may contaminate the direct response of the brain to TMS and confound its interpretation. While muscle artefacts need to be removed offline, strategies can be employed to reduce the auditory and somatosensory stimulation with the application of noise masking and a thin layer of foam between the coil and cap, respectively. Nevertheless, these strategies are imperfect, and disentangling the contributions of AEP and somatosensory stimulation from the signal reflecting direct cortical activation would strengthen the conclusions that can be drawn from TMS-EEG studies.

Previous investigations into the origins of the EEG activity produced by TMS (the TMS evoked potential or TEP) have produced conflicting results. Gordon and colleagues [7] showed significant differences between TEPs obtained by standard TMS and a sham stimulation designed to control for auditory and somatosensory stimulation. In contrast, a recent work by Conde and colleagues [8] used a similar study design and did not find significant differences in TEP components between sham and real stimulation; therefore, the authors suggested that TEP largely results from the auditory and somatosensory stimulation that is incidental in TMS.

These contrasting findings leave several questions open but may be partially explained by differing methodologies. As highlighted in a recent commentary by Belardinelli and colleagues [9], the studies differed in coil size, coil location and orientation, and intensity of stimulation in TMS and sham conditions. The TEP waveforms produced were also inconsistent across the studies, and analytically different approaches were taken such that one study performed a comparative analysis to look for similarities in the responses to different stimulation types, while the other simply looked for differences between conditions. Due to the divergent findings from these studies, the question whether the TEP reflect at least in part direct cortical activation still remains. Additionally, both studies employed a sham stimulation with simultaneous somatosensory and auditory input; while it is useful to consider this, as it mimics the combination of sensations that the TMS coil produces, it is also important to apply auditory and somatosensory stimulation separately to quantify their respective contribution to the TEP. Addressing these questions is essential to understand how to control for sensory stimulation in TMS-EEG and, ultimately, determine how the technique may be optimally used to understand cortical physiology.

Here we explore the contributions of confounding factors to the TEP waveform evoked from subthreshold stimulation over the primary motor cortex (M1). We compared conditions with varying contributions from somatosensory and auditory components: TEPs from real TMS stimulation with and without noise masking, pure auditory stimulation with and without noise masking, electrical stimulation of the scalp, and sham coil stimulation. To better assess possible residual AEP during TMS, due to incomplete suppression of the TMS click, we also correlated pure AEP with responses obtained with masked TMS, as done previously [8]. With this approach, we sought to isolate the contributions of auditory and somatosensory stimulation to the TEP waveforms in order to determine their respective contributions and to explore to what extent the AEP can be minimised by noise masking. Generally, we hypothesised that, while auditory and somatosensory stimuli would contribute to the TEP response, there would also be TEP components reflecting direct cortical activation. Our data suggest that, at least for subthreshold stimulation of the M1 hand area, it is possible to obtain genuine brain responses by using appropriate masking of the TMS click; in this location, the somatosensory stimulation by the TMS pulse contributes only marginally to the TEP. These results are further supported by additional experiments and analyses detailed in the Supplementary Material.

Materials and methods

Participants

Nineteen healthy right-handed individuals [10] (10 female, age: 29.7 ± 4.2) were enrolled in the study. Participants had no history of neurological or psychiatric diseases and were not taking drugs active at the central nervous system. All procedures were performed in accordance with the Declaration of Helsinki and approved by the human subjects review board of the University College London. Participants gave written informed consent prior to the experimental session.

Experimental design

Participants were sitting in a comfortable chair, in a quiet room, with forearms resting on a pillow placed on their lap. They were asked to fixate a white cross (6×6 cm) in the middle of a blank screen, to avoid eye movements during the EEG recordings. Participants wore earphones which, in some recording blocks (named “masked”, see below), continuously played a masking noise, composed of white noise mixed with specific time-varying frequencies of the TMS click, to minimize the AEP [11]. Unlike most TMS-EEG studies, subjects also wore ear defenders (SNR = 30) on top of the earphones, to further improve masking of the TMS click. The intensity of the masking noise was adjusted for each participant by increasing the volume (always below 90 dB) until the participant was sure that s/he could no longer hear the TMS click [12].

The experiment consisted of a single session in which subjects underwent a total of 7 blocks of EEG recording, each consisting of 120 trials, organised as follows (Fig. 1): 1) stimulation with a standard coil on a 5 cm thick pasteboard cylinder placed on the scalp without noise masking (TMS-AEP NOT MASKED; see below: responses in this block were similar to block 2, so it has not been used for statistical comparisons), 2) stimulation with a sham coil on the pasteboard cylinder placed on the scalp without noise masking (AEP NOT MASKED), 3) stimulation with a sham coil on the pasteboard cylinder placed on the scalp with noise masking (AEP MASKED), 4) stimulation with a sham coil placed directly on the EEG cap with noise masking (SHAM), 5) stimulation with a standard coil directly on the EEG cap and using noise masking (TEP MASKED), 6) stimulation with a standard coil placed on the cap without noise masking (TEP NOT MASKED), and 7) electrical stimulation of the scalp (ES).

The conditions in this study were designed to inform about the contribution of auditory, somatosensory and direct cortical activation to the TEP waveform by altering somatosensory and auditory stimulation in the various blocks. Briefly, the comparison between conditions where only auditory stimuli were provided, with or without noise masking, allowed the characterization of AEP and the extent to which the AEP could be cancelled by noise masking. Placing the sham coil on the head ensured current was not induced, while investigating the potential influence of vibration arising when the TMS coil is placed directly on the head. Comparing standard TMS blocks with and without noise masking allowed for the assessment of the contribution of AEP to the TEP and, importantly, allowed us to investigate specifically which TEP components were influenced by AEP. Finally, the ES block was designed to investigate somatosensory responses to scalp stimulation.

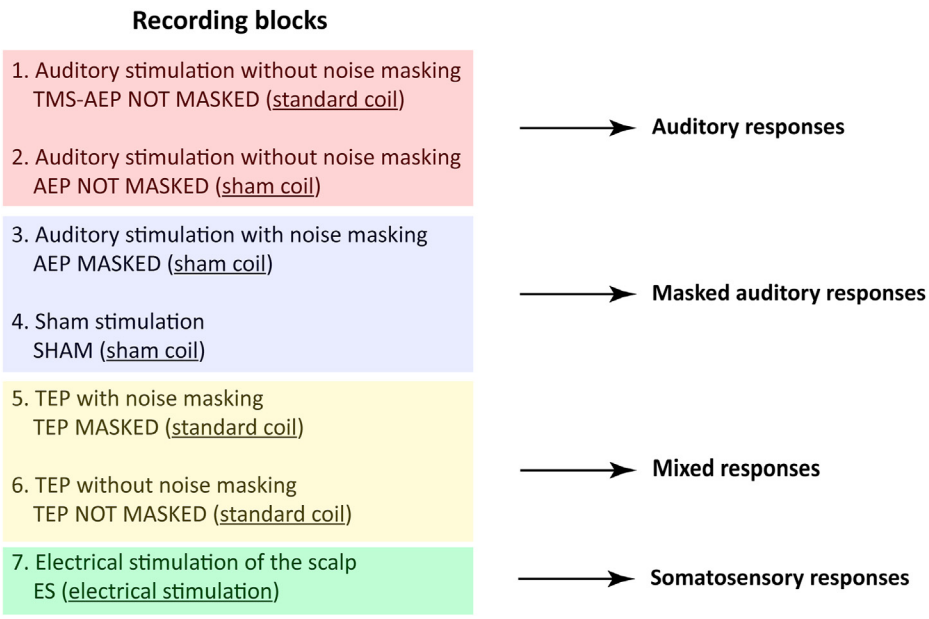

Fig. 1. Experimental protocol. Subjects underwent seven TMS-EEG recording blocks, in a randomised order: 1) stimulation with a standard coil on a 5 cm thick pasteboard cylinder placed on the scalp without noise (TMS-AEP NOT MASKED), 2) stimulation with a standard coil on the pasteboard cylinder placed on the scalp without noise masking (AEP NOT MASKED), 3) stimulation with a sham coil on the pasteboard cylinder placed on the scalp with noise masking (AEP MASKED), 4) stimulation with a sham coil placed directly on the EEG cap with noise masking (SHAM), 5) stimulation with a standard coil directly on the EEG cap and using noise masking (TEP MASKED), 6) stimulation with a standard coil placed on the cap and without noise masking (TEP NOT MASKED), and 7) electrical stimulation of the scalp (ES).

