BRIEF REPORT

REVISED Local brain-state dependency of effective connectivity: a pilot TMS–EEG study [version 2; peer review: 2 approved]

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v2 **First published:** 07 Apr 2022, **2**:45
https://doi.org/10.12688/openreseurope.14634.1
Latest published: 11 Jul 2022, **2**:45
https://doi.org/10.12688/openreseurope.14634.2

Abstract

Background: Spontaneous cortical oscillations have been shown to modulate cortical responses to transcranial magnetic stimulation (TMS). However, whether these oscillations influence cortical effective connectivity is largely unknown. We conducted a pilot study to set the basis for addressing how spontaneous oscillations affect cortical effective connectivity measured through TMS-evoked potentials (TEPs).
Methods: We applied TMS to the left primary motor cortex and right pre-supplementary motor area of three subjects while recording EEG. We classified trials off-line into positive- and negative-phase classes according to the mu and beta rhythms. We calculated differences in the global mean-field amplitude (GMFA) and compared the cortical spreading of the TMS-evoked activity between the two classes.
Results: Phase affected the GMFA in four out of 12 datasets (3 subjects × 2 stimulation sites × 2 frequency bands). Two of the observed significant intervals were before 50 ms, two between 50 and 100 ms, and one after 100 ms post-stimulus. Source estimates showed complex spatial differences between the classes in the cortical spreading of the TMS-evoked activity.
Conclusions: TMS-evoked effective connectivity seems to depend on the phase of local cortical oscillations at the stimulated site. This work paves the way to design future closed-loop stimulation paradigms.

Keywords

Transcranial magnetic stimulation; electroencephalography; brain state; effective connectivity

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 11 Jul 2022	✓ view	✓ view
version 1 07 Apr 2022	✗ view	✗ view

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Competing interests: P.L. has received consulting fees (unrelated to this work) from Nexstim Plc. R.J.I. is a minority shareholder of Nexstim Plc. The other authors declare no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Grant information: This project has received funding from the European Research Council under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 810377; and 945539 to M.R.); the Academy of Finland (Decisions No. 294625, 321631 and 327326); Jane and Aatos Erkko Foundation, the Finnish Cultural Foundation, and the Instrumentarium Science Foundation; by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), Project ERAPERMED2019-101, GA 779282 (to M.R.), and from the Tiny Blue Dot Foundation (to M.R. and M.F.).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Granö I, Mutanen TP, Tervo A *et al.* **Local brain-state dependency of effective connectivity: a pilot TMS–EEG study [version 2; peer review: 2 approved]** Open Research Europe 2022, **2**:45 https://doi.org/10.12688/openreseurope.14634.2

First published: 07 Apr 2022, **2**:45 https://doi.org/10.12688/openreseurope.14634.1

REVISED **Amendments from Version 1**

In this newer version, we have clarified further the reasons behind our chosen methodology for choosing our cortical targets, the stimulation intensity and the statistics to support our findings. We have also added some explanation and discussion about the small amount of subjects, which although may be small, it can be sufficient for demonstrating the influence of ongoing oscillations on effective connectivity as a possible effect.

Any further responses from the reviewers can be found at the end of the article

Introduction

The state of the brain affects the efficacy of transcranial magnetic stimulation (TMS;[1–10]) in eliciting cortical responses, such as those observed by means of TMS combined with electroencephalography (TMS–EEG). For instance, TMS–EEG can reveal effective connectivity patterns depending on sleep stage or deep sedation[1,4]. Noting that EEG signals provide a measure of brain state (projection of post-synaptic currents;[11,12]), we focus on the phase of oscillatory signals that reflect the local brain state and its impact on effective connectivity patterns.

Moreover, pre-stimulus oscillations can modulate TMS-evoked potentials (TEPs)[3,9,13,14], and if not accounted for, within-subject variability may mask meaningful changes in reactivity and measures of connectivity[15]. To address these challenges, brain-state-dependent and closed-loop stimulation paradigms are being developed[16–26]. To benefit fully from these novel techniques, we need to understand the basic mechanisms through which oscillations modulate cortical effective connectivity.

Both mu and beta rhythms (8–13 Hz, 13–30 Hz, respectively) in the frontal lobe can modulate TMS cortical and corticospinal responses[9,13,27–31]. In this preliminary work, we investigate the role of the phase of these two rhythms in effective connectivity when stimulating the left primary motor cortex (M1) and the right pre-supplementary motor areas (pre-SMA). As an indicator of effective connectivity, we investigate TMS-induced signal propagation, *i.e.*, the spatio-spectral patterns of TMS-evoked activity spreading across the cortex.

Methods

Data acquisition

Three healthy right-handed volunteer subjects (S1, female, 28 years old; S2, male, 41; S3, male, 43) were recruited. The Coordinating Ethics Committee of Helsinki University Hospital approved the study, and all subjects signed a written informed consent. During the experiment, the subject sat in a comfortable chair, fixating on a black cross 3 m away. To prevent the perception of the click sound produced by the TMS pulse, the subject wore earmuffs[4,32–34] over in-ear earphones that continuously played white noise combined with random bursts of recorded TMS click sounds[35].

