Journal of Visualized Experiments

Brain state-dependent brain stimulation with real-time EEG-triggered TMS

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE59711R2
Full Title:	Brain state-dependent brain stimulation with real-time EEG-triggered TMS
Keywords:	real-time; brain state-dependent stimulation; EEG-TMS; corticospinal excitability; motor cortex; human brain plasticity; phase; oscillation
Corresponding Author:	Ulf Ziemann Eberhard Karls Universitat Tubingen Tübingen, GERMANY
Corresponding Author's Institution:	Eberhard Karls Universitat Tubingen
Corresponding Author E-Mail:	ulf.ziemann@uni-tuebingen.de
Order of Authors:	Maria-Ioanna Stefanou
	David Baur
	Paolo Belardinelli
	Til Ole Bergmann
	Corinna Blum
	Pedro Caldana Gordon
	Brigitte Zrenner
	Ulf Ziemann
	Christoph Zrenner
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Tübingen, Baden-Württemberg, Germany

Response to the Reviewer Comments

Brain state-dependent brain stimulation with real-time EEG-triggered TMS

Maria-Ioanna Stefanou, David Baur, Paolo Belardinelli, Til Ole Bergmann, Corinna Blum, Pedro Caldana Gordon, Brigitte Zrenner, Ulf Ziemann*, Christoph Zrenner

Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, University of Tübingen, Germany.

*Corresponding Author

We appreciate the time and effort of the editor and referees put into reviewing this manuscript. We have addressed all issues indicated in the review report. Here we examine the suggestions one by one and discuss how we accordingly revised the manuscript.

Editorial comments:

Please see the attached word document. In-text comments have been made; these require your attention. Please address the comments by editing your manuscript/figures. Please maintain the current format and track all your edits.

Response to the Editor:

As suggested we have commented and adapted all of the raised issues directly in the manuscript. All commercial equipment was removed and added in the Table of materials. Furthermore, we wish to inform that for the filming of the video we plan a live TMS-EEG experiment in our laboratory, where the highlighted parts of the protocol are going to be used as narrative and all devices will be filmed during the experiment.

TITLE:

Brain State-Dependent Brain Stimulation with Real-Time Electroencephalography-Triggered Transcranial Magnetic Stimulation

AUTHORS & AFFILIATIONS:

Maria-Ioanna Stefanou[1,2], David Baur[1,2], Paolo Belardinelli[1,2], Til Ole Bergmann[1,2], Corinna Blum[1,2], Pedro Caldana Gordon[1,2], Brigitte Zrenner[1,2], Ulf Ziemann[1,2], Christoph Zrenner[1,2]

[1]Department of Neurology & Stroke, University of Tübingen, Germany
[2]Hertie Institute for Clinical Brain Research, University of Tübingen, Germany

Corresponding author:
Ulf Ziemann (ulf.ziemann@uni-tuebingen.de)

Email addresses of co-authors:
Maria-Ioanna Stefanou (maria-ioanna.stefanou@med.uni-tuebingen.de)
David Baur (d.baur@med.uni-tuebingen.de)
Paolo Belardinelli (paolo.belardinelli@uni-tuebingen.de)
Til Ole Bergmann (til.bergmann@uni-tuebingen.de)
Corinna Blum (corinna.blum@uni-tuebingen.de)
Pedro Caldana Gordon (pedrocaldanagordon@gmail.com)
Brigitte Zrenner (brigitte.zrenner@uni-tuebingen.de)
Christoph Zrenner (christoph.zrenner@uni-tuebingen.de)

KEYWORDS:

real-time, brain state-dependent stimulation, EEG-TMS, corticospinal excitability, motor cortex, human brain plasticity, phase, oscillation

SUMMARY:

This paper describes real-time electroencephalography-triggered transcranial magnetic stimulation to study and modulate human brain networks.

ABSTRACT:

The effect of a stimulus to the brain depends not only on the parameters of the stimulus but also on the dynamics of brain activity at the time of the stimulation. The combination of electroencephalography (EEG) and transcranial magnetic stimulation (TMS) in a real-time brain state-dependent stimulation system allows the study of relations of dynamics of brain activity, cortical excitability, and plasticity induction. Here we demonstrate a newly developed method to synchronize the timing of brain stimulation with the phase of ongoing EEG oscillations using a real-time data analysis system. This real-time EEG-triggered TMS in the human motor cortex, when TMS is synchronized with the surface EEG negative peak of the sensorimotor μ-alpha (8–14 Hz) rhythm, has shown differential corticospinal excitability and plasticity effects. The utilization of this method suggests that real-time information about the instantaneous brain state can be used for efficacious plasticity induction. Additionally, this approach enables personalized

EEG-synchronized brain stimulation which may lead to the development of more effective therapeutic brain stimulation protocols.

