The specific spatiotemporal evolution of TMS-evoked potentials reflects the engagement of cortical circuits

Matteo Fecchio[1,2,*], Simone Russo[3,4,*], Bruno Andry Couto[5,6], Ezequiel Mikulan[7], Andrea Pigorini[8], Giulia Furregoni[3,9], Gabriel Hassan[3], Sasha D'Ambrosio[7,10], Michela Solbiati[11], Alessandro Viganò[12], Sara Parmigiani[13], Simone Sarasso[3], Silvia Casarotto[3,12], Marcello Massimini[3,12,14], Adenauer Girardi Casali[6,**], Mario Rosanova[3,**]

*co-first authors
**co-last authors

Author Affiliations:

1. Center for Neurotechnology and Neurorecovery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02114
2. Department of Neurology, Harvard Medical School, Boston, MA, 02114
3. Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, 20157
4. Georgia Institute of Technology, Atlanta, GA, USA, 30332
5. Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark.
6. Institute of Science and Technology, Federal University of São Paulo, São José dos Campos, Brazil, 12247-014
7. Department of Health Sciences, University of Milan, Milan, Italy, 20146
8. Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy, 20122
9. School of Advanced Studies, Center of Neuroscience, University of Camerino, Camerino, Italy, 62032
10. Department of Clinical and Experimental Epilepsy, University College London, London, United Kingdom
11. Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy, 27100
12. IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy
13. Center for Translational Neuroscience (CTN), Department of Human Physiology, University of Oregon, Eugene, OR, USA
14. Azrieli Program in Brain, Mind and Consciousness, Canadian Institute for Advanced Research (CIFAR), Toronto, Ontario M5G 1M1, Canada

Corresponding authors:

Matteo Fecchio
Address:
Massachusetts General Hospital,
Center for Neurotechnology and Neurorecovery (CNTR),
101 Merrimac Street, Suite 310, Boston, MA 02114, United States.
E-mail: mfecchio@mgh.harvard.edu

Mario Rosanova
Address:
University of Milan,
Via Gian Battista Grassi, 74 - L.I.T.A. Vialba, Milano (MI), 20157, Italy.
E-mail: mario.rosanova@unimi.it

ABSTRACT

Transcranial Magnetic Stimulation (TMS) evokes electroencephalographic (EEG) responses that can last hundreds of milliseconds. Yet only the first 80 ms after the pulse are widely accepted to reflect genuine cortical responses to TMS, while later components have mainly been attributed to the effects of sensory co-stimulations. Here we reappraise this view by investigating the target-specificity of the spatiotemporal evolution of TMS-evoked potentials (TEPs). To this end, we compared TEPs elicited by TMS targeting the premotor and primary motor cortices in healthy subjects, under conditions designed to optimize the TMS effectiveness on the cortex while minimizing peripheral confounds. As a counterfactual, we conducted the same comparison on the EEG responses evoked by realistic sham TMS and high-intensity somatosensory scalp stimulation. We found that EEG responses to motor and premotor TMS can exhibit distinct spatiotemporal evolutions up to 300 ms both at the group and single-subject levels. These differences were absent or marginally present in both realistic sham TMS and high-intensity somatosensory scalp stimulation. Our findings suggest that, when effectiveness is optimized and peripheral confounds are controlled, TMS elicits specific long-lasting EEG responses that reflect genuine responses to the initial engagement of specific cortical targets. These results challenge previous assumptions and highlight how TMS-EEG can be reliably used to assess large-scale properties within corticothalamic networks.

Keywords: TMS-EEG; cerebral cortex; corticothalamic networks; reverberatory activity; confounding factors

INTRODUCTION

Transcranial Magnetic Stimulation combined with Electroencephalogram (TMS-EEG) allows probing cortical reactivity and connectivity directly and non-invasively, offering high potential for research and clinical applications [1–3]. However, its clinical adoption has been hindered by the lack of standardized procedures for collecting and analyzing TMS-evoked potentials (TEPs) [4–6].

Standardizing TMS-EEG measurements is challenging due to the variety of methods used to set stimulation parameters and data acquisition criteria. As an example, different research groups calibrate TMS intensity in various ways, either based on motor output, phosphenes perception, induced electric field strength, or EEG response amplitude, leading to inconsistent results [5,6]. Furthermore, biological confounds, such as TMS-related cranial muscle activations and auditory evoked potentials (AEP), are addressed either using offline signal processing methods [7–10] or applying preventive procedures during recording [11–14]. Finally, although several sham TMS protocols exist to account for auditory and somatosensory effects on TEPs [15], no standard approach has been agreed upon yet.

As a result, the TMS-EEG community is still debating the extent to which TEPs reflect genuine responses to direct cortical stimulation, unconfounded by sensory co-stimulations [16,17]. In a pivotal study, Conde and colleagues [18] found that EEG responses to both TMS and sham stimulations of different cortical sites shared common spatiotemporal features, indicating contamination by the effects of peripheral co-stimulation starting as early as 20 ms post-stimulus. Following studies suggest that genuine TEPs can last until 80 ms after the TMS [19–26], while others extend this limit to later latencies [27–32].

Here, we address this issue by comparing TEPs collected by targeting nearby cortical sites (i.e., the premotor (PM) and primary motor (M1) areas). We operate under the null hypothesis that they should not differ in spatial and temporal features. To test this hypothesis, the experiments were designed to optimize cortical activation by TMS (effective TMS, eTMS) while minimizing peripheral sensory co-stimulation. As a counterfactual, we also compared EEG responses to stimulation of the same targets in the absence of direct cortical engagement, using two types of sensory stimulation: a realistic sham (RS-TMS), combining electric scalp and auditory stimulations to replicate TMS-related sensory co-stimulations; and a high-intensity electrical scalp stimulation (HI-ES), delivering the highest tolerable scalp stimulation below pain threshold.

We found that TEPs from adjacent cortical targets show significant spatiotemporal differences lasting up to 300 ms, thus rejecting the null hypothesis. In contrast, EEG responses to sensory stimulation showed no or only marginal differences across the same targets. Taken together, these findings suggest that TEPs can reflect specific cortical activations even at later latencies, with little to no contribution from peripheral confounds.

MATERIALS AND METHODS

Study participants

We enrolled sixteen healthy subjects (n=16; 4 female, 3 left-handed, age: 25-51 years, Table S1). Exclusion criteria included neurological or psychiatric disorders, current CNS-active drugs use, substance abuse, and contraindications to TMS. The Ethics Committee Milano Area A approved the study, and all subjects provided written informed consent.

Experimental design and protocol

To test the hypothesis that TEPs are dominated by sensory components, especially at late latencies, we systematically compared the EEG responses between PM and M1 stimulation sites, at both group and single-subject level, and for each stimulation type: eTMS, RS-TMS, and HI-ES (Figure 1).