Transcranial magnetic stimulation and electromyography

Single-pulse, monophasic TMS was delivered using a Magstim 200^2 device connected to a 70-mm figure-of-eight coil held with the handle backwards at 45° to the midline, inducing current in the posterior-anterior direction (Magstim Company Limited, Whitland, UK). Sham stimulation was delivered with a dedicated sham coil (70 mm alpha sham coil, Magstim UK), which uses unique coil winding to impart a very small, shallow magnetic field and diverts part of the current to an inner device to produce the auditory click. Importantly, when placed on the EEG cap, the sham coil does not induce current in the brain, but retains the auditory component associated with the standard coil. TMS was delivered over the cortical hotspot, defined as the site within the left M1 where TMS elicited the largest motor evoked potential (MEP) in the right first dorsal interosseous (FDI) muscle. This location was sampled in the MNI space and the coil was maintained in the correct position throughout the stimulation by using a Brainsight neuronavigation system (Rogue Research Inc, Montreal, Canada) coupled with a Polaris Spectra optical measurement system (Northern Digital inc, Waterloo, Canada). An estimated individualised MRI scan in the MNI space was used for all the participants. Resting motor threshold (RMT) was calculated as the lowest stimulation intensity that produced a MEP of at least 50 μV in 5 out of 10 consecutive trials in the relaxed FDI [4,13]. When using the standard coil, the stimulation intensity was set at 90% RMT. In the recording blocks where the sham coil was used, the stimulation intensity was increased to match the sound generated by the standard coil. Surface EMG was recorded through a pair of Ag/AgCl 10 mm cup electrodes placed over the right FDI muscle in a belly-tendon montage using a Digitimer D360 (Digitimer Ltd. Welwyn Garden City, UK). Raw EMG signal was sampled at 5000 Hz, amplified (gain: 1000x), and bandpass filtered between 5 and 2000 Hz before being digitally converted with a CED 1401 analog-to-digital laboratory interface (Cambridge Electronic Design). Loudness of the TMS click was assessed by each participant by means of a visual analogue scale (VAS) ranging from 0 (no perception) to 10 (maximal perception) in the recording blocks where auditory masking was used (AEP MASKED, SHAM, TEP MASKED), and a possible correlation between VAS scores and RMT was investigated by means of the Spearman's correlation coefficient. A VAS assessment was also done to assess discomfort due to stimulation, and all blocks were considered. A Friedman's ANOVA was performed to investigate possible difference in the two variables across different blocks.

Electrical stimulation of the scalp

Electrical stimulation of the scalp was performed using 10 mm Ag/AgCl cup electrodes. The electrode placement was chosen to approximate the location of the coil and the direction of the current. In order to localise the position of the electrodes, the EEG cap was placed on the head, and measurements were made from Cz to the midpoint between electrodes C1, C3, CP1, CP3 (anode) and Cz, FCz, C1, FC1 (cathode). Stimulating electrodes were placed directly on the scalp and underneath the EEG cap at each of these two locations. Care was taken to keep the stimulating electrodes in the middle of the described recording electrode clusters, to minimize the stimulation artefact. Electrical stimulation intensity was set to be similar to the intensity of somatosensory stimulation evoked by the TMS stimulation. In order to determine this intensity, we first found the electrical stimulation threshold (eST), defined as the lowest electrical stimulation intensity at which the participant could consistently perceive the electrical pulse in 5 out of 5 trials (square-wave, 200 μs, Digitimer DS7A, Digitimer Ltd. Welwyn Garden City, UK). Next, TMS somatosensory threshold (tST), defined as the smallest TMS intensity at which the subject could consistently perceive the pulse, was measured. This was done with noise masking to ensure individuals were only focusing on the somatosensory feedback. Then, as TEP were evoked with a stimulation intensity of 90% RMT, we determined the ratio between the tST and 90% RMT, and this ratio was applied to the electrical stimulation. Therefore, the electrical stimulation intensity (eSI) was derived

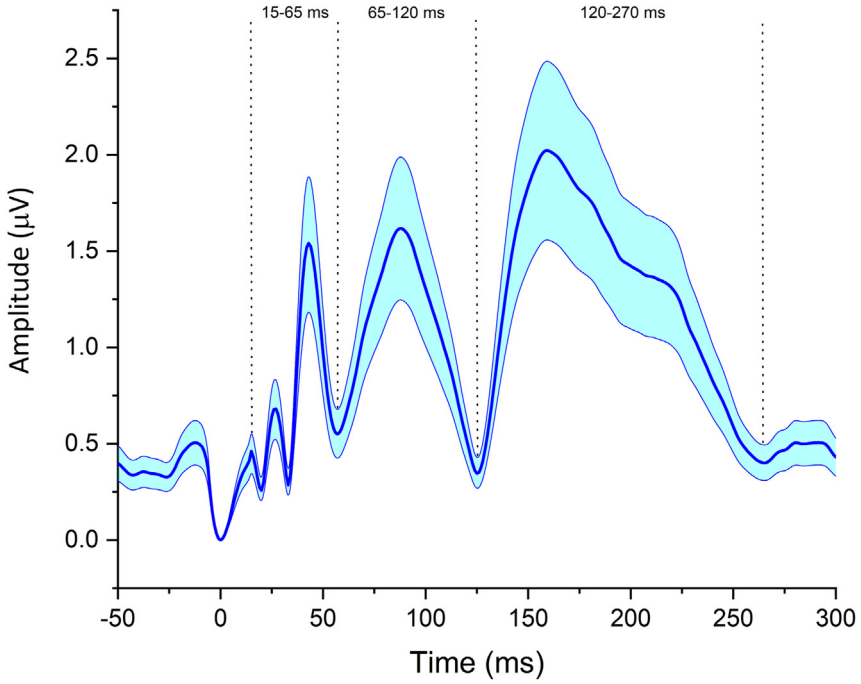

Fig. 2. Global mean field power averaged across subjects in block 6 (TEP NOT MASKED). Dotted lines represent boundaries of ToI (15–65 ms; 65–120 ms; 120–270 ms). Shadowing represents the standard error of the mean.

from the following formula: eSI = eST*(RMT*90/100)/tST). The equation relies on the assumption that somatosensory perception scales linearly with changes in both TMS and electrical stimulation intensity, at least in the range of 90–100% RMT. Compared to other methods used in previous papers [7,8], the present one has the advantage of calculating an individualised intensity for electrical stimulation which is not reliant on subjective matching of perceived somatosensory input from electrical and magnetic stimulation. At the end of the recording, participants were asked whether they could perceive any difference in the location or intensity between the TMS blocks where the standard coil was used and the electrical stimulation block.

Electroencephalographic recording and analysis

EEG was recorded using a DC-coupled, TMS-compatible amplifier (Actichamp, Brain Products, GmbH). Signals were recorded from 63 active electrodes mounted on a cap (actiCAP), in accordance with the international 10-10 system, including: Fp1, Fz, F3, F7, FT9, FC5, FC1, C3, T7, TP9, CP5, CP1, Pz, P3, P7, O1, FCz, O2, P4, P8, TP10, CP6, CP2, Cz, C4, T8, FT10, FC6, FC2, F4, F8, Fp2, AF7, AF3, AFz, F1, F5, FT7, FC3, C1, C5, TP7, CP3, P1, P5, PO7, PO3, POz, PO4, PO8, P6, P2, CPz, CP4, TP8, C6, C2, FC4, FT8, F6, AF8, AF4, F2. Recordings were online referenced to Oz and the ground electrode was placed on Fpz. In the offline analysis, an average reference was used. Skin impedances were kept below 5 kΩ and the sampling frequency during recording was 5000 Hz. When required, in order to mask the TMS-induced noise and avoid possible AEPs, participants wore in-ear headphones continuously playing a masking noise, as previously explained [14,15]. Additionally, a 0.5 cm foam layer was placed underneath the coil to minimize bone conduction of the TMS click and scalp sensation caused by coil vibration.

Offline EEG pre-processing was performed with EEGLAB 14.1.1 [16] with the addition of some functions included in the TMS-EEG signal analyser (TESA) toolbox [17] and in Fieldtrip open source MATLAB toolbox [18], all running in MATLAB environment (Version 2016b, MathWorks Inc., Natick, USA).

EEG signal recorded in all blocks was epoched (−1.3 to 1.3 s) using a baseline from −1000 to −10 ms. Epochs were visually inspected and those with excessively noisy EEG were excluded (remaining epochs were on average 116.4 ± 2.37, ranging from 113 to 120). A Friedman's ANOVA was performed to verify that there were no significant differences in the number of residual epochs in different blocks. The TMS artefact was removed from −5 to 10 ms around the trigger and interpolated by means of a cubic function. At this point, a first independent component decomposition analysis was run, using a fastICA algorithm. Only the 15 components explaining the largest variance were plotted, in a time window ranging from −200 to 500 ms, and those reflecting residual scalp muscle or decay were eliminated after visual inspection, based on time, frequency, scalp distribution and amplitude criteria. Albeit the use of ICA to remove these short-latency artefacts may lead to decrease of some of the TEP components, this effect has been demonstrated to be small [19] and, despite this limitation, ICA is widely used for the removal of early TMS-EEG artefacts [20,21]. ICA has also been used to remove artefacts caused by electrical stimulation of the scalp in an experimental setting similar to the present one [7]. After this, a band-pass (1–100 Hz) and a band-stop (48–52 Hz) fourth order Butterworth filter were applied. The signal was further epoched (−1 to 1 s) to reduce possible edge

Table 1
Summary of stimulation parameters. eST: electrical stimulation threshold; tST: TMS somatosensory threshold; eSI: electrical stimulation intensity, given by the formula eSI = eST*(RMT*90/100)/tST). (see text for details). AV = average; SD: standard deviation; SE: standard error.

	tST	TMS RMT	SHAM RMT	TMS/SHAM RMT	eST	eSI
	(% MSO)	(% MSO)	(% MSO)		(mA)	(mA)
AV	26.05	48.79	78.21	1.63	3.17	5.78
SD	4.18	9.66	12.32	0.03	1.09	2.20
SE	0.96	2.22	2.83	0.01	0.25	0.51

