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ORIGINAL PAPER

Single Pulse TMS-Induced Modulations of Resting Brain Neurodynamics Encoded in EEG Phase

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Abstract Integration of electroencephalographic (EEG) recordings and transcranial magnetic stimulation (TMS) provides a useful framework for quantifying stimulation-induced modulations of neural dynamics. Amplitude and frequency modulations by different TMS protocols have been previously investigated, but the study of stimulation-induced effects on EEG phase has been more limited. We examined changes in resting brain dynamics following single TMS pulses, focusing on measures in the phase domain, to assess their sensitivity to stimulation effects. We observed a significant, approximately global increase in EEG relative phase following prolonged (>20 min) single-pulse TMS. In addition, we estimated higher rates of phase fluctuation from the slope of estimated phase curves, and higher numbers of phase resetting intervals following TMS over motor cortex, particularly in frontal and centro-parietal/parietal channels. Phase changes were only significantly different from their pre-TMS values at the end of the stimulation session, which suggests that prolonged single-pulse TMS may result in cumulative changes in neural activity reflected in the phase of the EEG. This is a novel result, as prior studies have reported only transient stimulation-related effects in the amplitude and frequency domains following single-pulse TMS.

Keywords Transcranial magnetic stimulation · Electroencephalograpy brain dynamics · EEG phase

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Introduction

Transcranial magnetic stimulation (TMS) allows the delivery of a controlled, non-invasive input to the brain that can modify local brain activity and facilitate neural network adaptation. It can, therefore, be used in cognitive neuroscience, neurology and psychiatry, to assess non-invasively the recruitment, activation and coordination of cortical networks during a specific behavior, as well as disease-related modulations of these processes (Rossini et al. 2007; Walsh and Pascual-Leone 2003). Furthermore, TMS may have significant therapeutic potential in a wide range of disorders (Sokhadze et al. 2009; Fregni and Pascual-Leone 2007). However, in order to optimize its spatio-temporal application, quantitative measures of stimulation-induced neuromodulation are necessary. TMS pulses of sufficient intensity are presumed to depolarize neuronal ensembles in the targeted cortical volume and induce a burst-like activation of a large population of neurons followed by long-lasting depression, which can result in the functional disruption of information processing in the affected cortical region. In turn, this may result in the disruption of cognitive, sensory or

motor processes (Mariorenzi et al. 1991; Siebner et al. 2009; Silvanto et al. 2008). To date, robust quantitative measures of TMS effects on cortical networks are limited. The online integration of TMS and electroencephalography (EEG) is a relatively new approach (Miniussi and Thut 2010; Thut and Pascual-Leone 2010), and provides a useful framework for quantitatively assessing TMS-induced neuromodulations. EEG has superior temporal resolution in comparison to functional imaging, e.g., fMRI, and provides a highly quantitative approach for directly estimating dynamic effects of controlled stimulation on local and global brain dynamics. However, it is still unclear which EEG parameters vary robustly and specifically in response to TMS, and encode short- or long-term stimulation effects. Amplitude in the time domain, frequency and phase characterize a non-stationary signal, such as EEG, completely. Traditional time and frequency domain analyses have shown only transient single pulse TMS effects that do not persist following the end of the stimulation, and do not accumulate over time provided that single TMS pulses have an inter-stimulus interval of at least a few seconds (typically >5–10 s). TMS-EEG analysis in the phase domain has been relatively more limited but may provide insights into a different aspect of TMS-induced neuromodulation encoded in EEG.

We investigated the potential cumulative effect of single-pulse TMS, with an inter-pulse interval in the range 5–15 s, on multiple EEG phase parameters: (i) instantaneous wrapped and (ii) unwrapped phase (as well as the rate of phase accumulation from the slope of the latter), (iii) phase resetting and its frequency, i.e., length and frequency intervals of zero unwrapped phase slope, and (iv) relative phase as a measure of synchronization between brain regions. We studied 8 young healthy adults and recorded EEG continuously prior to, during and following single-pulse TMS, for approximately 60 min. Although no long-term stimulation effects have been previously reported for this stimulation protocol, we observed cumulative effects on phase parameters of individual EEG channels following ~25–30 min of single-pulse TMS (~50–80 TMS pulses). We also observed an almost global increase in relative phase between channels, which appeared to be randomly distributed in space. In contrast, relative phase prior to TMS had a clear spatial structure with lower phase differences in parietal and occipital channels and higher relative phase in frontal and central channels.