TEPs and EEG responses to RS-TMS and HI-ES were recorded in two separate sessions held on different days, both starting at 10 AM to minimize circadian effects. Each stimulation type required tailored experimental procedures that are detailed in Supplementary 1-5. Noise masking, whose parameters were adjusted on each subject's TMS "click" perception [33] (Supplementary 2), was played during all EEG recordings, regardless the stimulation condition. To collect TEPs, sensory confounders, such as AEP, scalp muscle contractions, and motor evoked potentials (MEPs) [34], were minimized (Supplementary 3 and 5).

Participants were seated comfortably during recordings and rated discomfort and pain on a Numerical Rating Scale (NRS; Supplementary 6, Figure S2, and Table S2) at the end of each measurement. At least 200 TMS pulses were delivered per each eTMS session, and 300 stimuli per each RS-TMS and HI-ES session. For all stimulation types, stimuli were randomly delivered at an interval jittering between 2000 and 2300 ms (0.5–0.4 Hz).

EEG recording and processing

EEG signals were recorded in direct current (DC) mode at 5 kHz using a 62-electrode cap connected to a TMS-compatible amplifier (BrainAmp DC, Brain Products GmbH). Electrodes were referenced to the forehead, with impedances kept below 5 kOhm. Preprocessing, including ICA for artifact removal, was performed as described in the Supplementary Material (Sections 7 and 8; Figure S3). To avoid bias in statistical comparisons, the first 135 consecutive good epochs (the minimum across all recordings) were used to compute evoked potentials. Grand-average evoked potentials were calculated by averaging single-trial responses across subjects for each target and experimental condition (eTMS, RS-TMS, and HI-ES).

Statistical analyses

For each experimental condition (eTMS, RS-TMS, and HI-ES), we compared evoked potentials across different targets at both the group- (grand-average) and single-subject levels using a non-parametric permutation test with cluster-based correction for multiple comparisons (FieldTrip Toolbox [35]; Supplementary 9). Significant differences between targets were considered only in channels and temporal samples with a significant evoked response for at least one target (Supplementary 10).

In addition, we extracted reproducible evoked components across trials and subjects using group-Task Related Component Analysis (gTRCA) [36], as described by Couto and colleagues [37]. For each condition and target, we retained the principal (i.e., most reproducible) gTRCA component together with its eigenvalue, which quantifies the degree of reproducibility at the group level. Statistical significance of each eigenvalue was assessed using the subject-based shifting method, with 500 permutations ($p < 0.05$).

Stimulation intensities were compared between targets for each condition using the paired Wilcoxon signed-rank test. A linear mixed-effects model (LMM) was used to assess the effects of experimental condition and stimulation target on the amplitude of late evoked responses and NRS outcomes (discomfort and pain). Condition and target were fixed factors, with a random intercept for each participant to account for repeated measures.

RESULTS

We recorded TEPs, and EEG responses to RS-TMS, and HI-ES while stimulating left PM and left M1 in 16 healthy subjects. Data recorded during RS-TMS and HI-ES in two subjects were discarded due to large decay artifacts that could not be removed without significantly corrupting the EEG response. After titrating noise masking (Supplementary 2), all subjects reported no auditory perception of the TMS "click" as confirmed by the absence of TMS-related AEPs (Figure S4) in TMS and RS-TMS conditions.

Stimulation intensities for eTMS, RS-TMS, and HI-ES are reported in Table S3 for each subject and target. Notably, eTMS intensity (expressed as the percentage of the maximal stimulator output), which affects coil vibration and hence tactile scalp stimulation, was not significantly different between targets ($p = 0.22$).

Subjectively, TMS resulted in low discomfort (NRS 0-4), and no pain (NRS = 0 in all but one subject, which scored 1). Notably, 13 out of 16 subjects reported a discomfort difference of 1 or less between PM and M1, with no significant differences in NRS values for discomfort or pain between targets. Despite RS-TMS intensity being matched to the TMS-related sensory stimulation of the scalp, discomfort and pain levels were higher during RS-TMS, with a maximum of 6 for discomfort and 3 for pain. Only three subjects reported discomfort and pain differences greater than 1 between targets. During RS-TMS stimulation, all subjects reported that the coil touching the scalp reduced discomfort, which otherwise was perceived as "too focal and pricking." Finally, as expected, subjects reported higher discomfort and pain during HI-ES, with maximum scores of 9 and 4, respectively. Only two subjects reported differences greater than 1 across targets (Figure S2 and Table S2).

eTMS evokes site-specific EEG responses

To test the hypothesis that TEPs are dominated by EEG responses to sensory co-stimulation—which are not expected to differ across targets—we first compared TEPs recorded during PM and M1 stimulation at the group level.

Figure 2A and 2B show the grand-average TEPs for PM and M1, respectively (TEPs collected in one subject are reported in Figure S5A). The scalp voltage distribution of the first TEP component is asymmetrical, with a more frontal distribution in PM than M1. The Global Mean Field Power (GMFP) calculated for significant evoked responses (Supplementary 10) shows different time-courses, with peaks at distinct latencies for the two targets, and diverging topographies. Specifically, late PM TEP peaked at 80, 112, and 213 ms, with slightly lateralized topographies involving frontal electrodes over the stimulated hemisphere, while M1 TEP peaked at 85, 128, 180, and 313 ms, mainly showing a clearly lateralized topography over the stimulated hemisphere. Cluster-based statistical tests confirmed these differences, identifying significant clusters in both amplitude and phase domains, mainly over the stimulated hemisphere, persisting beyond 300 ms (Figure 2C and 2D).

To confirm that the differences between targets are consistent at the individual level and not merely an artifact of averaging across subjects, we conducted two complementary analyses. Figure 3 shows the waveforms and average scalp maps of the principal (most reproducible) gTRCA components for PM and M1 TEPs (panels 3A and 3B, respectively; gTRCA eigenvalue = 2.21 and 2.66, both significantly reproducible at the group level, $p < 0.002$). These components exhibited a sequence of positive and negative deflections at different latencies (PM: 13, 39, 81, and 120 ms; M1: 13, 55, 123, 172, 262, and 354 ms). Both stimulations involved lateralized channels located more frontally for PM and more posteriorly for M1. On the other hand, Figure 3C and 3D shows the distributions of individual GMFPs. Most subjects exhibited significant responses lasting up to 308 ms for PM and 285 ms for M1, as indicated by the median GMFPs (blue traces). Figure 3E highlights timepoints where significant evoked responses differ between PM and M1 TEPs in each subject. In at least 50% of subjects, significant differences persisted for up to 262 ms, whereas only a small fraction of participants showed no differences between targets (orange area). These differences primarily involved frontoparietal EEG channels over the stimulated hemisphere (right side of Figure 3E). Phase analysis (Figure 3F) confirmed these results.

These findings show that eTMS to nearby cortical sites can evoke EEG responses characterized by spatiotemporal evolutions that differ up to about 300 ms at both group and single-subject levels.