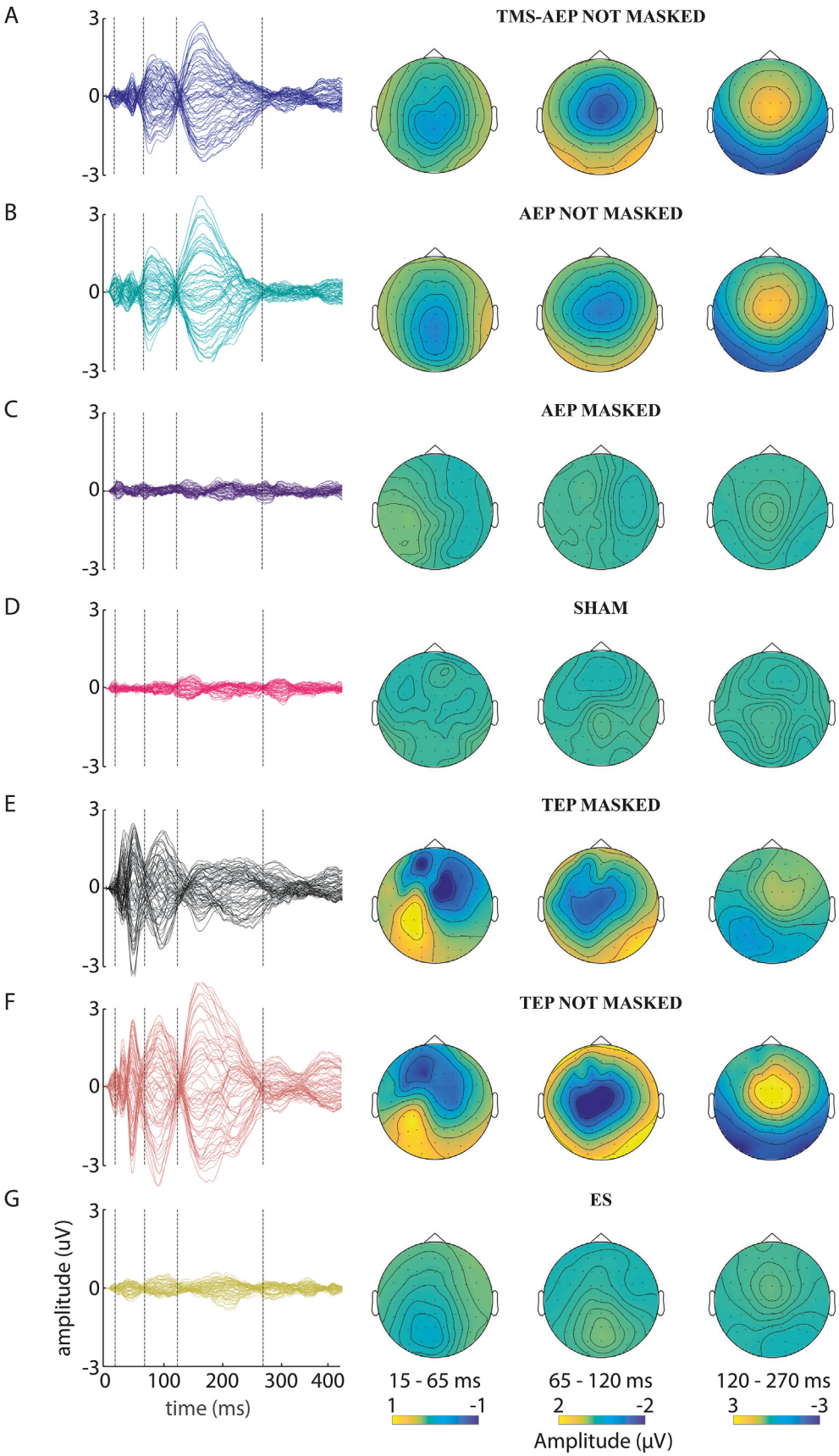

Fig. 3. Descriptive summary of results. Each of the seven rows correspond to a different recording block (A: block 1, auditory stimulation, standard coil, no noise masking (TMS-AEP NOT MASKED); B: block 2, auditory stimulation, sham coil, no noise masking (AEP NOT MASKED); C: block 3, auditory stimulation, sham coil, noise masking (AEP MASKED); D: block 4, sham stimulation (SHAM); E: block 5, TMS, standard coil, noise masking (TEP MASKED); F: block 6, TMS, standard coil, no noise masking (TEP NOT MASKED); G: block 7,

artefacts and a second round of fastICA was performed to remove residual artefacts (e.g. eyeblinks, continuous muscle activity, etc.). Lastly, EEG signals were rereferenced to the common average reference.

The TEP was first averaged in a cluster of electrodes surrounding the area of the stimulation (C1, C3, CP1, CP3) [14]. This allowed us to recognise the most common peaks described in the literature (N15, P30, N45, P60, N100, P200) [2] and to give a qualitative description of the signals at the stimulation site (Fig. 4, first column). We then calculated the global mean field power (GMFP), averaged across subjects, based on the following formula:

$$GMFP = \sqrt{\frac{\left[\sum_i^k \left(V_i(t) - V_{mean}(t)\right)^2\right]}{K}} \quad (1)$$

where t is time, K the number of channels, V_i the voltage in channel i and V_{mean} is the mean of the voltage in all channels [22]. To this purpose we used the recording obtained from block 6 (TEP NOT MASKED) since we anticipated that it would contain a larger signal compared to the other blocks, resulting from the sum of direct cortical activation, AEP and, potentially, SEP. Based on the GMFP waveform (Fig. 2), we selected three time regions of interest (ToI) for the following analysis (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms), as previously done [8]. In each ToI we calculated map-based statistics using the whole set of electrodes. This approach was chosen in an attempt to reduce bias due to the fact that TEP components can vary in terms of scalp distribution, the latter not necessarily corresponding to the stimulation site [2]. We used permutation statistics as implemented in Fieldtrip open source MATLAB toolbox [18]; correction for multiple comparisons was performed using a cluster-based algorithm [23]. We used t-tests for cluster-based statistics. To rank the found clusters, the sum of t values of all points in a cluster were computed. A p value of 0.05 was used to find clusters and the minimal number of channels per cluster was 1. The permutation was performed in the channel x time domains for the entire set of channels recorded and for the time intervals of interest. Corrected p values < 0.05 (two-tailed) were considered significant. By using the described procedure, in most of our analyses, we performed pairwise comparisons between the same ToIs of different conditions (Fig. 4). In a further analysis, aimed to define the duration of significant activity in the TEP MASKED condition, pairwise comparisons were performed between each ToI and a baseline of the same condition, of the same duration of the ToI compared and ending 5 ms before the TMS pulse.

We also performed two different correlation analyses, as described before [8]. In the first, designed to assess similarity between conditions at the scalp map level, correlation coefficients were calculated for each electrode and were averaged in the same ToI described before (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms), plus for the global time window (15–270 ms). In the second analysis, designed to give a more accurate assessment of correlation in time, correlation coefficients were calculated in each time point and averaged across channels. In both analyses, the coefficients' z-transform (Fisher's z-transform) were averaged and subsequently inverse z-transformed. To assess statistical significance, correlation coefficients were compared with those calculated in a baseline ranging from −400 to −100 ms by means of paired t-tests with false discovery rate correction for multiple comparisons. The analyses were performed with two different metrics: the Spearman's correlation coefficient, and the concordance correlation coefficient (CCC). The former is the non-parametric version of the Pearson's correlation coefficient and essentially quantifies association between distributions, based on covariance [24]. The CCC is a form of intraclass correlation coefficient optimally tuned to assess agreement between distributions and is calculated with the following formula:

$$CCC = \frac{2\sigma_{12}}{\sigma_1^2 + \sigma_2^2 + (\mu_1 - \mu_2)^2} \quad (2)$$

where σ_{12} is the covariance between two distributions, σ_x^2 is the variance of distribution x, and μ_x is the average of distribution x [25–27]. By using this procedure, we computed correlation and concordance in three pairs of conditions: 1) TMS-AEP NOT MASKED vs AEP NOT MASKED, to investigate the similarity between the AEP generated by the standard and the sham coil; 2) AEP NOT MASKED vs AEP MASKED, to assess the effectiveness of AEP suppression by noise masking; 3) TEP MASKED vs AEP NOT MASKED, to check a possible relation between potentials putatively generated only by direct cortical activation and pure auditory responses.

Results

The test session was well-tolerated and no participants reported any side effects. Thresholds and stimulation intensities are summarized in Table 1. TMS pulses induced a known pattern of negative and positive deflections [2,15,28]. Time-domain signals are represented as butterfly plots and scalp distributions in Fig. 3. Overall, evoked activity in the middle and late ToI was observed when auditory stimulation only was delivered (blocks 1 and 2, rows A and B respectively). These potentials were much smaller when the TMS click was masked (blocks 3 and 4, rows C and D respectively) and when only eletrical stimulation of the scalp was delivered (block 7, row G). A different pattern resulted when the standard coil was used, i.e. TEP in the early ToI were observed, with vertex potentials in late ToI being present only when noise masking was not used (blocks 5 and 6, rows E and F respecvitely). Subjects judged the location of TMS and electrical stimulation of the scalp to be roughly the same. Importantly, 10 subjects out of 19 reported that electrical stimulation resulted in stronger scalp sensation than TMS delivered with the standard coil, whereas the remaining 9 reported the opposite. Since there were no statistically significant differences between TMS-AEP NOT MASKED (Fig. 3, row A) and AEP MASKED (Fig. 3, row B) conditions, and the two showed very high correlation and concordance (Figs. 6 and 7), only the latter (block 2) was used for further comparisons.