Materials and Methods

Experimental Protocol, Data Collection and Pre-Processing

All data were collected at the Berenson Allen Center for Noninvasive Brain Stimulation and the Harvard-Thorndike Clinical Research Center at Beth Israel Deaconess Medical Center. Scalp EEG data from eight healthy subjects (four male and four female), age 20–25 years ($\mu = 21.8$, $\sigma = 2.4$), undergoing single-pulse TMS were analyzed. All subjects were healthy, right-handed, with a normal neurological exam, and no chronic medications. Subjects were seated in a comfortable chair with the elbow flexed at ~90°. Single, pseudo-randomly timed monopolar TMS pulses in with an inter-pulse interval of 5–15 s were delivered over left primary motor cortex (M1). Pulse intensity was at 120% of active motor threshold (AMT). The optimal scalp location for activation of the right abductor pollicis brevis (APB) muscle was determined as the location from which TMS-induced motor evoked potentials (MEPs) of maximum amplitude were measured in this muscle. Motor threshold intensity was determined according to the recommendation of the International Federation for Clinical Neurophysiology (Rossini 1994). A Magstim system, with a figure eight 70 mm coil (max magnetic field strength of 2.2 T was used (Kobayashi et al. 2009). Data were collected with a 32-channel system in the 10–10 configuration, and 1000 Hz sampling frequency. Electrode impedance was <5 kΩ. The TMS-EEG protocol was as follows: EEGs were recorded continuously for approximately 60 min (30 min pre-TMS delivery and 30 min during and following stimulation). Baseline EEG were first recorded for ~1–2 min ($\mu = 97.8$ s, $\sigma = 34.1$ s) where subjects were instructed to keep their eyes either closed (~30 s) or open (~30 s). In some subjects this eyes closed/open sequence was repeated twice. These baseline EEGs were the first segments of interest in this analysis. Following that, subjects were instructed to perform a visual task (involving image presentation) and a visually-guided motor task (keyboard key pressing). The details of these tasks are beyond the scope of this study which focuses on resting EEG. Between the two tasks and following their completion, resting EEGs were recorded (approximately 1–5 min long, $\mu = 181.8$ s, $\sigma = 117.7$ s across subjects, typically with eyes open). These segments were also analyzed, to assess the inter-interval resting EEG variability for each subject. Corresponding segments following 30–40 single TMS pulses under each baseline condition (eyes closed/open) and task completion under stimulation were also analyzed in the phase domain. Unwrapped phase may be thought of as a measure of phase accumulation, and therefore depends on the length of the signal from which it is estimated. Instead of maximum phase accumulation, we were instead specifically interested in the slope of unwrapped phase, a measure of the rate of signal fluctuation. Signals of different lengths may have the same phase slopes. We normalized signals by their lengths to facilitate interpretation of the results. All analysis was done using the software Matlab (Mathworks, Natick MA).

The 60 Hz powerline noise and its harmonics typically seen in EEG signals was suppressed in all data, using a second order elliptical stopband filter with 1.5 Hz bandwidth. The data were filtered in both directions to eliminate potential phase shifts associated with the non-linear phase of the filter. The high amplitude artifact associated with the application of TMS was removed by explicitly modeling the TMS input, as described in (Stamoulis and Chang 2009a, b). Muscle and eye blinking-related artifacts were suppressed using matched-filtering, as described in (Stamoulis et al. 2009a, b).

EEG Analysis

We investigated the following instantaneous phase parameters:

Wrapped phase $\phi(t)$ in the range $[-\pi, \pi]$, provides a measure of the instantaneous direction of a waveform/oscillation.

Relative phase between two oscillators i and j may be defined as the difference $|\phi_i(t) - \phi_j(t)|$, and is often used to quantify the coupling (or lack of) between pairs of oscillators. In the context of EEG analysis, relative phase may be used to quantify correlations between signals and consequently potential (de)synchronizations between brain areas (Breakspear 2002, Frankel and Kiemel 1993, Freeman 2004). Pairs of EEG electrodes are assumed to be phase synchronized if their phase difference does not increase with time, i.e., $|\phi_1(t) - \phi_2(t)| \approx K$, where K is a constant.

Unwrapped phase, which involves expanding phase beyond the $[-\pi, \pi]$ range, to include multiples of 2π, may be thought of as a measure of total phase accumulation in a signal and its slope provides a measure of the rate of phase fluctuation. For example, rapid fluctuations in phase may reflect a particular modulation of otherwise slowly-varying dynamics of the process measured by the signal of interest. Thus, potential changes in phase dynamics associated with TMS, including increased rate of oscillation or overall increase in noise levels in the brain, may be captured by this parameter.

Phase resetting refers to an interval of constant unwrapped phase (with approximately zero slope), and is thought to be potentially associated with a transition from one dynamic state to another. To estimate all these parameters, instantaneous phase was calculated from the analytic EEG signals obtained using the Hilbert transform (HT), which transforms a real-valued signal $x(t)$ into an analytical (complex) signal $z(t)$ (Cohen 1995, Freeman 2004). Phase is then the argument of this signal, i.e., $\phi(t) = \arg(z(t)) = tan^{-1}(\frac{Im(z(t))}{Re(z(t))})$.

Results

We first compared unwrapped baseline and resting phase prior to TMS stimulation for several segments per subject, to assess its variability, as well as phase at the end of the stimulation session (as well as in resting EEG segments between stimulation epochs and/or task completion). A representative example of unwrapped EEG phase and its variability in all EEG channels, for one subject, is shown in Fig. 1. Channels are grouped as frontal/fronto-central, central/centro-parietal/parietal, temporal and occipital. Left-side plots correspond to the eyes-open condition, and right-side plots to eyes-closed.