Biphasic TMS pulses were delivered through a figure-of-eight coil (70-mm radius; Cooled Coil, Nexstim Plc, Finland) connected to a Nexstim NBS 4.3 eXimia stimulator. Coil positioning was guided by neuronavigation software (Nexstim) based on the individual's T1-weighted magnetic resonance images (MRI). EEG signals were recorded with 60 Ag/AgCl-sintered electrodes and a TMS-compatible amplifier (36; eXimia EEG, Nexstim), bandpass-filtered at 0.1–350 Hz, and sampled at 1450 Hz. The scalp under the electrodes was scraped with conductive abrasive paste (OneStep AbrasivPlus, H + H Medical Devices, Germany) before the electrodes were filled with conductive gel (Electro-Gel, ECI, Netherlands). Each electrode's impedance was kept below 5 kΩ. The reference and ground electrodes were placed on the right mastoid and zygomatic bone, respectively. Motor-evoked potentials (MEPs) were recorded with a Nexstim electromyography (EMG) system. The EMG electrodes were fixed in a belly–tendon montage on the right *abductor pollicis brevis* (APB) muscle. Before the TMS–EEG experiment, we determined for each subject the optimal coil location and orientation producing the largest MEP with a fixed suprathreshold intensity[37,38]. At the optimal location, we estimated the resting motor threshold (RMT) as the intensity producing MEPs larger than 50 μV in 5 out of 10 times[39].

Single-pulse TMS was applied to the left M1 at the cortical representation site of APB and the right pre-SMA. For M1, we used an initial TMS intensity of 90% of RMT. We rotated and moved the coil to minimize any remaining peripheral responses (MEPs) and scalp muscle activations in the EEG[40]. Additionally, we used a dedicated real-time EEG readout[41] to fine-tune the stimulation intensity to obtain an early (<50 ms) response nearby the stimulated target with a peak-to-peak amplitude of 6–10 μV on average reference after averaging 20 trials. If MEPs were still present, we relocated the coil more medially within the motor knob. This resulted in stimulation intensities of 60 V/m for S1, 55 V/m for S2, and 90 V/m for S3[42].

The pre-SMA rough stimulation area was identified by individual anatomical landmarks as described earlier[34,43]. The final stimulation parameters were adjusted based on the output of a dedicated real-time EEG readout, a procedure followed as well for M1[41]. The final stimulation intensities at pre-SMA for subjects S1, S2, and S3 were 100, 80, and 125 V/m, respectively. The stimuli were given at random interstimulus intervals of 2–2.3 s; a block of 250 pulses was delivered to each target per subject. The sample-and-hold electronics of the EEG device[36], and the iterative process to adjust the coil location and orientation resulted in minimal TMS-related artifacts in the EEG recording for both stimulation locations.

Pre-processing

Data were pre-processed with custom-made MATLAB 2019a scripts[44] based on the EEGLAB toolbox[45]. The signals were first filtered at 1–45 Hz with a third-order zero-phase-shift Butterworth bandpass filter. Then, epochs were extracted with a time window of −1 to 1 s relative to the TMS pulse. After visual inspection, we removed trials heavily contaminated by eye blinks or scalp-muscle activations. Then, data were re-referenced to the average potential, and the baseline was corrected by subtracting the baseline average (−1000…−2 ms). Next, independent component analysis (ICA) separated the data into predominantly artefactual and neuronal components.

These components were visually inspected for every trial. Trials with highly distorted components were rejected, and then ICA was recomputed on the remaining data (number of remaining trials, after both trial-rejection steps: (mean±sd 233±11, range 218–244). Independent components generated by eye blinks, eye movements, continuous muscle artifacts unrelated to TMS timing, and electrode-movement artifacts were removed (mean±sd: 12±2 components were removed per dataset).

Phase evaluation

The trials were split semi-manually into positive- and negative-phase classes, separately for mu and beta bands, based on the pre-stimulus phase in each trial. First, the signals were bandpass filtered with a 4th-order zero-phase-shift Butterworth filter in the frequency band of interest. Then, a Hilbert transform was applied to determine the instantaneous phase at the time of the TMS pulse. Trials with a maximum deviation of 30° from the peaks were set into positive-phase or negative-phase classes, respectively. To correct for cases where the narrow-band signal did not correspond well to the broadband one, we manually inspected the choices made by the algorithm and corrected them in cases of clear misclassification. For this, both raw and the bandpass-filtered signals at the frequencies of interest were displayed from channel C3 (when stimulating M1) or F2 (when stimulating pre-SMA), together with the decision made by the algorithm. A trial was reclassified as positive- or negative-phase if the phase difference between the instantaneous phase at the TMS onset and the positive or negative peak, respectively, was less than 40°, and the unfiltered signal was qualitatively similar in waveform to the filtered one. Trials were excluded from further analysis if the signals greatly differed or TMS occurred at some other phase. We obtained for the analysis 72.6±20.5 (mean±sd) trials in each class and a total of 12 datasets (2 stimulus locations × 3 subjects × 2 frequency bands).