The Friedman's ANOVA performed on the number of residual epochs did not show significant effects (χ^2 (6) = 1.821, p = 0.935). The VAS scores for the perceived click intensity were 0.42 ± 0.69 (range 0–2) for the AEP MASKED condition, 0.42 ± 0.61 for the SHAM condition (range 0–2), and 0.37 ± 0.68 for the TEP MASKED condition (range 0–2), with the Friedman's ANOVA again not showing a significant effect ($\chi^2(2) = 0.286$, p = 0.867). There was a significant positive correlation, investigated with the Spearman's correlation coefficient, between the VAS score and RMT, in the three conditions where VAS for perceived click intensity was administered (AEP MASKED: r = 0.563, p = 0.012; SHAM: r = 0.795, p < 0.001; TEP MASKED: r = 0.666, p = 0.002). Since the VAS scores for discomfort were 0 for all subjects in all blocks, no statistical analysis was performed.

electrical stimulation of the scalp (ES)). Column 1 represents butterfly plots of time-domain signals from the whole set of electrodes. Columns 2 to 4 include scalp maps of time domain signals averaged in the early (15–65 ms), middle (65–120) and late (120–270) time regions of interest, respectively.

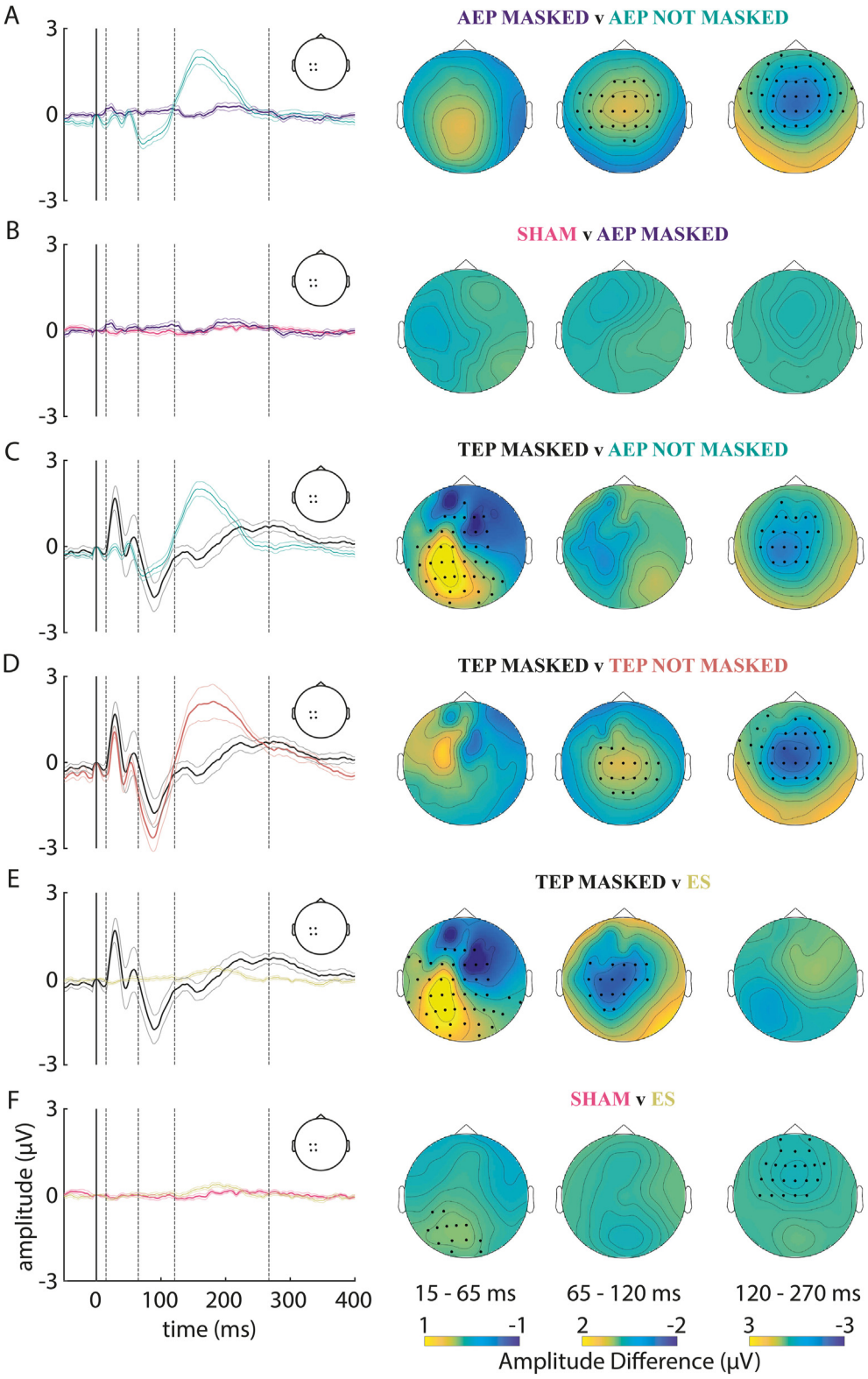

Fig. 4. Comparisons of several stimulation blocks. The first column represents time-domain signals averaged across four channels corresponding to the stimulation site (C1, C3, CP1, CP3). Shadowing indicates standard error of the mean. Vertical dashed lines indicate separation of the three ToI (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms). Columns 2 to 4 illustrate comparisons between two blocks at a scalp map level, averaged across the three ToI. Black dots represent significant clusters, either positive or negative. Note that, for graphical reasons, only one cluster for each map is shown, i.e. the one corresponding to the area of interest (see text and Table 2 for full details). **Row A**: comparison between AEP NOT MASKED (block 2, green line) and AEP MASKED (block 3, purple line) conditions. A significant positive cluster in the middle ToI (65–120 ms, $p = 0.001$) and a significant negative cluster in the late ToI (121–270 ms, $p = 0.001$) were found in the electrodes surrounding the vertex. **Row B**: comparison between SHAM (block 4, pink line) and AEP MASKED (block 3, purple line) conditions. No significant clusters were found. **Row C**: comparison between TEP MASKED (block 5, black line) and AEP NOT MASKED (block 2, green line) conditions. A significant positive cluster at the stimulation site in the early ToI (16–57 ms, $p = 0.001$) and a significant negative cluster around the vertex in the late ToI (121–203 ms, $p = 0.001$) were found. **Row D**: comparison between TEP MASKED (block 5, black line) and TEP NOT MASKED (red line, block 6) conditions. A significant positive

Table 2
Summary of results (see text for details).

Conditions compared	Blocks compared	Cluster direction	Early ToI (ms, p value)	Middle ToI (ms, p value)	Late ToI (ms, p value)
AEP NOT MASKED vs AEP MASKED	2 vs 3	Positive	–	65–120 ms p = 0.001	121–270 ms p = 0.001
		Negative	–	65–114 ms p = 0.001	121–264 ms p = 0.001
SHAM vs AEP MASKED	4 vs 3	Positive	–	–	–
		Negative	–	–	–
TEP MASKED vs AEP NOT MASKED	5 vs 2	Positive	16–57 ms p = 0.001	–	121–203 ms p = 0.001
		Negative	15–54 ms p = 0.001	–	121–203 ms p = 0.002
TEP MASKED vs TEP NOT MASKED	5 vs 6	Positive	–	65–106 ms p = 0.004	124–242 ms p = 0.001
		Negative	–	–	125–249 ms p = 0.001
TEP MASKED vs ES	5 vs 7	Positive	18–55 ms p = 0.001	67–120 ms p = 0.002	–
		Negative	18–59 ms p = 0.001	76–119 ms p = 0.009	–
SHAM vs ES	4 vs 7	Positive	22–54 ms p = 0.022	–	126–225 ms p = 0.005
		Negative	–	–	–
TEP MASKED vs BASELINE	5	Positive	17–65 ms P = 0.001	65–120 ms P = 0.004	147–270 ms P = 0.001
		Negative	17–54 ms P = 0.001	72–108 ms P = 0.004	191–264 ms P = 0.015

Results of map comparisons and cluster statistics are reported in Table 2 and briefly described here. In some cases where a highly significant cluster was found in the location of interest (either the stimulation site or the vertex), a further significant cluster in the same ToI was noted in the surrounding electrodes; the latter reflects an opposite dipole due to the use of an average reference. For the sake of clarity in the presentation of our results and given that these additional clusters surrounding the ones over the area of interest did not add any meaningful information, we chose not to graphically represent their significance in Fig. 4.

AEP NOT MASKED vs AEP MASKED

When comparing AEP NOT MASKED and AEP MASKED conditions (blocks 2 and 3; Fig. 4, row A), no significant clusters were found in the early ToI. In the middle ToI, a significant positive cluster was found in the central electrodes, with a negative one in the surrounding peripheral electrodes. In the late ToI, an inverse pattern was observed: a negative cluster in the central area and a positive one in the peripheral electrodes (Table 2, row 1). This result indicates that a clear scalp potential due to the TMS click is observed, including a negative peak around 100 ms and a positive peak around 200 ms, both distributed around the vertex. Importantly, these AEP components could be effectively suppressed with noise masking.

SHAM vs AEP MASKED

In the comparison between SHAM and AEP MASKED conditions (blocks 4 and 3; Fig. 4, row B) no significant clusters were found. This means that in both cases it was possible to effectively mask the AEP by using the masking noise, and that the position of the coil (directly over the cap or separated by means of a pasteboard cylinder) did not lead to differences in the recorded potentials.