There is differential phase accumulation across EEG channels. Specifically, the phase rate of centro-parietal channels appears higher than that of other channels. These results appear to be consistent across subjects, as shown in Fig. 2. In addition, temporal and occipital channels have the lowest instantaneous phase fluctuations and consequently phase accumulation over the time interval of interest. In

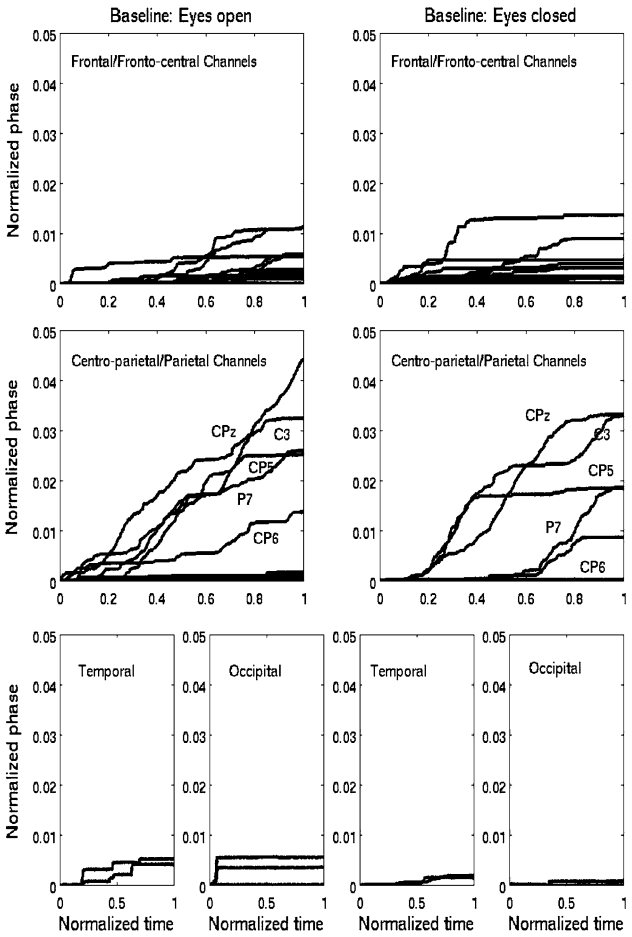


Fig. 1 Representative example of the variability of unwrapped phase across EEGs in one baseline segment (EC *open*, *left-side plots*) and a second baseline segment (EC *closed*, *right-side plots*)

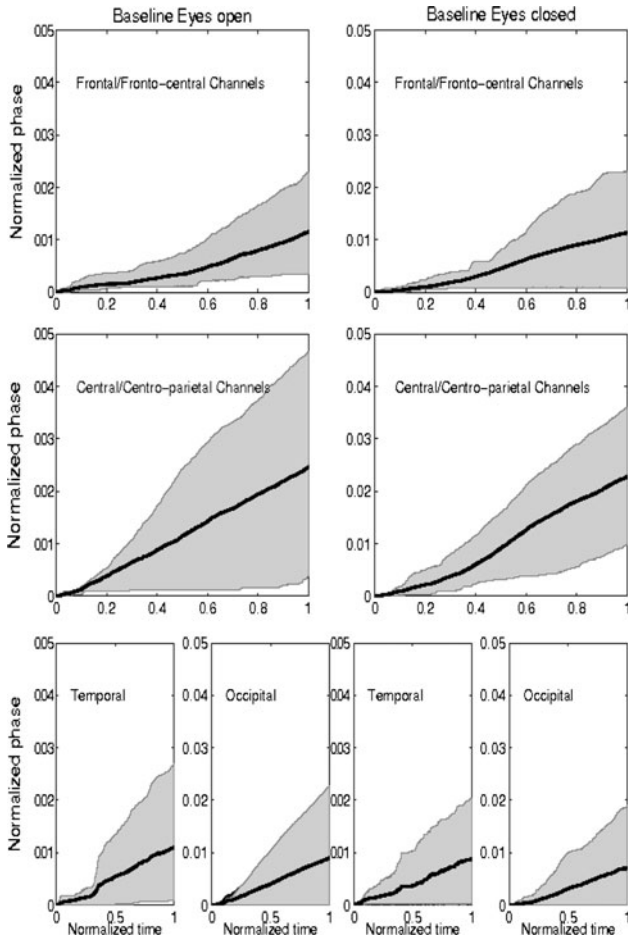


Fig. 2 Mean baseline unwrapped phase (*solid line*, averaged across channels within each channel group and across subjects), and superimposed inter-subject variability. *Left plots* correspond to eyes *open* and *right plots* to eyes *closed*