Correction of background oscillatory activity

Typically, the TMS-evoked responses are estimated as the mean across trials that have been delivered at randomized time intervals. The rationale is that, in this case, any background oscillations that are not time-locked to the stimulus are attenuated by the averaging process. However, in trials classified according to the pre-stimulus phase, such background oscillations are consistent across trials and are consequently present in the averaged signal. This effect, if not adequately addressed, may lead to incorrect interpretations. We removed the phase classification effect by extracting the pre-stimulus time period (−1000…0 ms) of each trial, sorting these non-stimulated trials according to phase at −500 ms, and subtracting their mean from the stimulated trials[14,46,47]. The stimulated trials were cut to a length of −500…500 ms when applying the correction to match the non-stimulated trials' length.

Source analysis

For each dataset, the global mean-field amplitude (GMFA[48,49]) was computed. To compare the two classes, we calculated the absolute difference in their GMFAs ($|GMFA_{positive\ phase} - GMFA_{negative\ phase}|$), and set a threshold based on 1000 random reassignments of the trials into new pseudoclasses. For each permutation, the maximum absolute difference between the pseudoclasses was calculated and stored. This procedure controls the within-dataset false discovery rate[50]. To keep the total false discovery rate below 0.05, we applied the Benjamini–Hochberg procedure[51] to set the threshold at the corresponding percentile of the permutation distribution for each dataset with $(1 - r * 0.05/12)$, where r is the rank of the dataset, and 12 is the total number of datasets. The rank was determined by the maximum difference in GMFA between the classes with respect to the permuted distributions. For time intervals where the differences in the GMFAs between the positive- and negative-phase classes in the post-TMS time period (0…300 ms) exceeded this threshold, we conducted source estimation. We averaged the mean EEG responses in these time intervals for both classes separately, which were then utilized for Tikhonov-regularized minimum-norm estimates (MNE)[52]. The obtained MNE maps were thresholded for visualization to show only the cortical area corresponding to at least 60% of the maximum MNE amplitude.

For source estimation, we calculated the lead fields that describe the sensitivity profiles of different EEG channels to neuronal activity in all the plausible cortical locations. First, the scalp, skull, and white-matter surfaces were extracted from the MRIs using the *headreco*[53–55] function of the SimNIBS software[56]. The surface meshes were imported to MATLAB, decimated to ~10,000 nodes, and cleaned from surface artifacts using the iso2mesh package[57]. The lead-field matrices were calculated with the boundary element method assuming conductivity values of 0.33, 0.0033 and 0.33 S/m for the intracranial cavity, skull and scalp, respectively[58]. Focal post-synaptic currents were modeled as current dipoles oriented normal to the white matter surface. For obtaining the cortical activity estimates, the Tikhonov-regularized MNE was used for projecting the TEPs to the source space[52] with a regularization parameter of 0.1.

Results

TEPs and GMFAs

We observed differences in GMFAs between the positive- and negative-phase classes in 4/12 comparisons that exceeded the threshold level. Two of the observed significant intervals were before 50 ms, two between 50 and 100 ms, and one after 100 ms post-stimulus (Figure 1 and Figure 2). Source estimates showed the most abundant differences close to the stimulation site. We observed large inter-individual variability in the spatial and temporal characteristics of the phase effects.

Signal propagation after M1 stimulation

The activation patterns and differences between the negative- and positive-phase classes are illustrated in Figure 1. The mu rhythm modulated responses in S1 and S3. In S1, the positive-phase condition elicited larger responses than the negative-phase condition at 102–105 ms post-stimulus. In S3, the negative-phase condition produced larger GMFAs at 21–19 and 135–142 ms post-stimulus. The beta rhythm modulated responses only in S3 at 30–32 ms post-stimulus, at the stimulation site and in the lateral right hemisphere, where the positive-phase condition produced larger GMFAs than the negative one.