RS-TMS evokes stereotypical EEG responses

We sought counterfactual evidence that TMS-related sensory co-stimulations can indeed result in EEG responses that, unlike TEPs, do not significantly differ over both space and time. To this aim, we first compared EEG responses evoked by RS-TMS of two scalp sites using the same procedure as for TEPs. Figure 4A and 4B show the grand average EEG responses to RS-TMS applied to PM and M1, respectively. RS-TMS evoked significant activity between 47-358 ms for PM and 48-342 ms for M1. Visual inspection of scalp topographies relative to GMFP peaks revealed similar activations, primarily over central electrodes, for both targets. This was confirmed by the cluster-based statistical test, which showed no significant differences in amplitude or phase (Figure 4C and 4D), and by the principal gTRCA components which were qualitatively similar. Unlike TEPs, these components were less reproducible at the group-level (gTRCA eigenvalue was 0.81 for PM and 0.85 for M1, both with $p<0.002$) slower and highly stereotyped across sites, peaking around central electrodes (Figure 4E and 4F). Notably, 3 and 6 subjects showed no significant responses to RS-TMS applied over PM and M1, respectively. Among the remaining participants, RS-TMS evoked small amplitude responses (Figure 4G and 4H, and Figure S5B showing EEG responses to RS-TMS for one subject), primarily between 80-250 ms. Late (100-400 ms) responses to RS-TMS were significantly smaller than TEPs ($p < 0.005$), as indicated by the significant GMFP (Figure S6).

We compared EEG responses to RS-TMS applied to PM and M1 using cluster-based analysis at the single-subject level. Only 5 of 14 subjects showed significant differences in voltage or phase domains (Figure 4I and 4J). In contrast to TEPs, over 70% of subjects showed no differences in spatiotemporal patterns between targets, as indicated by the sum of the blue (no response) and orange areas (responses not differing significantly between targets).

This second set of results shows that TMS-related sensory co-stimulation can indeed produce EEG responses whose spatiotemporal evolution is stereotypical, involves central electrodes and does not significantly differ between stimulation targets, both at the group and single-subject levels.

HI-ES evokes stereotypical EEG responses

Previous studies have shown that RS-TMS can result in evoked potentials of significantly lower amplitude compared to TEPs [29,38]. Thus, to maximize EEG responses to electrical scalp stimulation we performed HI-ES. Average responses to HI-ES emerged at late latencies, with amplitudes similar to late TEPs (Figure 5A and 5B). PM stimulation evoked significant responses between 43 and 490 ms, while responses to M1 stimulation occurred between 22 and 444 ms. Scalp topographies relative to GMFP peaks showed contralateral activations around 70 ms followed by central activations for both targets. Unlike RS-TMS, the cluster-based test identified significant amplitude differences between targets, but, unlike TEPs, these differences occur in a narrow time window (150–210 ms) and primarily over central electrodes (Figure 5C). Comparisons in the phase domain revealed significant clusters from 112 to 209 ms and 299 to 368 ms (Figure 5C and 5D), overlapping with the same scalp regions as the amplitude domain. Principal gTRCA components evoked by HI-ES (Figure 5E and 5F) were similar across targets, peaking around central electrodes, resembling those seen with RS-TMS (gTRCA eigenvalue = 1.73 and 1.62 for PM and M1, respectively, both with $p < 0.002$).

Analysis of GMFP at the individual level confirmed that HI-ES evoked larger responses than RS-TMS at late latencies (Figure S5B shows EEG responses to HI-ES for one subject), with amplitudes similar to TEPs (Figure 5G and 5H, and Figure S6). However, larger HI-ES responses did not result in differences between PM and M1. Specifically, the percentage of subjects with a significant response to HI-ES (orange area of Figure 5I and 5J) was similar to that observed with TMS (Figure 3I and 3J), while the percentage showing significant differences between targets (red area of Figure 5I and 5J) was comparable to that observed with RS-TMS (Figure 4I and 4J).

This third set of results shows that EEG responses to tactile scalp stimulation, even when maximized, involve central electrodes and do not significantly differ between stimulation targets at both the group and single-subject levels.

The hemispheric asymmetry of late evoked responses to eTMS, but not to RS-TMS nor HI-ES, discriminates between stimulation targets

Results so far show that TEPs from different, yet nearby, cortical sites exhibit distinct voltage waveforms and scalp topographies, even at late latencies. On the contrary, EEG responses to RS-TMS and HI-ES at late latencies are stereotyped and nonspecific, involving mainly central electrodes, regardless of the stimulation target. Results also suggest that lateralization of late responses is a key feature for differentiating between PM and M1 targets in TMS-evoked responses, but not in those evoked by RS-TMS or HI-ES. To test this, we compared potentials evoked in left and right electrodes and computed a simple index to quantify lateralization at early (0–100 ms) and late (100–400 ms) latencies (Supplementary 11). Figure 6 shows how the index distinguishes between stimulation targets, as quantified by Receiver Operating Characteristic (ROC) analysis for TEPs, with an area under the curve (AUC) of 0.69 for 0–100 ms, increasing to 0.91 for 100–400 ms. On the contrary, the lateralization of EEG responses to RS-TMS and HI-ES cannot distinguish between targets in both time windows (AUC for 0–100 ms: 0.51 for RS-TMS and 0.56 for HI-ES; AUC for 100–400 ms: 0.57 for RS-TMS and 0.51 for HI-ES).

DISCUSSION

The prevailing view in the TMS-EEG literature is that only early TEP components represent genuine cortical responses to the TMS pulse, whereas later components are primarily attributed to TMS-related sensory activations [19–22,24–28,38,39]. In this study, we asked whether the spatiotemporal evolution of TEPs, including their later components, can reflect a genuine cortical activation and hence provide specific information about the targeted cortical circuits. To address this question, we compared TEPs from one premotor and one motor cortical area in a group of healthy subjects, testing the null hypothesis that the spatiotemporal features of the resulting TEPs do not differ. As a counterfactual, we compared EEG responses from the same targets using realistic sham-TMS and high-intensity electrical scalp stimulation.

We found that TEPs differ significantly between the two adjacent cortical targets throughout a 300 ms time window and across large scalp regions. These differences are particularly evident over the stimulated hemisphere and detectable at the single-subject level (Figures 2, 3, and S5). Thus, they refute our null hypothesis. Conversely, both realistic sham-TMS and high-intensity electrical scalp stimulation evoked EEG responses that are either indistinguishable between targets or show differences that are restricted in both temporal and spatial extent (Figures 4 and 5, and S5). Together, these findings contrast with previous reports and suggest that sensory responses do not primarily drive TEPs even at late latencies. Various factors can explain these results.

Previous studies suggest that TEP components can be significantly affected by TMS-related AEPs [29,40], especially at late latencies. When noise masking is not applied or is ineffective, late TEP components are visibly influenced by the N100-P180 complex [7,17,41], which is usually prominent over central EEG electrodes, and reduces TEP site-specificity [10]. Consistent with this, we observed that sham TMS produces TMS-related AEPs with similar spatiotemporal features across stimulation sites (Figures S4A and S4B). We also confirm that TMS-related AEPs can be effectively abolished in both sham and real TMS conditions at the group level [29], and crucially, at the single-subject level [42] (Figures S4C and S4D) by optimizing noise-masking parameters [11,33] with ad hoc procedures during the recording (Supplementary 2).