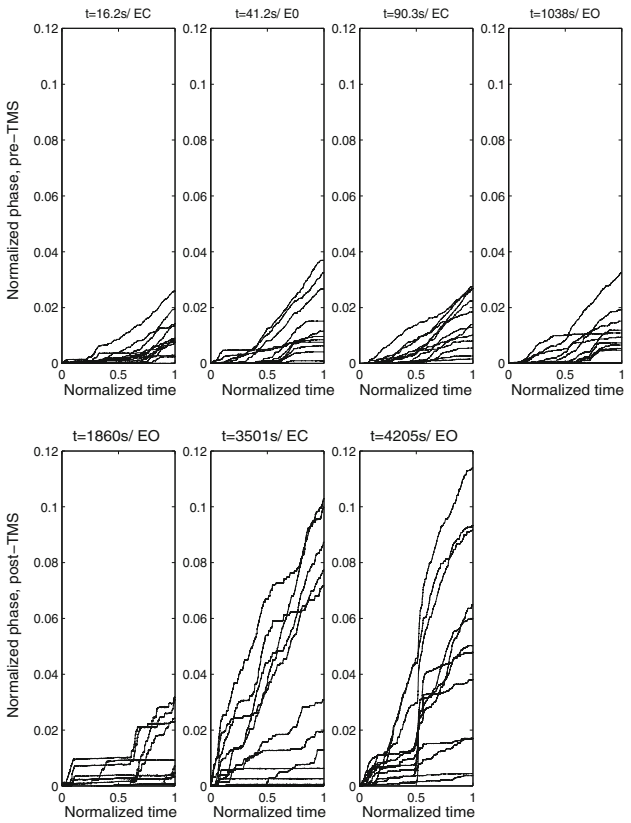


Fig. 3 Phase variability in central/centro-parietal channels prior to TMS (*top plots*) for four different segments and post TMS (*lower plots*) for three segments, at times $t = 0$ s, $t = 41$ s, $t = 90$ s, $t = 1038$ s, $t = 1860$ s, $t = 3501$ s and $t = 4205$ s

addition, occipital channels have the lowest number of phase resettings, i.e., epochs of constant phase (zero phase rate) which may potentially correspond to dynamic state transitions in the brain. We investigated the temporal statistics of these transitions separately. Note that since the occurrence of phase resettings vary between subjects, and are typically not temporally aligned, averaging over subjects eliminates subject-specific phase transitions. EEG is highly variable and despite insignificant changes in underlying neurodynamics, both varying noise levels and potentially random fluctuations in inter-channel synchronization may affect EEG phase. However, in the absence of significantly noisier signals, the overall phase rate (slope of the unwrapped phase curve) may be robust to that variability, as shown in Figs. 2 and 4.

We examined several pre- and post-stimulation segments for each subject, under both eyes-closed and eyes-open resting conditions. A representative example of unwrapped phase at four segments before TMS and 3 segments between TMS applications and following the completion of the stimulation session, for one subject, specifically in central/centro-parietal channels, is shown in Fig. 3. In this example we focused on this particular subset of channels as they often showed the highest rate of phase accumulation, pre- and post-TMS (see Fig. 1), consistently across subjects. We also examined the inter-subject variability of pre- and post-TMS phase fluctuations across subjects, which are shown in Fig. 4.

There is insignificant variability in phase accumulation and rate ($p > 0.3$) at different times prior to TMS and at the beginning of the TMS session (t = 1860 s, measured from the start of the recording session corresponds approximately to the first few minutes of TMS single-pulse stimulation). There is significant ($p < 0.001$), bilateral increase in phase accumulation and rate in a subset of central/centro-parietal channels in the last $\sim$11 min of stimulation (lower middle and right plots correspond to EEGs approximately 11 min apart), suggesting a possible cumulative effect of TMS on instantaneous phase fluctuations. The stimulation session was on average 25–27 min long.

Similar effects were estimated across subjects, with insignificant inter-segment changes in unwrapped phase

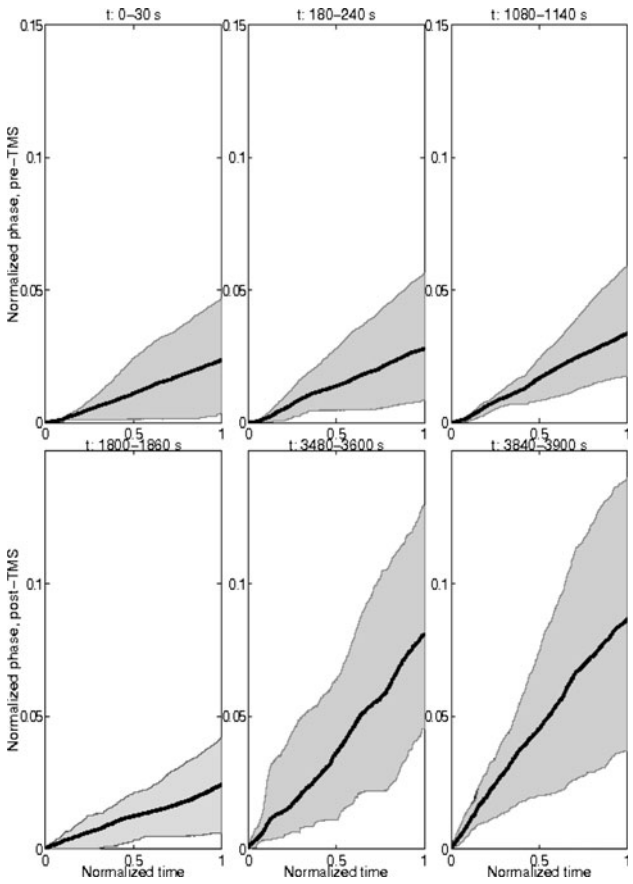


Fig. 4 Inter-subject variability of unwrapped phase in central/centro-parietal channels prior to TMS (*top plots*) for three different segments and post TMS (*lower plots*) for three segments. *Solid lines* correspond phase averaged over all subjects and the *shaded areas* represent the superimposed min/max variability