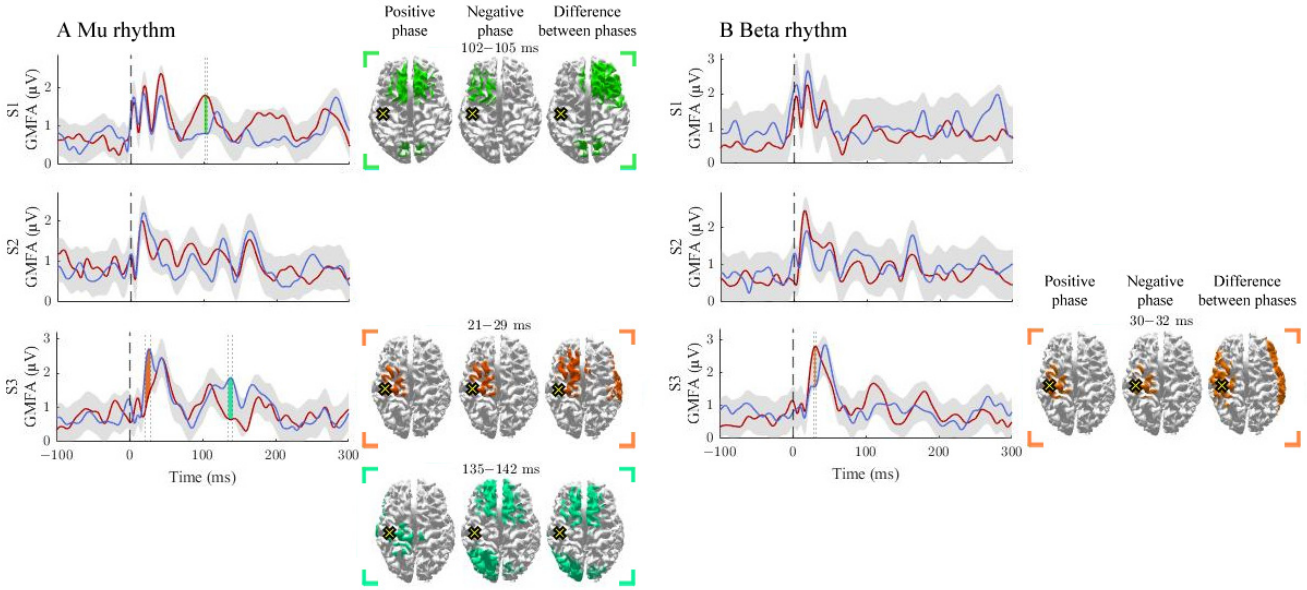

Figure 1. The effect of the positive and negative phases on TEPs when stimulating M1. The **A** and **B** panels summarize the effects of mu and beta rhythms, respectively. The curves show the global mean-field amplitudes (GMFA) of the positive-phase (red) and negative-phase (blue) conditions. The cortical maps illustrate the source estimates for the significant differences between the phase conditions. The shaded areas indicate the average GMFA over the two conditions ± the threshold for meaningful changes. Time intervals that exceed the threshold are marked with different colors. For each time interval, the corresponding time-averaged source estimates are shown on the right in the same color. For each time interval, only sources stronger than 60% of the maximum amplitude are shown. The dark dashed vertical line indicates the time of the TMS pulse. The cross marks the stimulation site.