In addition to the contribution of TMS-related AEPs, the lack of control over TMS effectiveness in engaging its cortical target may explain discrepancies in the literature [16]. In this work, we used a real-time TEP

visualization tool [11] to ensure that the initial (<50 ms) cortical response was largest in EEG channels underneath the TMS coil with a preset peak-to-peak amplitude >6 µV after averaging 20 trials (Figure S1). These high-amplitude early and local TEP components represent an initial prerequisite (i.e. the independent variable) to effectively activate the target neuronal population [43–46] and to eventually engage circuit-specific chains of interactions [47,48](i.e., the dependent variable of interest).

We found that TEPs spatiotemporal evolution significantly differed between the two cortical targets, extending up to 300 ms at the single-subject level in both amplitude and phase. This is notable, as both targets are in close proximity (approximately 3 cm apart) and are part of the motor circuit [49,50]. Nevertheless, the consistent differences observed between nearby targets are reinforced by a novel analytical tool that extracts the most reproducible TEP components across trials and subjects for given stimulation parameters. These components show striking waveform and topographic differences between the two cortical targets (Figure 3). Moreover, they align with previous studies showing that TEPs exhibit lasting differences in waveforms, frequency content, and cortical activation patterns depending on the stimulation site [30–32,34,51–56]. These site-specific differences likely reflect the engagement of distinct neural circuits, as shown by a recent study combining TMS targeting different cortical areas with intracranial recordings [57] and cortical source modelling integrated with connectivity modelling [58]. Motor cortex stimulation produced slower (Figure S5A) and more lateralized activations ipsilateral to the stimulation site, even at late latencies, consistent with previous studies using subthreshold intensities [29]. These late activations are unlikely due to sensory activations as they are ipsilateral to the stimulation target and may rather result from cortico-thalamic interactions, as seen in TMS-EEG studies in patients who had undergone thalamectomy [59]. Moreover, these TMS-evoked late oscillations can be modulated by coil orientation [60] and motor tasks [61]. Also, the slow, late TEP components (around 200 ms) following premotor stimulation predominantly involve central electrodes, resembling those elicited by sensory stimulation. It is worth considering the nature of these components. A recent study comparing cortical responses to premotor cortex activation either obtained with TMS or with intracortical electrodes suggests that they may reflect the engagement of specific neural circuits by the TMS pulse. Specifically, this study showed that late components (about 200 ms latency) evoked by premotor cortex stimulation are most likely underpinned by thalamic rebound activity, with their amplitude significantly modulated by motor tasks that alter the state of thalamocortical neurons [62]. More generally, late components can be also observed following intracortical stimulation, which, by definition, is devoid of sensory co-stimulation [63] and thus are likely due to the actual engagement of target circuits.

After demonstrating significant, long-lasting differences in premotor and motor TEPs both in time and space, we examined counterfactual evidence. Late TEP components have often been attributed to somatosensory-evoked potentials [64], arising from craniofacial muscle contractions or activation of scalp somatosensory receptors and nerve branches [65]. Importantly, TMS intensity, expressed as percentage of the maximal stimulator output, which accounts for TMS-related scalp stimulation, was not significantly different between the two cortical targets (Table S3). Furthermore, to mimic the somatosensory stimulation from TMS coil discharge, realistic sham TMS combined scalp electrical stimulation (matched to the TMS-related somatosensory perception) and TMS in a sham position preserving its vibratory component [41]. Under this condition, EEG responses were dominated by an N100-P200 complex in central regions, with no significant differences between premotor and motor sites. Notably, nearly half of the participants showed no significant EEG responses to realistic sham TMS (Figure 4). This finding is consistent with previous studies using sham-TMS [29,38] and may be explained by the low density of somatosensory fibers in the scalp [66]. In this regard, it is worth recalling previous TMS-EEG studies in brain-injured patients with preserved somatosensory pathways that found no significant EEG responses when sensory confounds were controlled and TMS was targeted over cortical lesions [67,68].

We raised the intensity of electrical scalp stimulation to the maximum tolerable level below pain perception, eliciting significant responses for both premotor and motor targets in all but one participant. Unlike TEPs, but

similar to realistic sham, EEG responses to high-intensity electrical scalp stimulation showed stereotypical components (Figures 4, 5, and S5) that were invariant across stimulation targets (Figure 6). The average waveform presented a component around 70 ms, with a topography slightly contralateral to the stimulation site, consistent with sensory responses to electrical stimulation of the forehead [69]. This was followed by two later components resembling the N100-P200 complex, typical of multimodal vertex responses [70]. These later components were slightly larger for the premotor target, possibly reflecting greater cortical responsiveness to stimulation of scalp regions closer to the face [71]. Significant differences between the two targets, when stimulated with high-intensity electrical stimulation, were confined to central scalp regions around 200 ms (Figure 5). These findings suggest that, unlike TMS, high-intensity somatosensory scalp stimulation activates the same neural network for detection and processing of salient stimuli, regardless of the stimulation site.

Lastly, it has been suggested that TMS-related somatosensory activations may involve scalp or muscle nociceptors [65]. In our study, the scalp muscle contractions were controlled by coil adjustment. Accordingly, only one out of 16 participants perceived pain during eTMS over the motor cortex (Figure S2), which rules out significant contributions of pain-related evoked potentials to late TEP components.

Our experimental design, along with the lack of a standardized TMS sham procedure, prevents us from fully ruling out the possibility that somatosensory responses to TMS-related scalp stimulation may still affect later TEP components. However, taken together, our findings suggest that if such a contribution exists it is negligible, provided that rigorous experimental procedures to minimize artifacts and to maximize the signal-to-noise ratio are employed. First, ROC analysis demonstrated that the lateralization of the responses evoked by eTMS, but not by RS-TMS nor HI-ES, successfully discriminated between targets (Figure 6), with the greatest discriminative power found in the late components (between 100 and 400 ms).

To address our scientific question, we applied a specifically designed approach aimed at minimizing confounding factors and controlling for the immediate impact of TMS on the cortex prior to recording. In addition, our approach reduces TMS-related artifacts during the experimental session, thereby lowering the need for extensive preprocessing to extract TEPs and helping to minimize bias in the results [72,73] (Figure S3). It is important to recognize that this approach may not easily apply to all TMS-EEG studies, for example, those targeting lateral, such as the dorsolateral prefrontal cortex, or posterior structures, such as the cerebellum. In these cases, spurious activations should be considered and eventually addressed with off-line artifact rejection tools.

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Author Contributions

Conceptualization: MF, SR, AC, MR

Data acquisition: MF, SR, GF, GH, SDA, MS, AV, SP, MR

Formal analysis: MF, BC, AC

Methodology: MF, SR, AC, MR

Software: MF, SR, BC, AC

Writing – original draft: MF, SR, AC, MR

Writing – review and editing: MF, SR, BC, EM, AP, SP, SS, SC, MM, AC, MR

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Supervision: MF, SC, SS, MM, AC, MR

Conflict of Interest

MM is a co-founder and shareholder of Intrinsic Powers, a spin-off of the University of Milan. MR, SC, SS, are advisors and shareholders of Intrinsic Powers. SR is the Chief Medical Officer of Manava Plus. These affiliations in no way affect the content of this article. The other authors report no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data that support the findings of this study as well as individual and group-level data are available upon request.