prior to TMS and the beginning of stimulation and significant differences in phase slope ($p < 0.0001$) after prolonged TMS. Figures 3 and 4 focus only on channels in central/centro-parietal regions. We also investigated the rate of phase fluctuation, and thus the slope of unwrapped phase, across channels. For this purpose we fitted first order regression models to each EEG phase curve, in order to estimate their overall slope, i.e., without taking into account changes in slope following phase resetting, as these changes were in general small (<10°). Intra-subject variability was assessed based on all analyzed EEG segments, i.e., independently of eyes open/closed conditions, since only small changes in phase parameters were observed between the two conditions. The results are summarized in Fig. 5, for all segments per subject (left plot) and all subjects (right plot). The left-side plot shows mean *intra-segment* phase rate (slope) pre- and post-TMS and its variability across segments. The right-side plot shows the corresponding mean *inter-subject* phase rate and its variability across subjects.

Despite the localized application of TMS over the optimal scalp location for induction of motor potentials in the contralateral APB, phase changes occurred across large areas of the brain. Specifically, following prolonged single-pulse TMS, we observed an increase in instantaneous phase fluctuations across subjects, with highest phase rate increase in fronto-central and centro-parietal/parietal regions, as shown in Fig. 5. Although phase slope variability is small across channels prior to TMS (black curves), there is a very large increase in phase slope in FC, P, and CP channel subsets following TMS. This implies that there are more rapid phase fluctuations in corresponding brain regions following stimulation over motor cortex.

In addition to phase rate we also examined intervals of approximately constant phase, or phase resetting, which are in general thought to be associated with dynamic state changes in a system. Spontaneous state changes at baseline have been observed in previous studies (Breakspear 2002, Freeman 2004). State changes may be random and highly variable between baseline EEGs, even for the same subject. We examined the distributions of both the timing and duration of these constant phase transitions within and across subjects. These intervals also corresponded to rapid (wrapped) phase reversals ($\pm\pi$), as shown in the example in Fig. 6, for one EEG segment from one subject.

The onset time for each phase resetting was used as the time marker for estimating their frequency in each channel and EEG segment, and characterizing them statistically. The probability distribution functions (pdf) of the frequency of phase resettings prior to and following TMS, and their corresponding variability across EEG segments for all subjects are summarized in Fig. 7a. The solid curves represent the pdfs for the frequency phase transitions averaged over all corresponding pre- or post-TMS segments and subjects, and dotted lines represent the inter-segments variability of these distributions. Pdfs were estimated non-parametrically by using a Gaussian kernel (Parzen 1962). An example showing the difference in the frequency of occurrence of phase resettings pre- and post-TMS is shown in Fig. 7b.

A statistically significant increase ($p < 0.001$) in the number of estimated phase resetting intervals was observed in EEG segments at the end of the TMS session, as shown in Fig. 7a. On average 2–6 resettings were estimated prior to TMS, with higher frequency in frontal and central channels and lower frequency in occipital and temporal channels. In contrast, a high number of much shorter phase-resettings (on average 8–20) were observed in post-TMS EEGs, with a higher frequency in frontal and centro-parietal/parietal channels. The number of phase resettings did not significantly change in baseline EEGs at the beginning of the stimulation, indicating that these transitions may result from a cumulative stimulation effect.