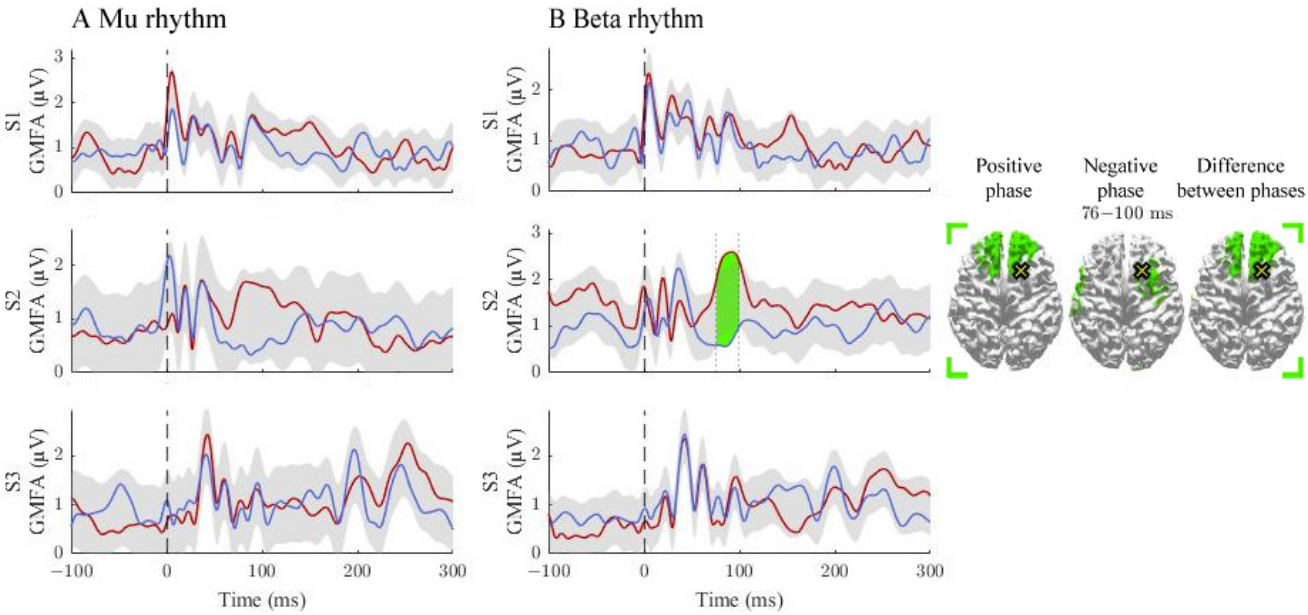

Figure 2. The effect of the positive and negative phases on TEPs when stimulating pre-SMA. The **A** and **B** panels summarize the effects of mu and beta rhythms, respectively. The curves show the global mean-field amplitudes (GMFA) of the positive-phase (red) and negative-phase (blue) conditions, whereas the cortical maps illustrate the source estimates for the significant differences between the phase conditions. The shaded areas indicate the average GMFA over the two conditions ± the threshold for meaningful changes. The time interval which exceeds the set threshold is marked with color. For the time interval, the corresponding time-averaged source estimates are shown on the right in the same color. Only sources stronger than 60% of the maximum amplitude are shown. The dark dashed vertical line indicates the time of the TMS pulse. The cross marks the stimulation site.

Signal propagation after pre-SMA stimulation

The activation patterns and differences between the classes are illustrated in Figure 2. For the mu rhythm, no supra-treshold time-intervals were found. The beta rhythm modulated responses in S2 at 76–100 ms post-stimulus, where the positive-phase condition elicited stronger responses than the negative one. The source estimates revealed differences close to the stimulation site.

Discussion

We found that the phase of spontaneous cortical oscillations at the TMS pulse instant seems to affect the post-stimulus effective connectivity pattern. It is proposed that the state of the post-synaptic neural population modulates the efficacy of the synaptic transmission[59]. Such mechanisms can play a role in multiple places in the signaling cascade, determining where and when the responses differ from each other. We observed high variability between subjects, which could be credited to, *e.g.*, differences in the cortical folding, inter-individual differences in stimulated circuits, and inter-individual cortical connections.

To highlight meaningful changes due to the phase of ongoing EEG oscillations on TEPs, we analyzed differences in GMFA that are unlikely to reflect purely changes in the background activity. In this preliminary study, we observed supratreshold differences in 4 out of 12 datasets already with this small number of trials. More data would likely show more subtle phase effects not distinguishable with this trial number. Our *post hoc* power analysis[60] indicated that assuming a short-lived (20 ms) 1-μV difference in GMFA, we would need over 100 trials in each phase class to show this difference statistically with 80% power. It is also important to note that three subjects is a relatively small sample size and our interpretations may not be generalizable for a larger population. Nonetheless, three subjects are sufficient for demonstrating the methodology and at a single subject level a possible effect of ongoing oscillations on the effective connectivity. Thus, in future studies, we need to collect a higher number of trials per phase in a larger group of study participants to consolidate our observations.

Other pre-stimulus indices than the phase have also been shown to modulate effective connectivity in the human corticocortical circuits[30,61–64]. These same factors could also play a role in corticocortical effective connectivity. For example, high pre-stimulus mu power has been shown to reduce MEP amplitudes[30,63], although more research is still needed[65]. Power has also been suggested to interact with the phase, resulting in power-dependent phase modulation[29]. Therefore, further control of the power in phase-dependent stimulation will be important in future works.

Conclusions

Our results suggest that TMS-induced effective connectivity is dependent on the pre-stimulus phase of the local oscillations. Our findings open new avenues for further research and support the progress of brain-state-dependent and closed-loop stimulation paradigms.

Institutional Review Board Statement

Ethics and Consent: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by The Coordinating Ethics Committee of Helsinki University Hospital (protocol code: HUS/1198/2016, date of approval: 21.7.2017). Written informed consent was obtained from all subjects involved in the study.

Data availability

The data presented in this study are available upon reasonable request from the corresponding author as long as the confidentiality requirements are strictly followed. We are not allowed to make physiological or anatomical data publicly available according to our ethical permission statement.

Author contributions

Conceptualization, P.L.; methodology, I.G., T.M., A.T., J.N., V.S., M.F., M.R. and P.L.; software, I.G. and T.M.; validation, I.G., T.M. and V.S.; formal analysis, I.G., T.M., V.S. and M.F.; investigation, I.G., A.T., J.N., M.R. and P.L.; resources, R.I.; data curation, I.G. and A.T.; writing—original draft preparation, I.G., T.M. and P.L.; writing—review and editing, I.G., T.M., A.T., J.N., V.S., M.F., M.R., P.L. and R.I.; visualization, I.G., T.M., A.T., J.N., V.S. M.F., M.R., P.L., and R.I.; supervision, T.M. and P.L.; project administration, P.L., and R.I.; funding acquisition, T.M., A.T., J.N., M.F., M.R., R.I. All authors have read and agreed to the published version of the manuscript.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 04 August 2022

https://doi.org/10.21956/openreseurope.16165.r29686

✔ **Keiichi Kitajo**
Division of Neural Dynamics, Department of System Neuroscience, National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan