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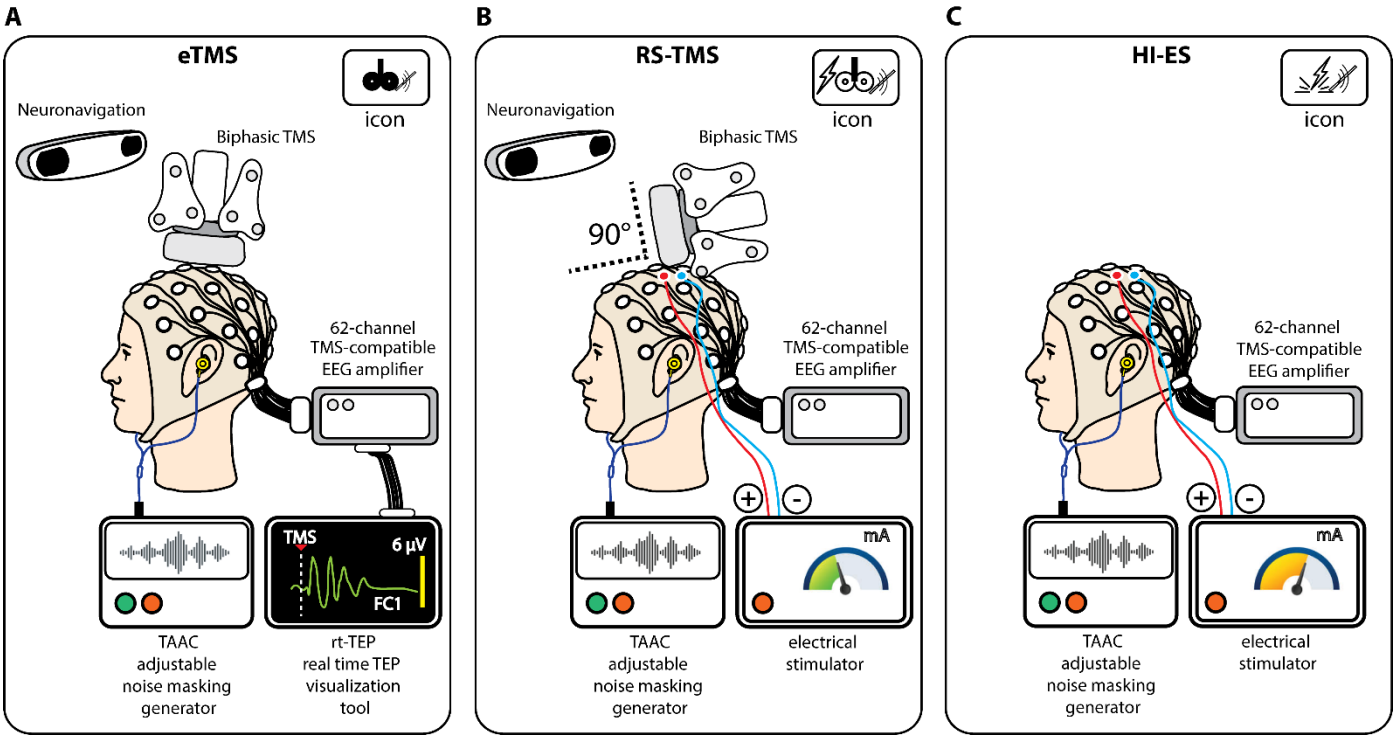

Figure 1. Schematic representation of the experimental setup for effective TMS, realistic sham TMS, and high-intensity electrical stimulation. In each panel, the icon representing the stimulation type is shown in the top-right corner. EEG was acquired with a 62-electrode cap connected to a TMS-compatible amplifier. Noise masking was played in all conditions (bottom left of each panel). **(A)** Biphasic TMS pulses were delivered over the left PM and M1 cortical areas using a neuronavigated figure-of-eight TMS coil. Rt-TEP, a real-time visualization tool (bottom right) was used to optimize TMS parameters and evoke early TEP components (< 50 ms) with amplitudes ≥ 6 µV beneath the coil (see also Figure S1). **(B)** RS-TMS was designed to mimic scalp and auditory sensations associated with TMS without actual cortical stimulation. Electrical stimulation was applied to scalp sites overlying the same cortical areas targeted with TMS. Pairs of stimulating electrodes (blue and red), spaced 2.5 cm apart [18], were positioned to match the TMS coil location and the direction of the TMS-induced electric field. The TMS coil, rotated 90° in the sagittal plane, was kept in contact with the scalp over the target area, a position known to effectively eliciting TMS-related AEPs and preserving coil vibrations [35]. TMS and electrical stimuli were delivered simultaneously, with intensity adjusted to match the scalp sensation of TMS based on participant's reports (Supplementary 4, Table S3). **(C)** High intensity electrical stimulation was applied to the same scalp sites used for RS-TMS, but without the TMS coil. Stimulus intensity was gradually increased to the highest level tolerated by each participant without inducing pain (Supplementary 4, Table S3).