TEP MASKED vs AEP NOT MASKED

TEP MASKED condition induced a different scalp response than AEP NOT MASKED (blocks 5 and 2; Fig. 4, row C). There was a significant positive cluster in the early ToI around the left central area, corresponding to the site of the stimulation, with a significant negative cluster in the right frontal region. This corresponds to an early cortical activation at the TMS site, which was not present with auditory stimulation alone. Although a clear negative potential was found at the site of the stimulation in the middle ToI, indicating a negative response around 100 ms caused by TMS, this did not reach statistical significance. In the late ToI there was a large significant negative cluster at the vertex and a large positive one in the peripheral electrodes, indicating a positive component at the vertex evoked by auditory stimulation (Table 2, third row). Overall, this indicates that standard TMS elicits early TEP components larger than auditory stimulation, whereas the late central positive component induced by the latter is not present when standard TMS is delivered during noise masking.

TEP MASKED vs TEP NOT MASKED

When comparing TEP MASKED and TEP NOT MASKED conditions (blocks 5 and 6; Fig. 4, row D), there were no significant clusters in the early ToI, meaning that early TEP components were

cluster in the middle ToI (65–106 ms, p = 0.004) and a significant negative cluster in the late ToI (125–249 ms, p = 0.001) were found in the electrodes surrounding the vertex. **Row E**: comparison between TEP MASKED (block 5, black line) vs ES (block 7, yellow line) conditions. A significant positive cluster in the early ToI at the stimulation site (18–55 ms, p = 0.001) and a significant negative cluster between the stimulation site and the vertex in the middle ToI (76–119 ms, p = 0.009) were found. **Row F**: comparison between SHAM (block 4, pink line) and ES (block 7, yellow line) conditions. A significant positive cluster in the posterior-left electrodes in the early ToI (22–54 ms, p = 0.022) and a significant negative cluster in the late ToI (126–255 ms, p = 0.005) in the central-anterior electrodes were found. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

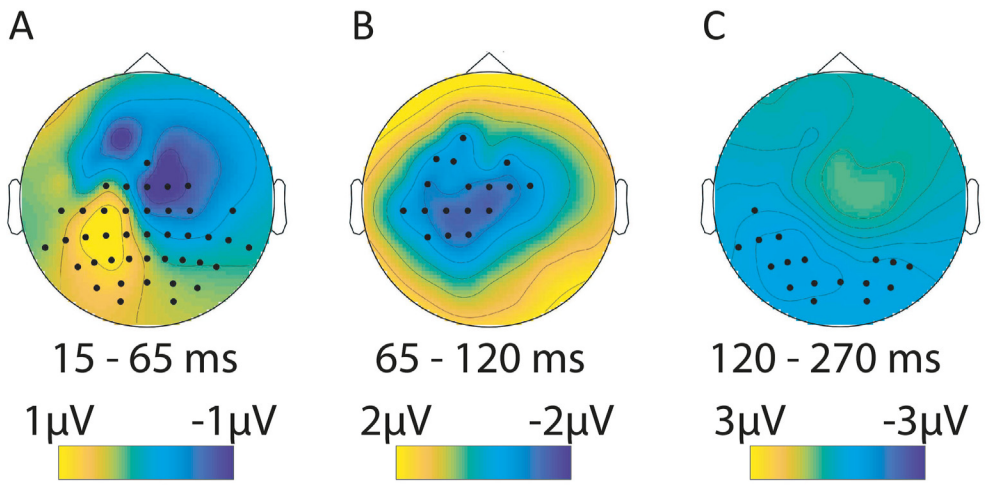

Fig. 5. Comparisons of activity in each ToI in the TEP MASKED condition with a baseline of the same condition, of the same duration of each ToI compared and ending 5 ms before the TMS pulse. Note that, for graphical reasons, only one cluster for each map is shown, i.e. the one corresponding to the area of interest (see text and Table 2 for full details). **Panel A**: early ToI. A significant positive cluster (17–65 ms, $p = 0.001$, plotted) and a significant negative cluster (17–54 ms, $p = 0.001$, plotted) were found. **Panel B**: middle ToI. A significant positive cluster (65–120 ms, $p = 0.004$) and a significant negative cluster (72–108 ms, $p = 0.004$, plotted) were found. **Panel C**: late ToI. A significant positive cluster (147–270 ms, $p = 0.001$) and a significant negative cluster (191–264 ms, $p = 0.015$, plotted) were found.

not significantly affected by the TMS click. The middle ToI showed a significant positive cluster around the vertex, indicating a larger negativity when noise masking was not applied. In the late ToI there was a large significant negative cluster at the vertex and a large positive one in the peripheral electrodes, indicating a strong positive component at the vertex evoked by auditory stimulation (Table 2, fourth row).

TEP MASKED vs ES

The comparison between TEP MASKED and ES conditions (blocks 5 and 7; Fig. 4, row E) resulted in a significant positive cluster in the early ToI around the left central area, at the site of the stimulation, with a negative cluster in the right frontal region. In the middle ToI there was a large, significant negative cluster close to the stimulation site, surrounded by a significant positive cluster at the posterior periphery of the right hemisphere. This indicates that TMS, differently from electrical stimulation, was able to activate the cortex at the site of stimulation not just early after the stimulus but also around 100 ms. Notably, the latter activation is different from the negative peak around the same latency induced at the vertex by auditory stimulation (Fig. 4, row A). There were no significant clusters in the late ToI, again indicating that noise masking effectively suppressed the positive peak at the vertex around 200 ms (Table 2, fifth row).

SHAM vs ES

In the early ToI, the comparison between SHAM and ES conditions (blocks 4 and 7; Fig. 4, row F) showed a weakly significant positive cluster in the posterior midline-left electrodes, reflecting a posterior negative potential present in the ES condition. There were no significant differences in the middle ToI, whereas in the late ToI we found a weakly significant negative cluster at the vertex, indicating a small positive component evoked by the scalp ES (Table 2, sixth row). It should be noted that the statistical significance of these clusters was weaker than those listed in the other comparisons and would not hold if a Bonferroni correction for multiple comparisons was applied to control for inflation of type I error.

TEP MASKED vs BASELINE

When comparing activity in each ToI with baseline in the TEP MASKED condition (Fig. 5), we found a significant positive cluster in the left central area, at the site of the stimulation, with a negative cluster in the right frontal region. The same analysis on the middle ToI showed a significant negative cluster close to the stimulation site, surrounded by a significant positive cluster at the posterior periphery of the right hemisphere. In the late ToI, we observed a significant negative cluster in the left central-posterior electrodes, and a significant positive one in the central-anterior electrodes of the right hemisphere. Statistics are summarized in Table 2. Overall, significant and lateralized activity was observed in all the ToI, suggesting that direct brain activation due to TMS lasts for the whole time window considered.

Correlation analyses

AEP obtained with the standard and sham coil (TMS-AEP NOT MASKED and AEP NOT MASKED conditions, blocks 1 and 2) showed statistically significant correlation (Fig. 6) and concordance (Fig. 7). In analysis 1, the correlation was clear in all three ToI, and particularly in the middle and late ones, where higher correlation and concordance values were found in electrodes around the vertex, corresponding to the main potentials following auditory stimulation (N100/P200). In analysis 2, significant correlation and concordance were obtained for time points ranging between 15 and approximately 250 ms, with islands of significance up to 400 ms. By contrast, AEP NOT MASKED and AEP MASKED conditions (blocks 2 and 3) showed no correlation (Fig. 8) or concordance (Fig. 9); this suggests that auditory responses were effectively suppressed by noise masking and confirms the results obtained in the comparison between the two conditions (Fig. 4, row A). Similarly, there was no correlation (Fig. 10) or concordance (Fig. 11) between conditions TEP MASKED and AEP NOT MASKED (blocks 5 and 2), indicating that potentials in the two blocks have independent sources; this further support the conclusion that AEPs were effectively suppressed by noise masking.