The authors have addressed all of my concerns. I think that the manuscript is acceptable.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nonlinear neural dynamics, Computational Neuroscience, Cognitive Neuroscience

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 02 August 2022

https://doi.org/10.21956/openreseurope.16165.r29685

✔ **Florinda Ferreri**
Department of Neuroscience, University of Padua, Padova, Italy

I have read the new version of the paper. It can be indexed as is.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: TMS, TMS-EEG

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 June 2022

https://doi.org/10.21956/openreseurope.15803.r29330

Florinda Ferreri
Department of Neuroscience, University of Padua, Padova, Italy

In the paper “Local brain-state dependency of effective connectivity: a pilot TMS–EEG study” the Authors aimed to conduct a pilot study to set the basis for addressing how spontaneous oscillations affect cortical effective connectivity as measured through TMS-evoked potentials (TEPs).” They applied TMS to the left primary motor cortex and right pre-supplementary motor area of three subjects while recording EEG. They conclude that TMS-evoked effective connectivity appears to depend on the phase of local cortical oscillations at the stimulated site.

The paper is interesting, but 3 subjects are not enough even for a pilot study. I have some further comments:

- It is not so clear the phrase “we rotated and moved the coil to minimize both peripheral responses (MEPs) and scalp muscle activations”. At the 90% of RMT no MEPs are expected.
- After stimulation of M1, TEPs have been clearly described: which component is expected to have a peak-to-peak amplitude of 6–10 μV after averaging 20 trials?
- In TMS-EEG experiments Independent component analysis (ICA) can cancel genuine brain responses. The Authors should comment further on this point.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: TMS, TMS-EEG

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 05 Jul 2022

Pantelis Lioumis, Aalto University School of Science, Espoo, Finland

In the paper "Local brain-state dependency of effective connectivity: a pilot TMS–EEG study" the Authors aimed to conduct a pilot study to set the basis for addressing how spontaneous oscillations affect cortical effective connectivity as measured through TMS-evoked potentials (TEPs)." They applied TMS to the left primary motor cortex and right pre-supplementary motor area of three subjects while recording EEG. They conclude that TMS-evoked effective connectivity appears to depend on the phase of local cortical oscillations at the stimulated site.

The paper is interesting, but 3 subjects are not enough even for a pilot study. I have some further comments:

Answer: We thank the reviewer for the interest in our paper. Regarding the number of subjects; yes, 3 is a small number. However, many discoveries have been made with 3 or even fewer subjects. In this case, we wanted to demonstrate a phenomenon and propose a methodology rather than to obtain statistics about how common this type of finding is on a population level. We have now addressed this in the Discussion section: "It is also important to note that three subjects is a relatively small sample size and our interpretations may not be generalizable for a larger population. Nonetheless, three subjects are sufficient for demonstrating the methodology and at a single subject level a possible effect of ongoing oscillations on the effective connectivity. Thus, in future studies, we need to collect a higher number of trials per phase in a larger group of study participants to consolidate our observations."

It is not so clear the phrase "we rotated and moved the coil to minimize both peripheral responses (MEPs) and scalp muscle activations". At the 90% of RMT no MEPs are expected.

Answer: MEPs can be present even with 90% of RMT; they are simply appearing more seldomly and with smaller amplitudes. This is what we observed in some of the subjects, and this is why we applied this procedure to minimize any remaining MEPs as well as scalp

muscle activations. We have clarified this in the Data acquisition section: “We rotated and moved the coil to minimize any remaining peripheral responses (MEPs) and scalp muscle activations in the EEG.”

After stimulation of M1, TEPs have been clearly described: which component is expected to have a peak-to-peak amplitude of 6–10 μV after averaging 20 trials?

Answer: This aim of 6–10 μV peak-to-peak amplitudes refers to the earliest distinguishable components. For M1, that would be the N15–P30 peak-to-peak amplitudes in most subjects. We defined this criterion as peak-to-peak amplitudes before 50 ms for it to be applicable also to other stimulation sites, where peak latencies can vary, such as in pre-SMA in our case (usually around P20 and N40).

In TMS-EEG experiments Independent component analysis (ICA) can cancel genuine brain responses. The Authors should comment further on this point.

Answer: Yes, this is completely correct. Independent component analysis (ICA) attempts to separate events in the data that are independent in time. Brain signals can especially mix with artefacts time-locked to the TMS pulse in ICA, as these do not meet the requirement of independence (Metsomaa *et al.*, 2014). Therefore, we only used ICA to remove events that are independent to the timing of the TMS pulse, such as ocular artefacts and continuous scalp muscle activations. ICA, when used with caution, can be a useful tool for removing certain types of artefacts, and is widely utilized in TMS–EEG analysis. It is also important to note that, when running the experiments, we applied procedures to minimize artefacts such as scalp muscle activations by fine tuning the stimulation parameters, as explained in the Data acquisition section. This way we guaranteed the safe use of ICA as we did not need to remove such artefacts with it. Metsomaa, J., Sarvas, J., Ilmoniemi, R.J., 2014. Multi-trial evoked EEG and independent component analysis. J. Neurosci. Methods 228, 15-26. doi:10.1016/j.jneumeth.2014.02.019

Competing Interests: No competing interests were disclosed.