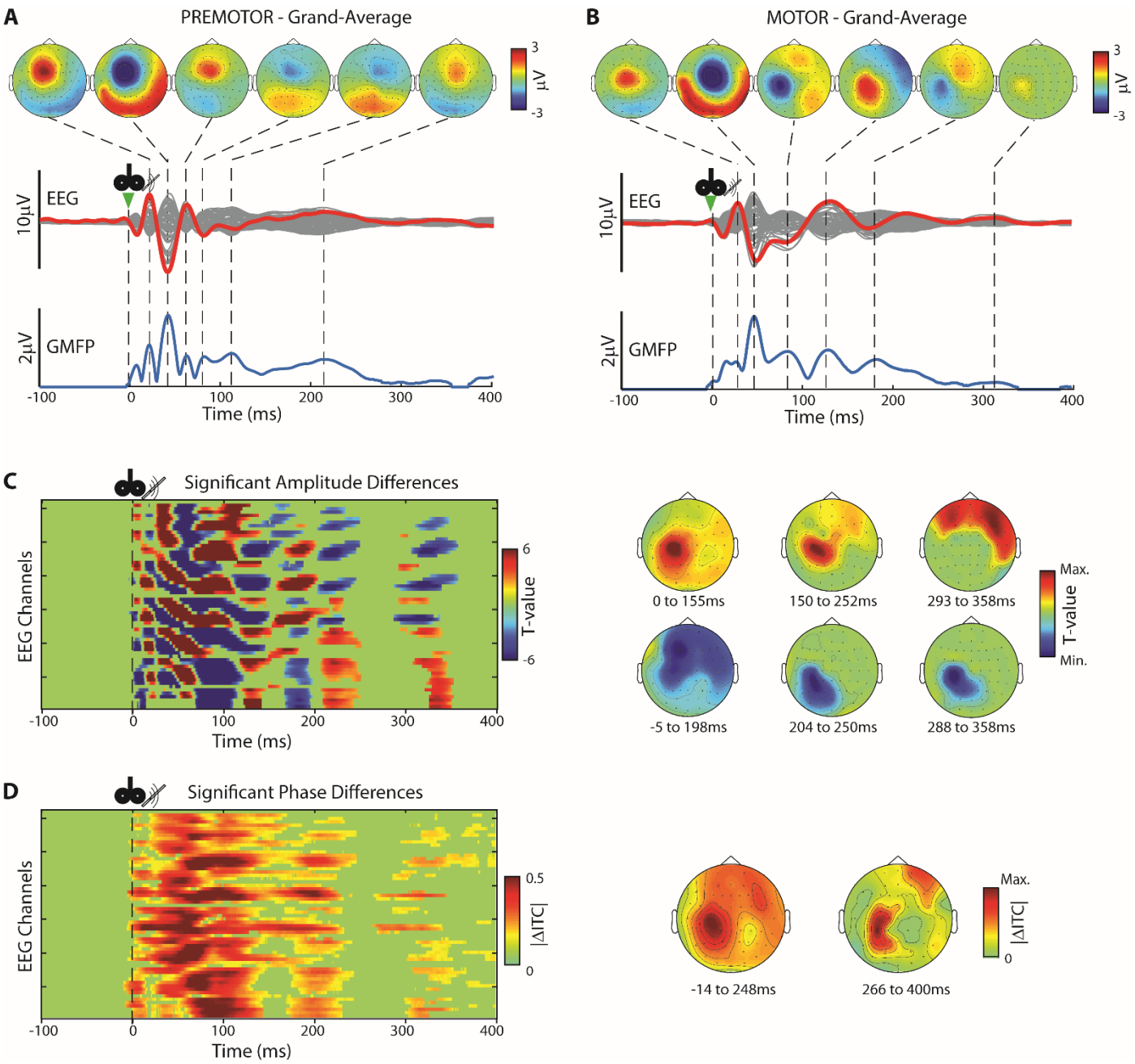

Figure 2. Grand average EEG responses to eTMS of premotor and motor sites differ significantly across multiple scalp regions and over long latency periods.

(A, B) TEPs averaged across subjects following stimulation of the premotor (A) and motor (B) cortices are shown. The butterfly plot (middle row) shows all EEG channels superimposed (gray), with the red trace highlighting the EEG response of the channel closest to the stimulation site (FC1 for premotor and C1 for motor). GMFP values were calculated from significant TEPs and are displayed at the bottom row (blue). The topographical scalp voltage distributions (top row) are presented at the times of the GMFP peaks (dashed vertical lines). **(C, D)** Cluster-based statistical tests performed on the grand average TEPs reveal significant differences in amplitude (C) and phase (D) between responses evoked by premotor and motor stimulation. On the left, heatmaps showing the statistical results (T-values for amplitude and absolute difference in inter-trial coherence for phase) of significant clusters (blue and red for panel C, red for panel D) across EEG channels and times are displayed. Average topographical distributions of individual clusters are exhibited on the right, along with the corresponding time intervals. Six clusters are identified from amplitude comparisons, extending up to 358 ms, and two clusters from phase comparisons, extending up to 400 ms post-stimulation.