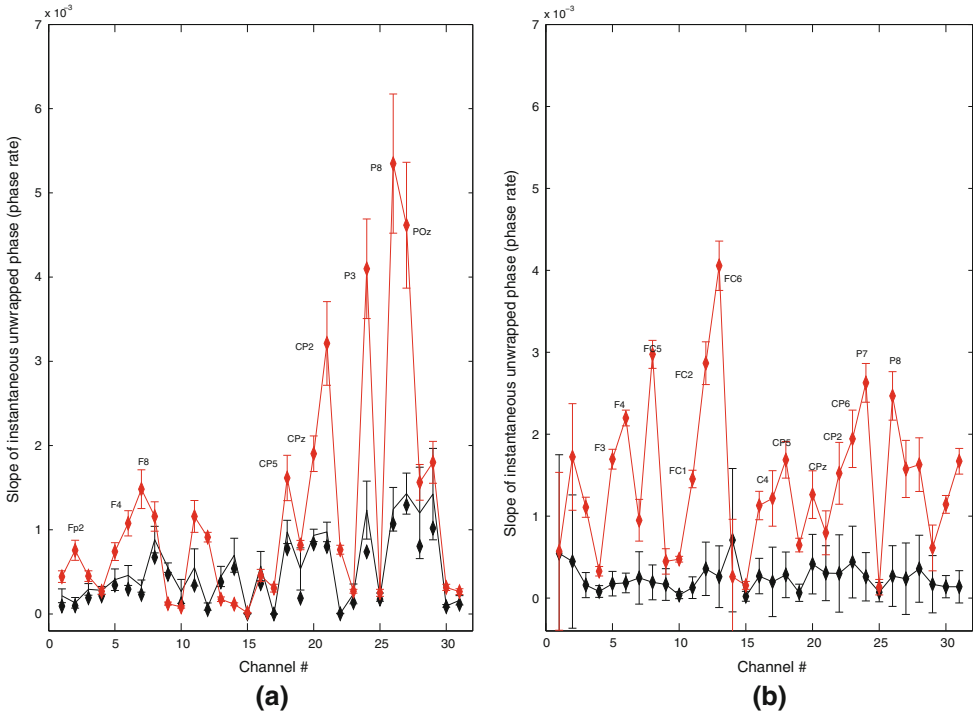


Fig. 5 Instantaneous phase rate pre-stimulation (*black*) and post-stimulation (*red*). **a** Intra-subject phase slope variability pre-TMS (*black*) and post-TMS (*red*). **b** Inter-subject (averaged over baseline segments) phase slope and its variability (Color figure online)

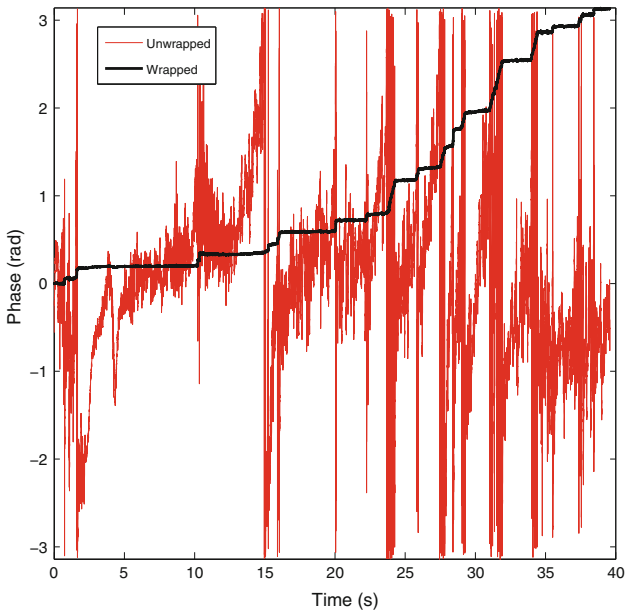


Fig. 6 Example of unwrapped phase (*black*) and superimposed wrapped instantaneous phase (*red*) of one EEG channel (Color figure online)

We finally examined *relative phase* as a measure of synchronization between EEG signals from distinct brain areas. Phase synchrony has been investigated in a number of previous studies as a mode of reciprocal interaction between neuronal ensembles, but much less in studies involving TMS. Overall, we observed a spatially diffused and random (approximately global) relative phase increase in EEGs at the end of the stimulation session, but not at the beginning. In contrast, baseline and resting pre-stimulation EEGs had an identifiable spatial structure with higher relative phase in frontal and central regions but lower relative phase in parietal, occipital (bilateral) and centro-parietal/ fronto-central regions (unilaterally). Relative phase between EEGs, in the range $[-\pi, \pi]$, is shown in Fig. 8, at baseline prior to TMS (top plot), at the end of the first 5 min of TMS (middle plot) and at the end of the stimulation session (bottom plot). These results represent an average phase over time within the segment, and then averaged over all subjects, where the average was taken over all all subjects for EEG segments at the three time points, i.e., beginning of the entire recording session (baseline), $\sim$5 min following the first TMS pulse, and right after its completion. Note that relative phase is shown and thus the phase matrix is anti-symmetric with $\Delta\phi_{i,j} = -\Delta\phi_{j,i}$.

We focus on either the lower or upper-triangular parts of the phase matrix, given the anti-symmetry of the matrix. Mean relative phase prior to TMS was on average statistically identical to relative phase after the first few minutes of stimulation. In contrast, almost spatially global and statistically significant ($p < 0.001$) increase in relative phase was observed in all EEGs approximately 22–30 min after the beginning of the stimulation, indicating a cumulative decorrelation effect, presumably due to prolonged single-pulse TMS. Note that relative phase increased