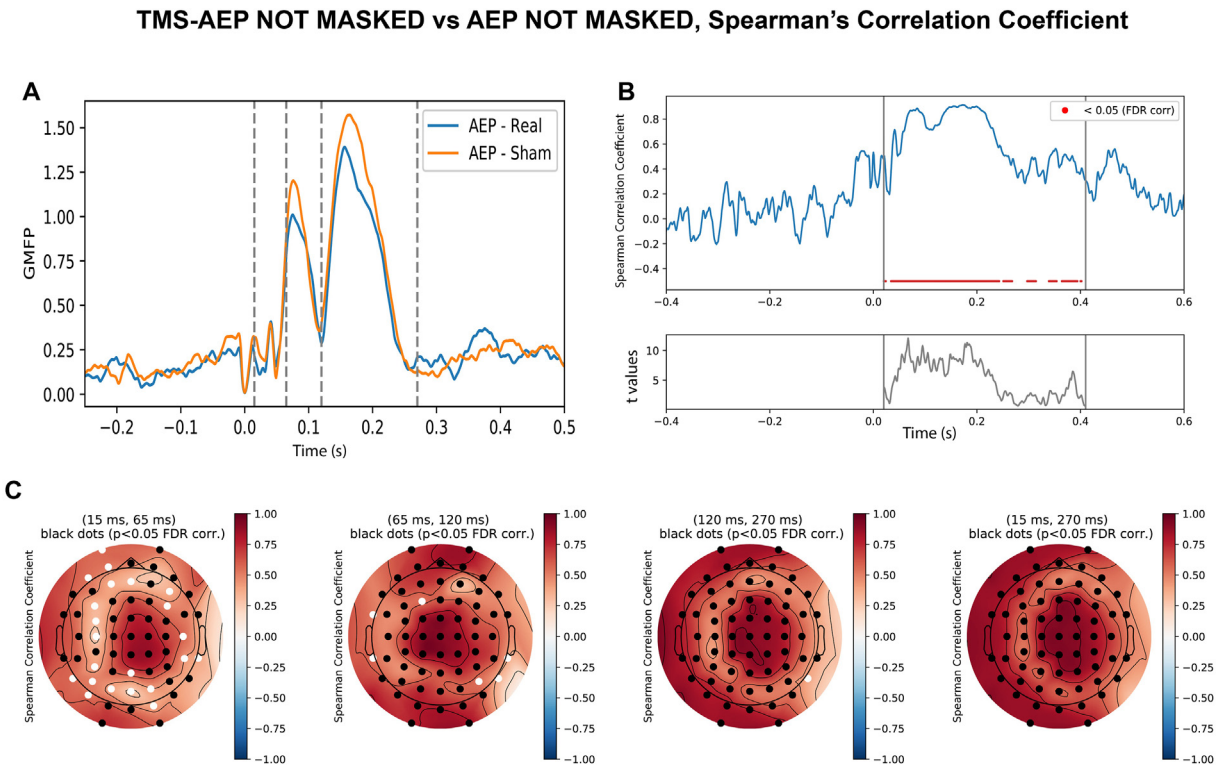

Fig. 6. Similarity between conditions TMS-AEP NOT MASKED and AEP NOT MASKED, investigated with the Spearman's correlation coefficient. **Panel A**: global mean field power of the two conditions (AEP - Real = TMS-AEP NOT MASKED; AEP - Sham = AEP NOT MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; the red bar shows statistically significant correlation between the two conditions, in the range of 15–400 ms. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Discussion

We evaluated the EEG responses due to auditory and somatosensory stimulation produced by TMS and measured their impact on the TEP. Overall, we found that standard TMS evoked a clear and pronounced early cortical activity at the stimulation site, which was not contaminated by AEP, provided that appropriate masking of the TMS click is performed. A negative peak around 100 ms was observed close to the stimulation site, which was not suppressed by noise masking and likely represents another direct cortical response. Auditory stimulation alone induced potentials between 100 and 200 ms; however, it was possible to suppress these components by appropriate noise masking. Electrical stimulation of the scalp alone did not induce SEP, as one would have expected if there had been substantial activation of the somatosensory pathways. Overall, our data suggest that, by careful control of confounding sources, it is possible to obtain a good estimate (over the first 300 ms or so) of the "true" brain responses produced by TMS on M1. These responses show a high degree of lateralization, whereas responses distributed around the vertex mostly derive from indirect brain activation caused by auditory and somatosensory input.

Contribution of auditory stimulation to the TEP

The first interesting finding is the clear definition of scalp responses generated by the TMS click. AEP are generally described in terms of early, middle and late, based on their latency. The early ones reflect brainstem activation; they last for a few ms after the stimulation [29] and are not considered here, since pre-processing of TMS-EEG data usually removes data in that time window [21,30]. Using both a standard and sham coil, we reproduced the known pattern of middle- (N15, P30, N40, P50) and late-latency responses (N100, P200) [3,31]. Since the two sets of responses showed no significant differences and exhibited very high and significant correlation and concordance values, we propose that, at least in this context, psychophysically matched sound intensity is the most important factor in generation of AEP, regardless of the coil used. Interestingly, with noise masking, middle- and late-latency AEP were suppressed. This demonstrates that, when stimulating at 90% RMT, with appropriate noise masking, it is possible to obtain TEPs that are not contaminated by AEP. Additionally, the same result was obtained in the sham condition, where the coil lay directly over the EEG cap. Both these results indicate that bone conduction of sound was effectively suppressed by the use of the foam layer, as demonstrated in a previous report [31]. The contribution of the AEP to the TEP was clarified by two other observations. The first comes from the comparison between TEP obtained by using a standard coil with noise masking and pure auditory stimulation without noise masking. In this case, it was clear that the first elicited larger early responses, especially the P30 peak; by contrast, the second induced very small amplitude early responses, but substantial N100 and P200 components around the vertex. This is confirmed by the comparison between the TEP obtained using the standard coil, with and without noise masking. Here, there was no difference in the components earlier than 60 ms, and again this makes it unlikely that they received a significant contribution from the AEP. The N100 was larger and located in the midline central electrodes when no noise masking was used; by contrast, the N100 present in the block with noise masking is smaller and lateralized (as can be further noticed in the comparison between TEP with noise masking

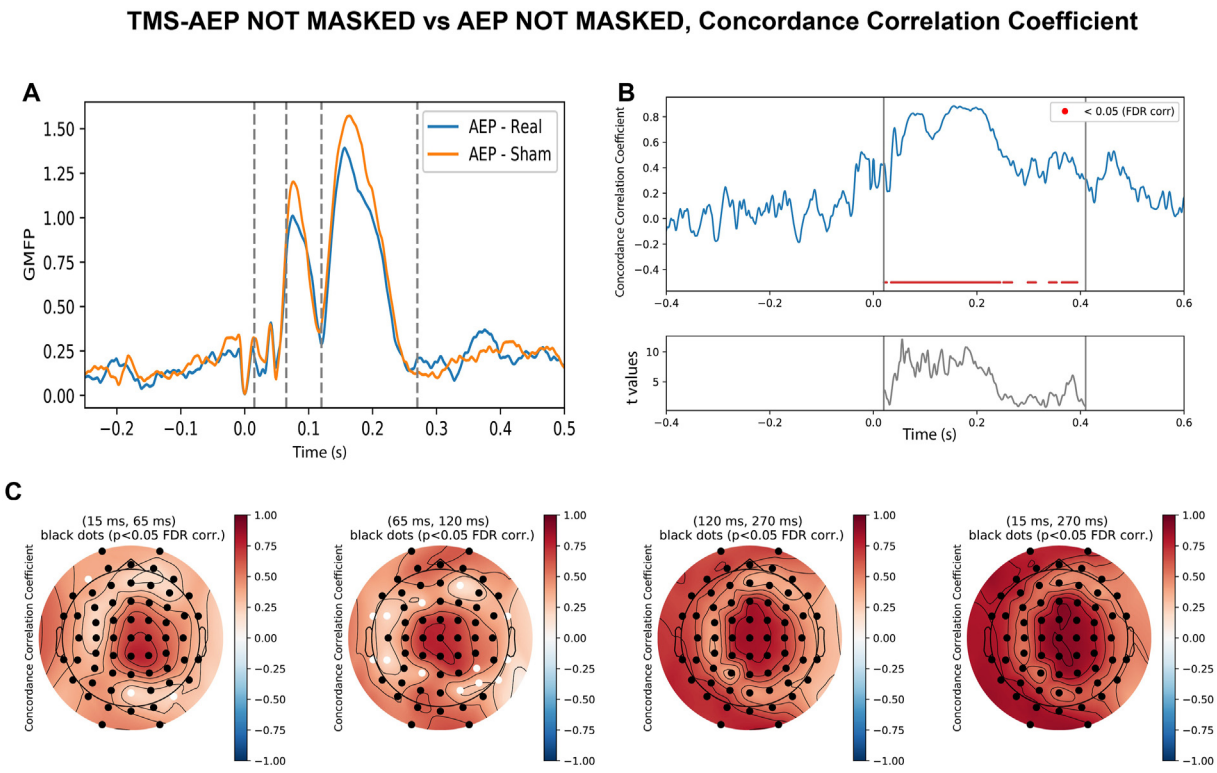

Fig. 7. Similarity between TMS-AEP NOT MASKED and AEP NOT MASKED, investigated with the concordance correlation coefficient. **Panel A**: global mean field power of the two conditions (AEP - Real = TMS-AEP NOT MASKED; AEP - Sham = AEP NOT MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; the red bar shows statistically significant correlation between the two conditions, in the range of 15–400 ms. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and electrical stimulation of the scalp). Finally, the central P200 was present only when no noise masking was used.

Overall, the present data demonstrate the presence of two distinct negative peaks, around 100 ms, that likely involve different mechanisms. The first is close to the stimulation site and likely reflects direct cortical activation by TMS; the second, by contrast, has a wider distribution around the vertex and is followed by a P200 with the same distribution. This conclusion is based on several independent arguments: a) the auditory N100 is suppressed by noise masking, as indicated by the significant difference (row A of Fig. 4) and by the absence of correlation or concordance (Figs. 8 and 9) between AEP MASKED and AEP NOT MASKED conditions. By contrast, the TMS N100 is not suppressed by noise masking, as it is present in a condition where masking noise is used (TEP MASKED); b) the auditory N100 is always followed by a vertex P200 (see Fig. 3, rows A and B, and supplementary figure 3), whereas the TMS N100 is not (see TEP MASKED condition); c) the latency of the TMS N100 is significantly longer than that of the auditory N100 (supplementary figure 1); d) there is no correlation or concordance between conditions TEP MASKED and AEP NOT MASKED (Figs. 10 and 11). This finding suggests that the N100 observed in the TEP MASKED condition was not significantly contaminated by the AEP.