Reviewer Report 25 April 2022

https://doi.org/10.21956/openreseurope.15803.r28993

Keiichi Kitajo
Division of Neural Dynamics, Department of System Neuroscience, National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan

The authors report the effects of the instantaneous phase of EEG oscillations on the effective

connectivity as assessed by the global cortical spread of TMS-evoked responses targeting two local areas, right M1 or pre-SMA. The research question is sound. Although this study uses cutting-edge techniques to measure and assess TMS-evoked responses, it is hard to conclude things using data from three participants. Still, it will be beneficial for readers to read this manuscript who are interested in the methodological aspects of TMS-EEG recordings and phase-dependent neural dynamics. I, therefore, suggest the authors provide more technical details of the study.

How did the authors determine the 90% RMT?

On page 3, For M1, we applied an initial TMS intensity of 90% of RMT. We rotated and moved the coil to minimize both peripheral responses (MEPs) and scalp muscle activations.

I don't quite get the exact procedure to determine 90% of RMT. I suppose that the regular procedure is as the following. First, the coil orientation and location should be optimized to get the most prominent MEP at a TMS intensity higher than the RMT. Next, the RMT should be determined by some adaptive procedure reducing the TMS intensity. Finally, the intensity of TMS should be reduced to 90% of RMT, keeping the orientation and location of the coil. Is the authors' procedure different from this?

How was the TMS intensity for pre-SMA optimized for each individual?

I think that one of the challenges in TMS-EEG studies is artifact reduction. However, I don't see details on the online and offline methodology of artifact reduction except for auditory artifact reduction using in-ear earphones and earmuffs. Were the lead wires rearranged relative to the coil orientation to reduce TMS-induced electromagnetic artifacts?

How were the TMS-evoked artifacts, such as the decay artifact, removed in the offline analysis? Did the EEG amplifier system have any online artifact reduction protocol? Please explain these details more explicitly.

On page 4, I understand that the authors conducted trial-wise permutation tests to analyze the GMFA differences between two target phase conditions within each subject. And the authors corrected the results by the number of multiple comparisons, which the authors think is 12, i.e., the number of datasets. However, if the authors conduct the permutation test at every time point, the number of comparisons will be much larger considering the number of time points. What is the rationale for ignoring the number of time points? Another idea is to go for the cluster-based permutation test (Maris *et al.* 2007[1]) considering the cluster. I am not sure if there is a single-subject version of the statistical test, though.

Figures 1 and 2. The titles for the figures look exactly the same. I guess the authors forgot to put M1 and pre-SMA.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nonlinear neural dynamics, Computational Neuroscience, Cognitive Neuroscience

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 30 Apr 2022

Pantelis Lioumis, Aalto University School of Science, Espoo, Finland

The authors report the effects of the instantaneous phase of EEG oscillations on the effective connectivity as assessed by the global cortical spread of TMS-evoked responses targeting two local areas, right M1 or pre-SMA. The research question is sound. Although this study uses cutting-edge techniques to measure and assess TMS-evoked responses, it is hard to conclude things using data from three participants. Still, it will be beneficial for readers to read this manuscript who are interested in the methodological aspects of TMS-EEG recordings and phase-dependent neural dynamics. I, therefore, suggest the authors provide more technical details of the study.

Answer: We thank the reviewer for the positive comments and for an encouraging review. Below, we answer each point separately. How did the authors determine the 90% RMT? We answer this question below the next comment.

On page 3, For M1, we applied an initial TMS intensity of 90% of RMT. We rotated and

moved the coil to minimize both peripheral responses (MEPs) and scalp muscle activations.

I don't quite get the exact procedure to determine 90% of RMT. I suppose that the regular procedure is as the following. First, the coil orientation and location should be optimized to get the most prominent MEP at a TMS intensity higher than the RMT. Next, the RMT should be determined by some adaptive procedure reducing the TMS intensity. Finally, the intensity of TMS should be reduced to 90% of RMT, keeping the orientation and location of the coil. Is the authors' procedure different from this?

Answer: Yes, a very similar procedure to what the reviewer describes was used. First, the motor hotspot was defined as the cortical target producing the largest MEP amplitudes. Then, the motor threshold was determined as the intensity producing MEPs of 50 μV or larger 5 out of 10 times. Finally, the intensity was reduced to 90% of the RMT. However, if MEPs were still present, or the early TEP components were small (below 6 μV), or covered by muscle or decay artifacts, the coil was moved and/or rotated and the intensity adjusted to satisfy the following condition: [no MEPs] & [no artifacts after 15 ms] & [early TEP components bigger than 6 μV (before 50 ms)]. Details on the procedure can be found in Fecchio et al., 2017. We have clarified this procedure in the Data acquisition section: "Before the TMS–EEG experiment, we determined for each subject the optimal coil location and orientation producing the largest MEP with a fixed suprathreshold intensity [37], [38]. At the optimal location, we estimated the resting motor threshold (RMT) as the intensity producing MEPs larger than 50 μV in 5 out of 10 times [39]."