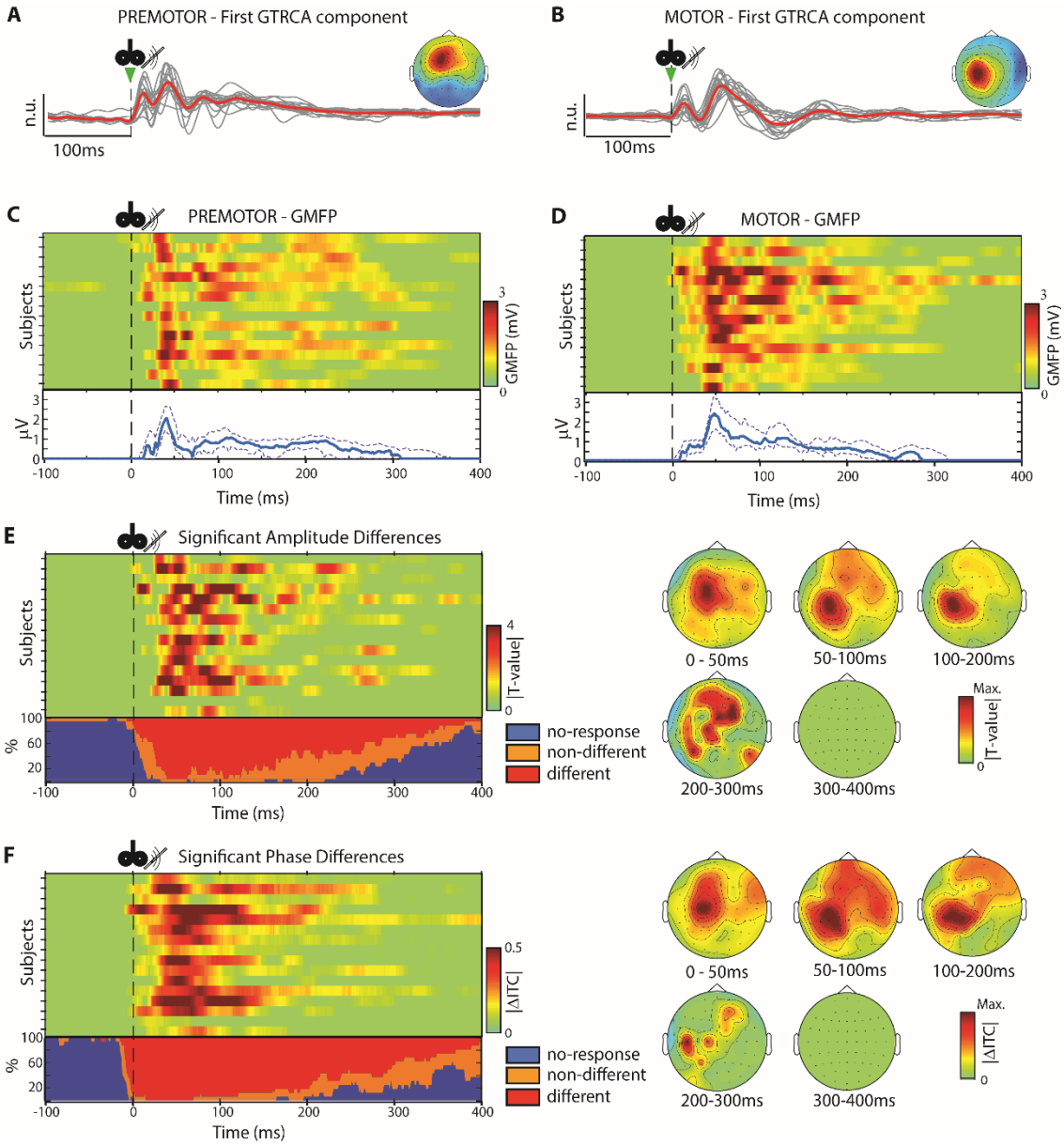

Figure 3. Significant differences in amplitude and phase between premotor and motor TEPs are confirmed both at the group and individual levels. (A, B). Waveforms of the most reproducible component at the group level, as extracted by gTRCA, for both premotor (A) and motor (B) TEPs, along with their average scalp maps representing the strength and direction of the component at each EEG channel. Gray lines are the principal gTRCA components of individual subjects, and the red line is the average component across subjects. **(C, D)** GMFP of significant TEPs evoked by premotor (C) and motor (D) stimulation for each subject (n=16). Solid blue traces represent median GMFP values, with dashed lines indicating interquartile ranges. **(E, F)** Results of the cluster-based statistical tests for each subject. Heatmaps show the time courses of average significant amplitude (E) and phase (F) differences between EEG responses to premotor and motor stimulation. Each line represents the average absolute T-values (E) and ITC differences (F) across significant clusters for one subject. The bottom plots illustrate, for each time sample, the percentage of subjects presenting evoked responses, in amplitude (E) and phase (F), that are significantly different between premotor and motor stimulation (red), evoked responses that are not significantly different between targets (orange), and the percentage of subjects with no evoked response (blue). Topographical distribution of absolute T-values (E) and ITC differences (F) for significant clusters (median across subjects), averaged

over different time windows (0-50 ms, 50-100 ms, 100-200 ms, 200-300 ms, 300-400 ms), are shown on the right.

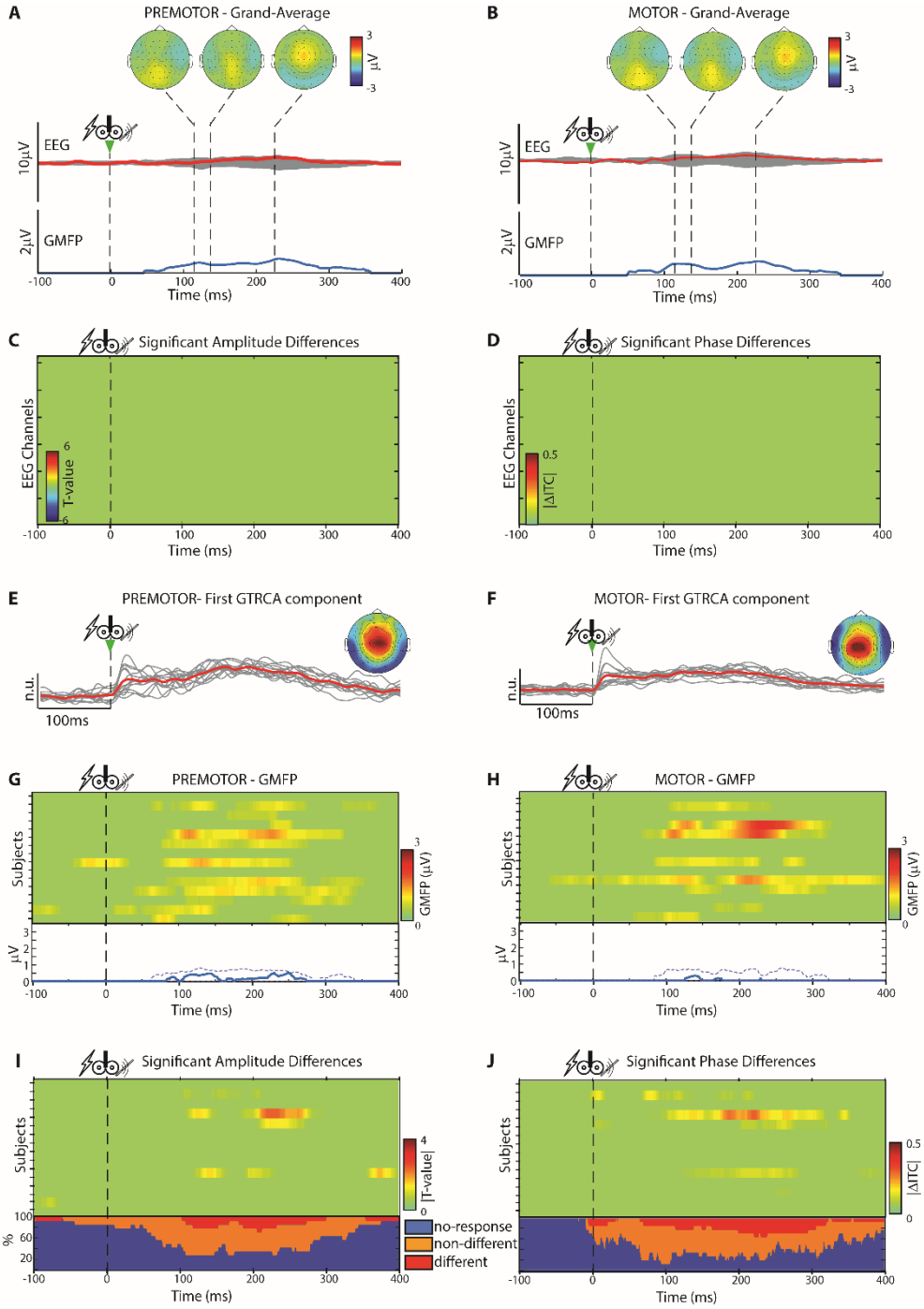

Figure 4. Realistic sham TMS evokes stereotypical EEG responses. This figure replicates the analyses from Figures 2 and 3 using EEG responses evoked by RS-TMS. **(A, B)** Grand-average evoked responses following RS-TMS for premotor (A) and motor (B) stimulation, including butterfly plots, highlighted channels, GMFP traces, and topographical maps at GMFP peaks, as in Figures 2A and 2B. **(C, D)** There are no significantly different clusters in either amplitude (C) or phase (D) when comparing grand-average EEG responses evoked by premotor and motor RS-TMS. **(E, F)** Waveforms of the principal gTRCA component, along with their average scalp maps, as in Figures 3A and 3B. (G, H) GMFP of significant evoked potentials for each subject (n=14), including median and interquartile GMFP values, as in Figures 3C and 3D. **(I, J)** Results of the cluster-based statistical tests for each subject, as in Figures 3E and 3F. Throughout all time points, only a minority of subjects (maximum 5 out of 14) show responses to RS-TMS that are significantly different between premotor and motor stimulation.