In conclusion, considering previous literature, it is likely that the N100 found at the stimulation site reflects direct cortical activation by TMS [2], whereas the central N100 and P200 represent saliency-related multimodal responses (SRMR), which are non-specific responses, possibly linked to arousal and/or attentional reorientation following an external stimulus, regardless of its modality [32].

Our set of results appear different from those obtained by Conde and coworkers [8], who found a substantial correlation between cortical responses obtained by using a standard coil and those following a "realistic sham" stimulation, similar to the one used here (see Supplementary Material). A possible reason for this is that masking of the TMS click was more effective in the present study: indeed, in the paper by Conde and coworkers, VAS scores for residual TMS click were much higher than in the present study (respectively up to 3.82 on average, with values of 8 in some subjects, against an average of 0.42, with maximal individual values of 2). At least part of this difference might be due to the use, in our study, of ear defenders on top of the earphones playing the masking noise, which probably helped in suppressing the AEP.

Contribution of electrical stimulation of the scalp to the TEP

It has previously been suggested that somatosensory activation due to TMS could contribute to the TEP waveform. However, previous reports [7,8] were focussed on the impact of simultaneous somatosensory and auditory stimulation and did not assess the possible effects of electrical stimulation alone; the latter represent a novel contribution of the present work. To the best of our knowledge, only one study described responses to electrical scalp electrical stimulation. However, only one subject was tested, and details of the stimulation, such as intensity and location, were not mentioned [12]; thus, a comparison with the present findings is difficult. An advantage of our protocol is that we measured individual thresholds for electrical stimulation and scaled the stimulation intensity according to the ratio between somatosensory and motor thresholds to TMS, in an attempt to give an electrical pulse

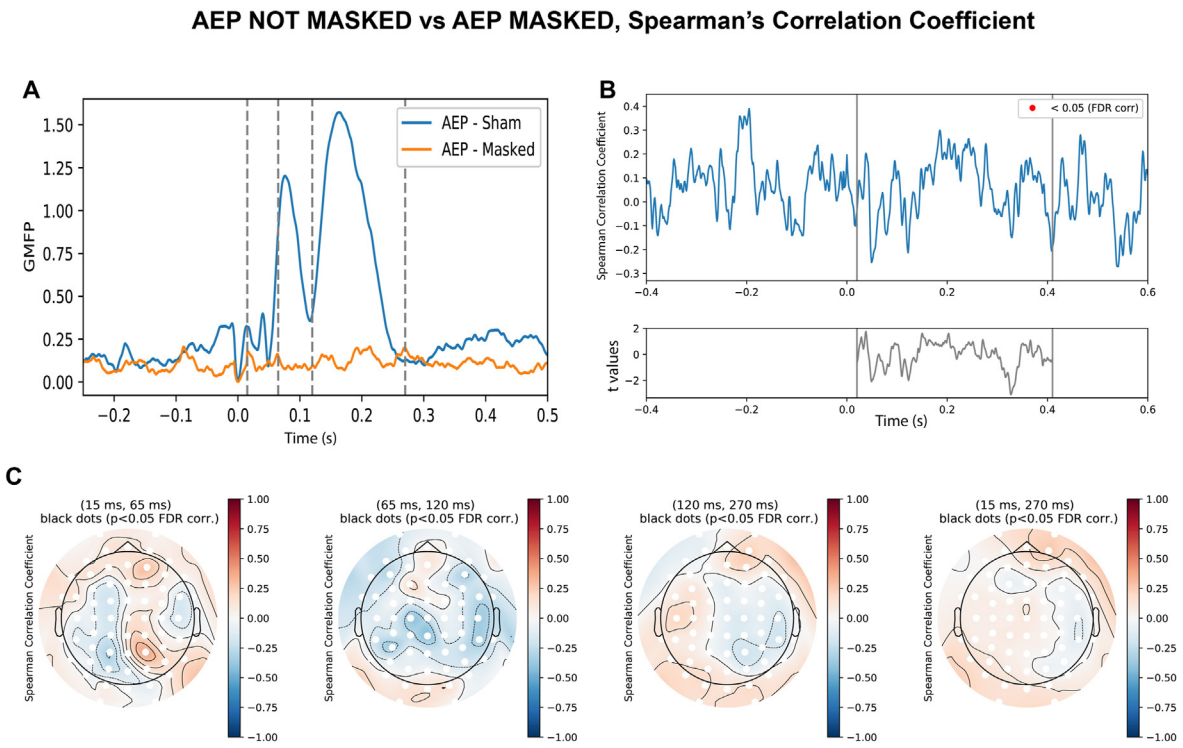

Fig. 8. Similarity between conditions AEP NOT MASKED and AEP MASKED, investigated with the Spearman's correlation coefficient. **Panel A**: global mean field power of the two conditions (AEP - Sham = AEP NOT MASKED; AEP - Masked: AEP MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; no statistically significant correlation was found. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white.

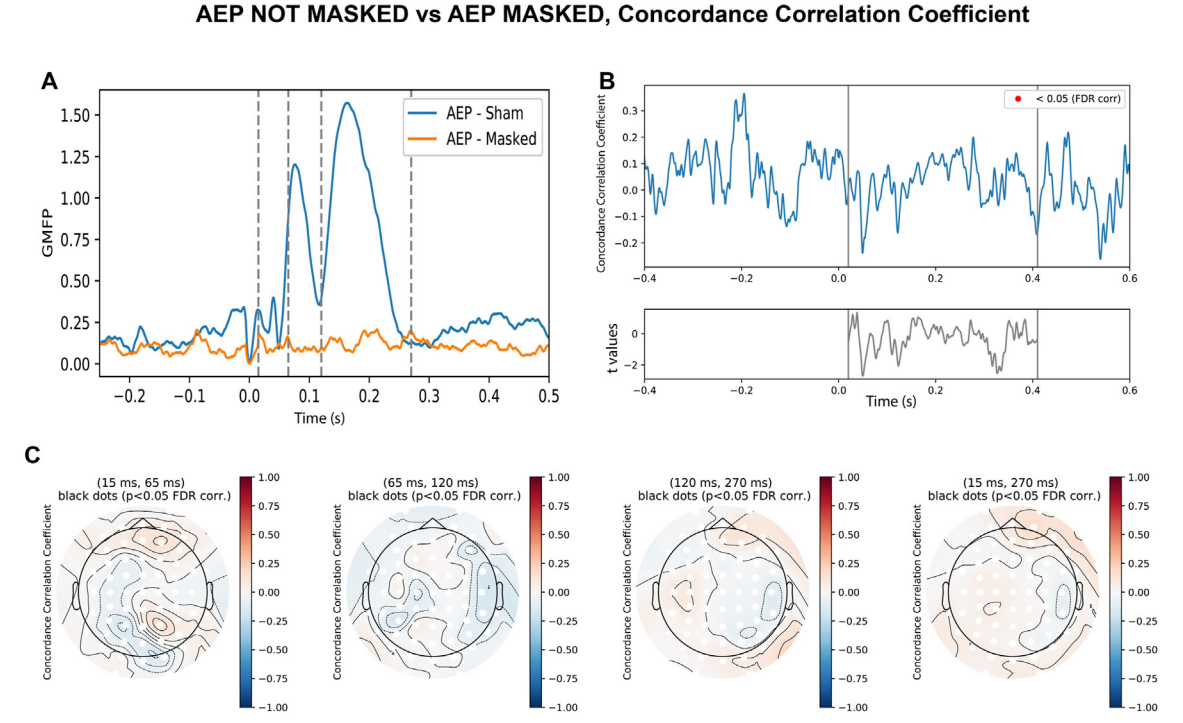

Fig. 9. Similarity between conditions AEP NOT MASKED and AEP MASKED, investigated with the concordance correlation coefficient. **Panel A**: global mean field power of the two conditions (AEP - Sham = AEP NOT MASKED; AEP - Masked: AEP MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; no statistically significant correlation was found. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white.

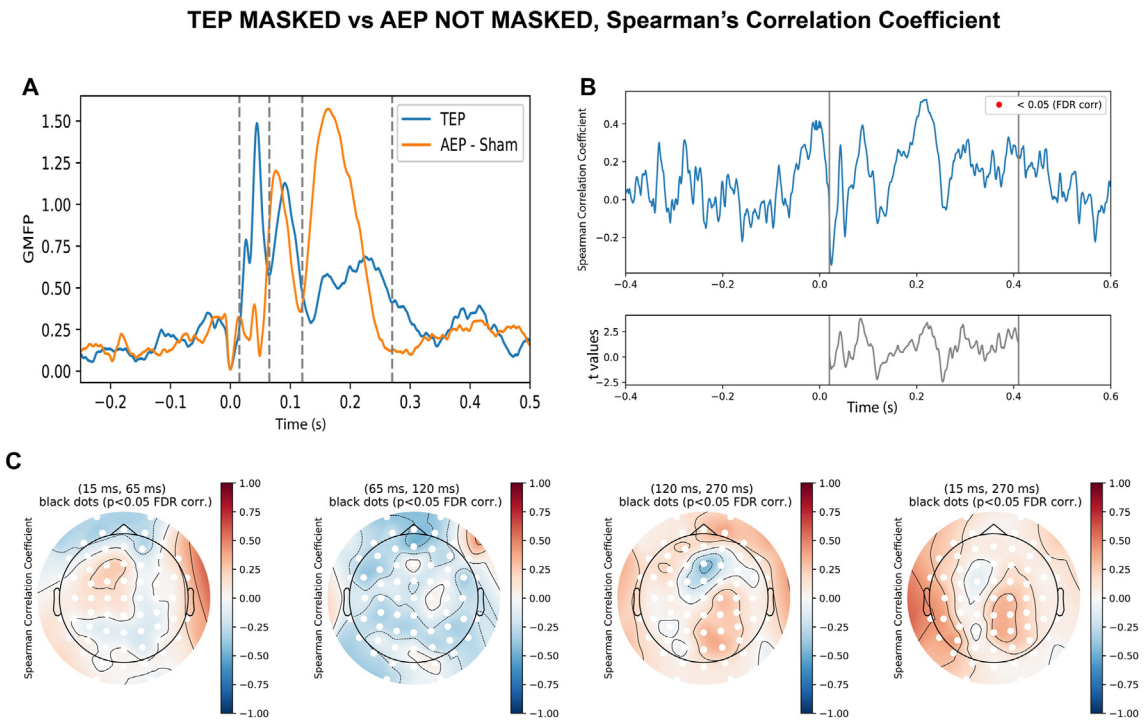

Fig. 10. Similarity between conditions TEP MASKED and AEP NOT MASKED, investigated with the Spearman's correlation coefficient. **Panel A**: global mean field power of the two conditions (TEP = TEP MASKED; AEP − Sham = AEP NOT MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; no statistically significant correlation was found. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white.