How was the TMS intensity for pre-SMA optimized for each individual?

Answer: The TMS intensity was set to produce peak-to-peak amplitudes of 6–10 μV in the first 50 ms in the EEG signal. To achieve this, we used a graphical user interface (rt-TEP) to check in real-time for the quality of TEPs before starting the actual data acquisition (Casarotto et al., 2022). We utilized a functional mapping approach to set the stimulation parameters by delivering a few TMS pulse to check for the presence of TMS-evoked scalp muscle activations. If these were present, the stimulation parameters were adjusted to minimize the evoked muscle activity. Specifically, the coil was initially rotated, and if this procedure was not sufficient, the intensity was lowered. If also the last step was effective, we slightly translated the coil from the initial position. When TMS muscle activity was not detected, we proceeded delivering 20 pulses and checked for the presence of TEPs. The parameters of the stimulation were adjusted to obtain a signal with the first components lateralized (different from left to right), and with an amplitude in the channels under the stimulation site of 6–10 μV in the first 50 ms. This way, we could control both artefacts (described in more detail below and also in Casarotto et al., 2022)) and TEP amplitudes in real time. Please note that a similar procedure was followed for M1 targeting, although in this case we started from the hotspot location with the 90%MT and then, if the aforementioned conditions were not satisfied, we adjusted to new location and intensity as described a few lines above. We have clarified this information in the Data acquisition section:

"The final stimulation parameters were adjusted based on the output of dedicated real-time

EEG readout, a procedure followed as well for M1 (Casarotto et al., 2022)."

I think that one of the challenges in TMS-EEG studies is artifact reduction. However, I don't see details on the online and offline methodology of artifact reduction except for auditory artifact reduction using in-ear earphones and earmuffs. Were the lead wires rearranged relative to the coil orientation to reduce TMS-induced electromagnetic artifacts? How were the TMS-evoked artifacts, such as the decay artifact, removed in the offline analysis? Did the EEG amplifier system have any online artifact reduction protocol? Please explain these details more explicitly.

Answer: Most importantly, artifacts were avoided and reduced in the experimental session. The sample-and-hold amplifier cuts out the TMS pulse artifact, and there were no decay artifacts in the data. Muscle artifacts were avoided by careful coil placement, achieved by mapping as explained in the previous point. If present, decay artefacts were individuated by means of the rt-TEP tool and reduced/abolished by re-performing the impedances or rotating the position of the wires. Mapping was done by delivering 20 pulses and utilizing a graphical user interface (rt-TEP) to observe artifacts and peak-to-peak amplitudes (Casarotto et al., 2022). If the recorded TEPs were still affected by artifacts, the coil was rotated or moved to reduce them, and another 20 pulses were delivered. This was iterated until acceptable TEPs with minimal artifacts and large enough peak-to-peak amplitudes were achieved. Due to the careful methodology in the experiment, very clean data was recorded. Therefore, little needed to be removed in the offline analysis. We have added a clarifying sentence in the last paragraph of the Data acquisition section:

"The sample-and-hold electronics of the EEG device [36], and the iterative process to adjust the coil location and orientation resulted in minimal TMS-related artifacts in the EEG recording for both stimulation locations."

On page 4, I understand that the authors conducted trial-wise permutation tests to analyze the GMFA differences between two target phase conditions within each subject. And the authors corrected the results by the number of multiple comparisons, which the authors think is 12, i.e., the number of datasets. However, if the authors conduct the permutation test at every time point, the number of comparisons will be much larger considering the number of time points. What is the rationale for ignoring the number of time points? Another idea is to go for the cluster-based permutation test (Maris *et al.* 2007[1]) considering the cluster. I am not sure if there is a single-subject version of the statistical test, though.

Answer: A separate multiple-comparison correction was utilized in the time domain than over the datasets. In the time domain, the correction was part of the trial-wise permutations, as the *maximum* differences between GMFA traces for each permutation was used to set the threshold for the whole time-window. Therefore, each sample is compared against the maximal statistic across time, thus controlling the family-wise error rate. This is a common method described, for example, by Holmes and colleagues (1996). We have now clarified this in the source analysis section: "This procedure controls the within-dataset false discovery rate (Holmes et al. 1996)."

Figures 1 and 2. The titles for the figures look exactly the same. I guess the authors forgot to put M1 and pre-SMA.

Answer: Thank you for pointing this out. We have now specified the corresponding stimulation locations to each figure caption.

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Competing Interests: No competing interests were disclosed.