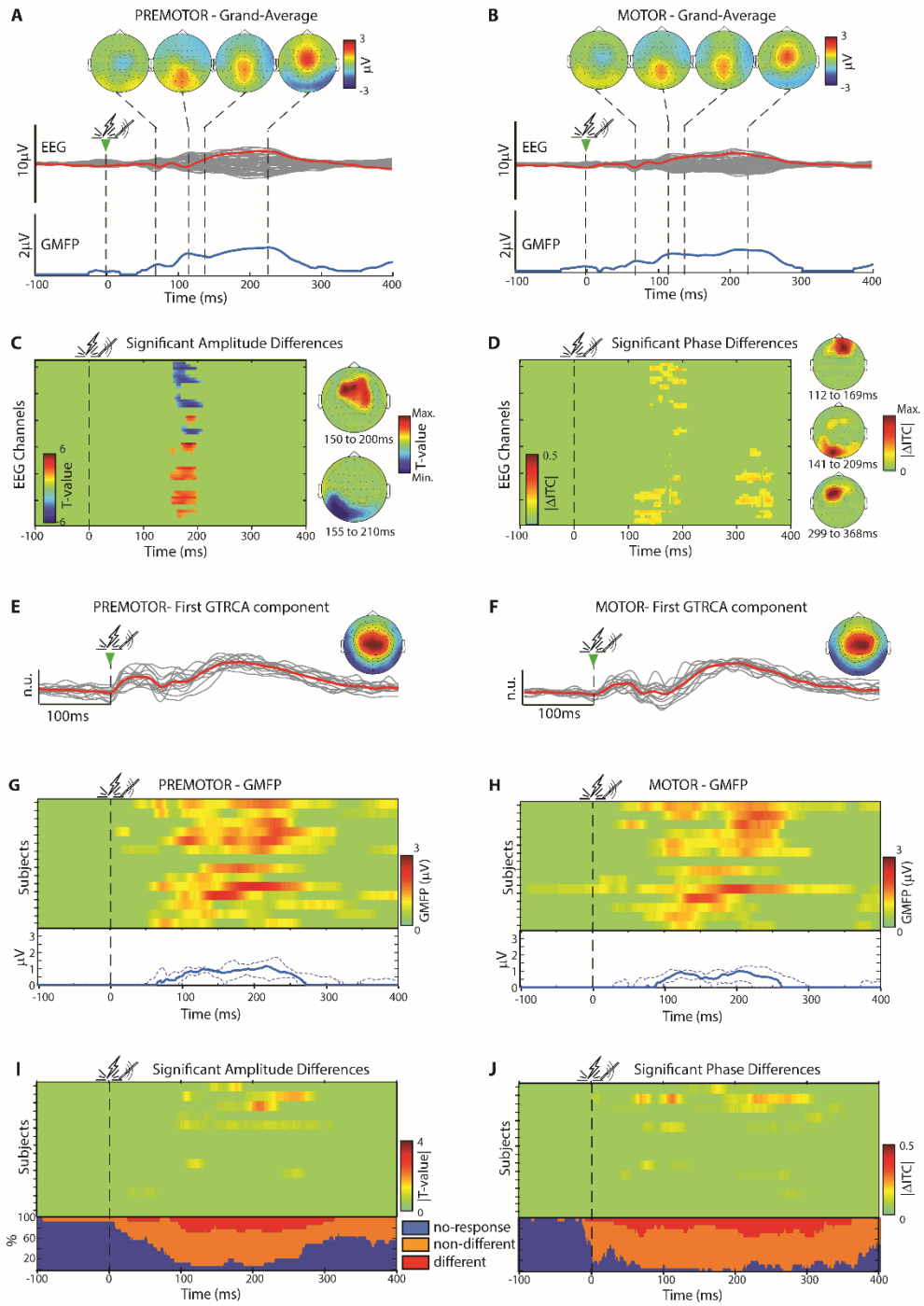

Figure 5. High-intensity electrical stimulation elicits only marginal amplitude and phase differences between premotor and motor stimulation. This figure shows the results of the same analyses from the previous figures using the responses evoked by HI-ES. **(A, B)** Grand-average evoked potentials for premotor (A) and motor (B) stimulation, including butterfly plots, highlighted channels, GMFP traces, and topographical maps at GMFP peaks, as in Figures 2A and 2B. **(C, D)** Two significantly different clusters were found in amplitude (C) and three in phase (D) when comparing grand-average EEG responses evoked by premotor and motor HI-ES. These panels show significant clusters' heatmaps and topographical distributions as in Figures 2C and 2D. **(E, F)** Waveforms of the principal gTRCA component evoked by HI-ES, along with their average scalp maps, as in Figures 3A and 3B. **(G, H)** GMFP of significant potentials evoked by HI-ES for each subject (n=14), including median and interquartile GMFP values, as in Figures 3C and 3D. **(I, J)** Results of the cluster-based statistical tests for each subject, as in Figures 3E and 3F. Similarly to RS-TMS (Figure 4), consistent differences between premotor and motor stimulation are observed in only a minority of subjects (a maximum of 5 out of 14; red area panels I and J).

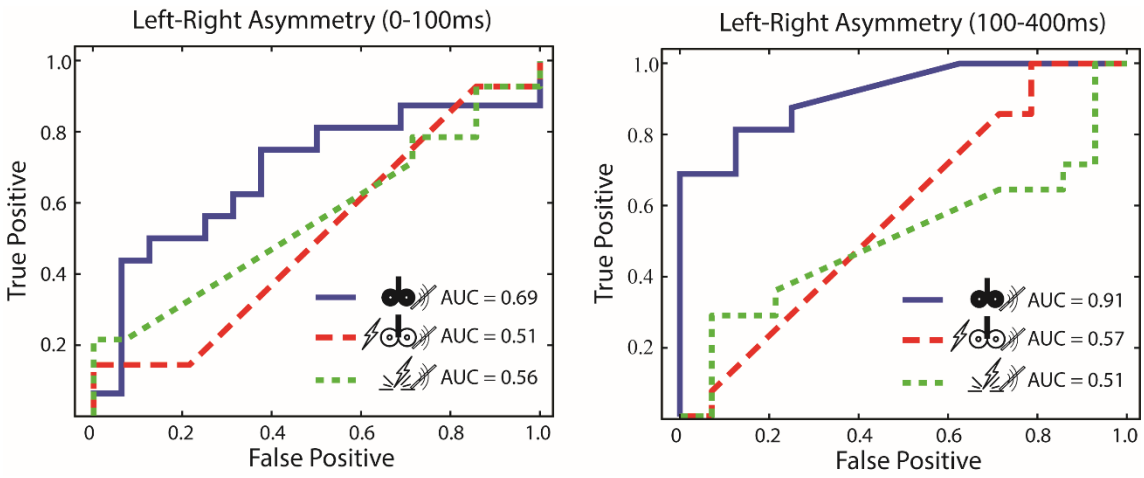

Figure 6. Hemispheric asymmetry of late evoked responses discriminates between motor and premotor stimulation in TEPs but not in peripheral evoked potentials. For each recording session, significant differences in the mean field power between left and right EEG channels were used to compute an asymmetry index representing the lateralization of the evoked response (Supplementary 12). This figure displays the results of the receiver operating characteristic (ROC) analysis for discriminating between premotor and motor evoked responses using the asymmetry index averaged across early (0-100 ms) and late latencies (from 100 to 400 ms). ROC curves are shown for eTMS (solid blue line), RS-TMS (dashed red line), and HI-ES (dashed green line), along with the corresponding values of the area under the curve (AUC).

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