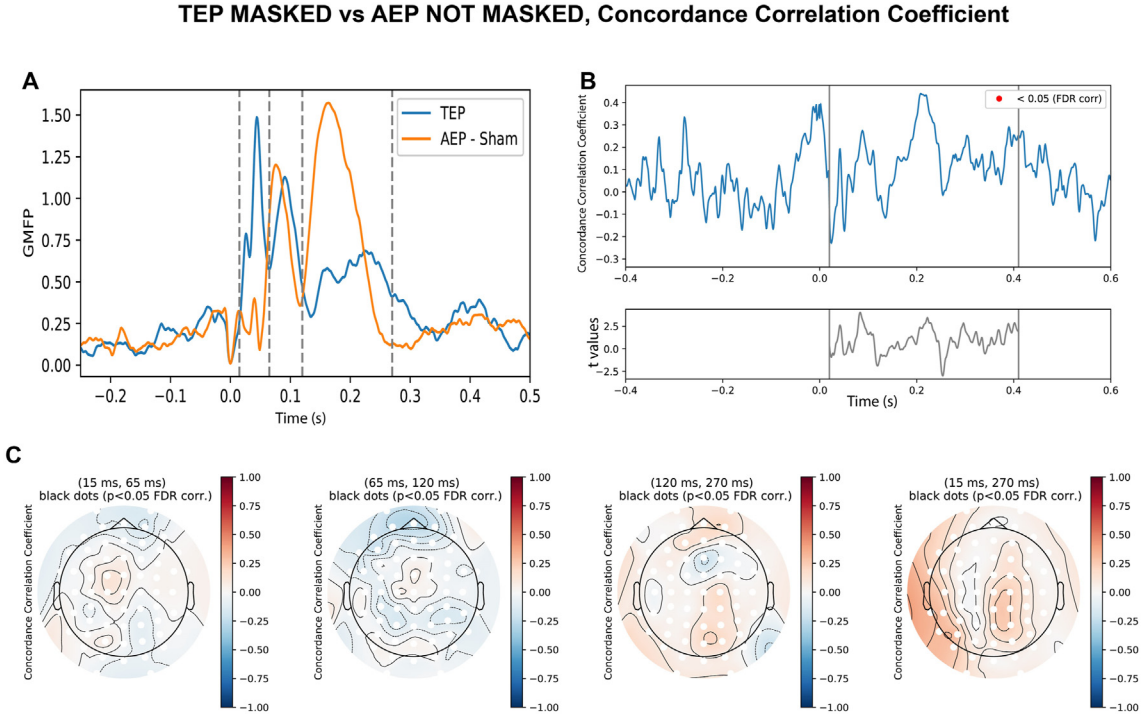

Fig. 11. Similarity between conditions TEP MASKED and AEP NOT MASKED, investigated with the concordance correlation coefficient. **Panel A**: global mean field power of the two conditions (TEP = TEP MASKED; AEP − Sham = AEP NOT MASKED). **Panel B**: *upper chart:* correlation coefficient, averaged across all channels and calculated in each time point; no statistically significant correlation was found. *Lower chart*: t statics (see text for details). **Panel C**: correlation investigated in all electrodes, in four different ToI, one in each column (early: 15–65 ms; middle: 65–120 ms; late: 120–270 ms; global: 15.270 ms). Electrodes with statistically significant correlations are plotted in black, while those not showing statistically significant correlations are plotted in white.

which, in terms of intensity, closely resembled the intensity perceived with TMS. The attempt was successful and confirmed by the fact that participants were evenly split over whether they perceived the electrical stimulus to be stronger or weaker than the TMS. Electrical stimulation of the scalp alone elicited a very small response compared to other blocks where standard TMS was used (Fig. 3). When compared to sham stimulation, there was a significant negative potential in the posterior electrodes in the early ToI, around 40 ms. This probably represents a resetting of the resting alpha rhythm, as observed with stimuli of other sensory modalities [33]. A second weak potential was found in comparison to sham, represented by a positivity around 200 ms in the central electrodes. Again, this is interpretable as SRMR [32], although its amplitude was considerably less than that after auditory stimulation. We can conclude that a positive wave recorded around 180–200 ms and distributed around the vertex represents a SRMR due to peripheral input [32]; thus, it is shared by several of the experimental conditions in our work (see Fig. 3 and supplementary figure 2). This finding is also in line with a previous report, where a similar vertex activation was found with electrical stimulation in the shoulder region [34].

It is very important to note that EEG responses following electrical stimulation of the scalp were not observed at the site of stimulation. This was expected since the intensity necessary to obtain direct cortical responses with electrical stimulation is much higher than that used here [35]. We also did not obtain SEP, making it unlikely that the potentials observed were due to activity in the primary somatosensory cortex . This is not surprising considering two lines of evidence. First, EEG signals following stimulation in the cranio-facial region are difficult to obtain. Clear SEP have been observed only by stimulating branches of the trigeminal nerve with needle electrodes [36,37]; this is very different from TMS, where stimuli are not applied on a nerve trunk. Secondly, somatosensory threshold at the scalp level is much higher compared to other body areas, due to the low density of cutaneous receptors [38]. For these reasons, we believe that our electrical stimulation was not sufficient to elicit a synchronous afferent volley large enough to elicit a SEP.

Limitations

It is important to note that the present results have been obtained using a 90% RMT stimulation intensity. The use of a higher intensity might lead to changes in the TEP waveform due to incomplete suppression of AEP by noise masking and the introduction of reafference potentials that accompany peripheral EMG activity; thus, caution should be used in extending the present results to different experimental conditions, such as stimulation of different scalp sites and the use of larger currents. Additionally, stimulation of peripheral nerves supplying the scalp by the sham coil used in the present paper is not comparable to that induced by the standard coil; hence, the need to control for somatosensory activation by using electrical stimulation of the scalp in several recording blocks. Finally, the present work relies on the assumption that ICA can be used to remove artefacts associated to TMS stimulation, with reasonable preservation of the underlying EEG signal. While this approach has theoretical limitations, its applicability has been empirically demonstrated [21,39].

It should also be noted that TMS applied over M1, as in the present work, results in a substantial artefact due to scalp muscle activation, which needs to be removed to properly assess the TEP. ICA has proven to be efficient to achieve this and, importantly, other methods have not been shown to be definitely superior [21,34]. As a consensus on the matter has yet to be reached [40], further studies comparing and validating different approaches to TMS-EEG artefacts removal are certainly warranted.

Conclusions

In conclusion, the present findings suggest that, when TMS is applied over M1 with an intensity slightly lower than motor threshold, it is possible to obtain genuine cortical EEG responses which are lateralized, specific for the stimulation site and last about 300 ms, provided that procedures to minimize indirect cortical activation (noise masking and foam layer) are properly adopted. These responses include a positive peak around 30 ms, a negative one around 100 ms, and a shallower negativity peaking around 250 ms, whereas other TEP components appear less reliable. This topography is replaced by non-specific central components (in particular, a prominent positivity around 200 ms) in conditions where the brain is only indirectly activated by peripheral stimulation (AEP NOT MASKED, TMS-AEP NOT MASKED, ES and control experiments listed in the Supplementary Material). The same pattern becomes prominent when TMS is performed with a standard coil, without noise masking (TEP NOT MASKED condition). Thus, sensory evoked components, if present, are associated with a late, negative-positive waves with symmetrical central distribution, compatible with a SRMR [32]. Importantly, these components, particularly the auditory N100, can contaminate signals generated by direct brain activation, if masking procedures of sensory stimulation are not carefully applied.

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CRediT authorship contribution statement

Lorenzo Rocchi: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Alessandro Di Santo:** Investigation, Writing - original draft, Writing - review & editing. **Katlyn Brown:** Investigation, Writing - original draft, Writing - review & editing. **Jaime Ibáñez:** Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Elias Casula:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Vishal Rawji:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Vincenzo Di Lazzaro:** Conceptualization, Writing - review & editing. **Giacomo Koch:** Conceptualization, Writing - review & editing. **John Rothwell:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.10.011.

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