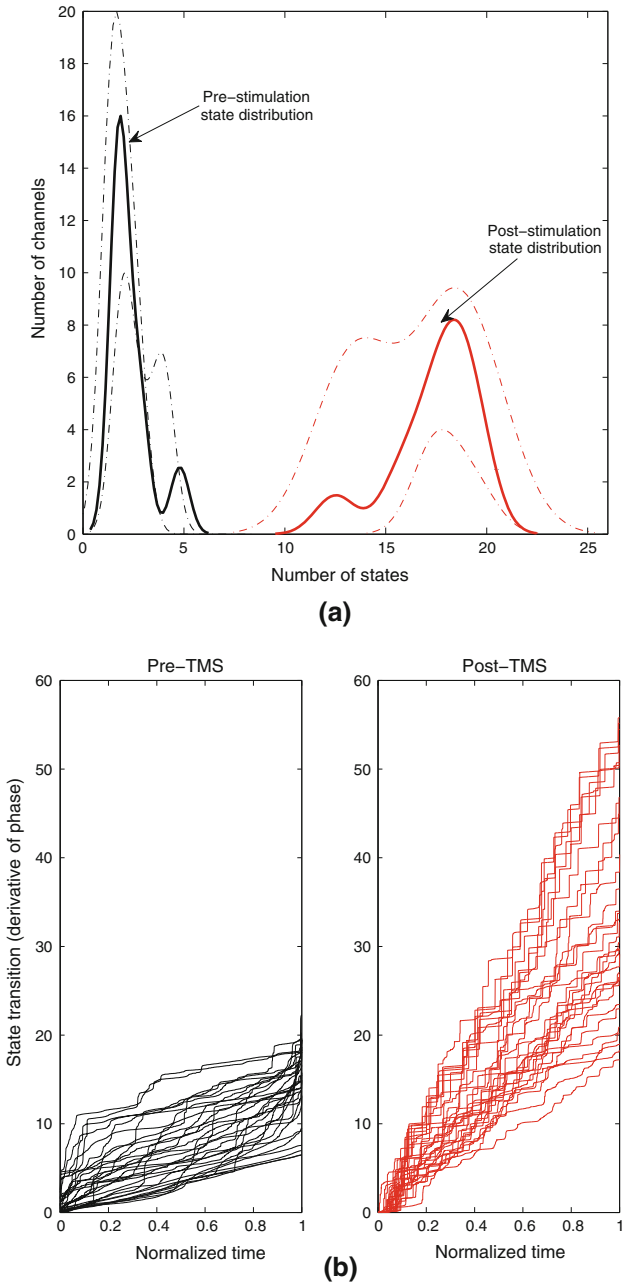


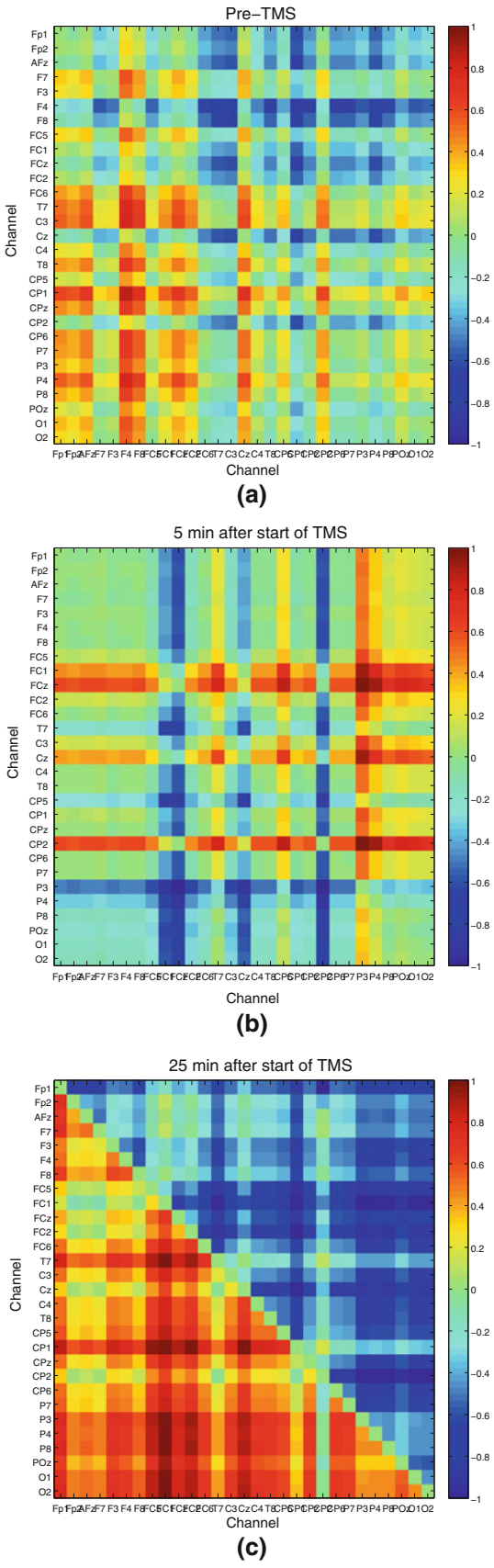
Fig. 7 Statistics of phase resetting. **a** Distribution of phase resettings estimated from unwrapped phase, prior to (*black*) and following TMS (*red*). *Dotted lines* denote max, min variability of the distribution. **b** Example of the frequency of phase resetting prior to (*left plot*) and following TMS (*right plot*). All channels are superimposed (Color figure online)

almost uniformly across channels, though fronto-central/central and parietal channels had slightly higher relative phase changes. Other variations appeared random. Finally, although prior to or at the beginning of the TMS session clusters of channels had either positive or negative relative phases, indicating spatial correlation/synchrony or de-correlation, at the end of the TMS session, almost all channels had positive relative phases.

Discussion

We have investigated the sensitivity of EEG phase parameters in response to prolonged single-pulse TMS, with variable inter-stimulus interval in the range 5–15s. The study involved combination of TMS and EEGs with the goal to quantify stimulation-induced changes in the dynamics of the resting brain. Specifically, we examined single channel wrapped and unwrapped phase, phase rate as a measure of dynamic signal fluctuation, phase resetting as a potential measure of transition between dynamic brain states, encoded in the EEG, and relative phase as a measure of inter-channel synchronization. We have found that changes in these parameters following prolonged single-pulse TMS are quantifiable in the EEG and may reflect cumulative stimulation-induced effects rather than random dynamic EEG fluctuations. Increased phase variability following long-term single-pulse TMS suggests that stimulation may transiently increase the flexibility or spatial de-coupling of the brain. Decoupled baseline oscillations are an intrinsic property of the healthy brain, possibly reflecting the brain's ability to adapt to novel inputs and selectively synchronizing specific networks. Another possible interpretation is that TMS increases the overall high-frequency noise levels in the brain, resulting in increased phase accumulation and phase differences, i.e., signal decorrelations (de-coupling). Both potential mechanisms have important implications for the optimization of the spatio-temporal application of TMS.

A significant increase in transient phase resettings was also observed following TMS, possibly reflecting the ability of this stimulation protocol to induce transient changes in dynamic brain states. Although it is unclear from this analysis whether these phase jumps indeed correspond to state changes, and whether the latter are dynamically stable or unstable, these results provide evidence that single-pulse TMS cumulatively modulates some aspect of the neurodynamics of brain encoded in the EEG. Phase rate and frequency of phase resetting were higher in frontal and centro-parietal/parietal channels. We also estimated relative phase prior to and following TMS. Although relative phase appeared to have a clearly identifiable spatial structure both prior to and in the first few minutes of stimulation, with higher phase differences (lower correlation) in frontal and centro-parietal channels, a global, spatially non-specific increase in relative phase occurred in all subjects following TMS. The underlying mechanism of this change is unclear. The multi-sensory effects of TMS may contribute to these distributed effects. Propagation via cortico-cortical or subcortical connections may be one of the mechanisms that facilitates spreading of TMS effects in large areas of the brain. Regardless of the exact mechanism, increased (cumulative) desynchronization between



◀ **Fig. 8** Relative phase prior to and following TMS. Intensity levels are in radiants. **a** Max. relative phase: pre-TMS. **b** Max. relative phase at the end of 5 min of single-pulse TMS. **c** Max. relative phase at the end of 25 min of single-pulse TMS

brain regions at rest using prolonged single-pulse TMS may have important therapeutic implications, e.g., in epilepsy or in neuro-developmental disorders possibly associated with abnormal hyper-synchrony of the resting brain.

In summary, we have presented novel results of cumulative effects of single-pulse TMS, at least in terms of phase changes in the EEG, and in a small number of healthy subjects. Although the exact mechanism of phase modulation by TMS is unclear, we have presented evidence that such modulation exists and results from the prolonged but not short term application of single-pulse TMS. Although we assume that the observed effects of prolonged single-pulse TMS are due to the impact of stimulation on the brain, we cannot rule out that the observed effects may be induced by non-specific, extra-cranial effects of TMS. Furthermore, it is possible that the observed EEG phase modulations following TMS and task completion may, in part, be due to the task, and thus associated with the active brain (rather than the resting brain). However, note that phase was also estimated following task completion in the control condition, i.e., without stimulation (for example, see Fig. 3, panel 3 of the top plots). Estimated phase fluctuations in the active, but not stimulated brain were not statistically different from those at baseline. Therefore, the task alone did not induce the observed phase changes, though a complex interaction between TMS and the preceding task cannot be ruled out. In addition, our design protocol did not include suitable experiments to assess and control for possible effects associated with (1) loud (often > 120 dB) clicking sound due to copper winding within the TMS coil and (2) startle effects resulting in increased, multi-sensory effects and corresponding brain activations, not directly associated with the stimulation, and other possible effects of TMS. The loud clicking sound may cause activation of auditory cortex, in addition to TMS-induced activations and needs to be taken into account, particularly in studies of stimulation-related modulations of neurodynamics across the entire brain. A simple sham control study would involve recreating the clicking sound and measuring resulting cortical activation with EEG. Similarly, the effect of startle on cortical activation may also be assessed using a sham experiment. Therefore, in addition to providing initial results on potential cumulative modulations of brain neurodynamics by single-pulse TMS, this study also highlights necessary modifications to the design of TMS studies, to include experiments to assess secondary effects unrelated to the stimulation and

subsequently control for these effects, in order to quantify true stimulation-induced modulations of brain dynamics. The effects of fatigue on these parameters may also be assessed through additional experiments. Although fatigue is another plausible mechanism of distributed changes in EEG parameters, we would also expect to observe changes in the frequency content of the EEG, which were not apparent in these recordings. Finally, validation of these results in a larger study is important, and potential correlation between such cumulative effects with behavioral changes may have important implications on the choice of stimulation protocol. Prolonged single-pulse TMS may be safer than repetitive TMS (inter-stimulus interval of <10 s), including theta-burst stimulation (TBS), and may thus be in some cases appropriate for therapeutic purposes.

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