INTRODUCTION:

TMS is a well-established method for noninvasive brain stimulation, which enables the specific modulation of ongoing network dynamics and studies of corticocortical and corticospinal neural pathways with high spatiotemporal precision[1]. When stimulating the primary motor cortex (M1), the neural response can be quantified as motor-evoked potentials (MEPs), as well as TMS-evoked EEG potentials. MEPs can be recorded by electromyography (EMG) of target muscles, and their amplitude reflects corticospinal excitability when stimulating the primary motor cortex[2].

Despite the unique potential of noninvasive brain stimulation as a scientific tool to investigate and modulate brain networks in healthy study participants and in patients, TMS studies suffer from large trial-to-trial and intra- and interindividual variability of evoked responses[3–5]. Specifically, in TMS studies of corticospinal excitability and plasticity, MEP responses, as well as induced long-term potentiation (LTP)- or long-term depression (LTD)-like plasticity, exhibit high intrinsic variability, even when the stimulus parameters are carefully controlled[3,4]. However, evidence from animal studies indicates that the observed variability of responses is not attributable to "random noise" but is instead related to the fluctuating brain states at the time of stimulation[6]. Accordingly, by combining TMS with EEG in a real-time brain state-dependent stimulation paradigm (i.e., EEG-triggered TMS), the fluctuating instantaneous brain state can be used to optimize stimulus timing[7–10].

Several studies have related the instantaneous phase of ongoing neural oscillations to neuronal excitability using TMS-compatible EEG systems[11,12]. Modern EEG amplifiers can handle the large electromagnetic TMS artifacts, and increasingly well-established experimental protocols exist for the combination of EEG with TMS[13,14] and the post hoc removal of TMS-related EEG artifacts[15,16]. While the influence of the prestimulus brain state as assessed by EEG on TMS-evoked responses can be assessed with randomly applied TMS stimuli that are sorted post hoc[17,18], the repetitive application of TMS in a predefined brain state requires real-time EEG-triggered TMS[11,19].

Here, a custom millisecond-resolution EEG-triggered TMS setup is used to synchronize TMS pulses with a predetermined phase of ongoing brain oscillations[11], demonstrating that the negative EEG deflection of the μ-alpha rhythm corresponds to a higher cortical excitability state (leading to larger MEP amplitudes) as compared to the positive EEG deflection[8,11,12,20]. In this manuscript, we present a method for conducting real-time EEG-triggered TMS protocols to study human brain networks.

PROTOCOL:

All experimental procedures described in the following sections have been approved by the Institutional Ethics Committee following the guidelines of the Declaration of Helsinki, and all participants provided written informed consent prior to study enrollment.

1. Study participants

1.1. Subject recruitment

1.1.1. Recruit study participants based on predefined inclusion criteria. Screen candidates for contraindications, such as the presence of implanted medical devices (e.g., cardiac pacemaker), according to TMS safety guidelines[21], or for neurological or psychiatric diseases and the use of drugs that act on the nervous system.

1.1.2. For studies requiring MRI, assess the potential study participants for possible contraindications to MRI according to radiological safety standards[22]. Perform a power analysis to ensure that the study sample is sufficient for statistical analysis.

1.1.3. Optionally, preselect subjects having a prominent oscillation of interest in the signal extracted by the chosen EEG montage in order to improve the accuracy of the phase detection.

NOTE: In this experiment, the C3-centered Laplacian (C3 references to the average of the surrounding electrodes CP1, CP5, FC1, and FC5) was used to extract the sensorimotor μ-rhythm with the subject at rest and eyes open. Preselected were subjects having a single peak in the alpha band (8–14 Hz) which contains >25% of total power in the current source density (CSD) power spectrum. This criterion ensured that the oscillation amplitude was sufficiently large in comparison to the background noise (good signal-to-noise-ratio [SNR]) to enable the algorithm to estimate the instantaneous phase of the trigger signal with sufficient accuracy and increased the likelihood of observing a significant excitability effect[11,12,28,29,30].

1.2. Subject information

1.2.1. Provide the subjects with the study-related informed consent form. Provide printed TMS and MRI safety screening questionnaires.

NOTE: These documents and the study protocol, as well as the use of personal data (e.g., from questionnaires) and identifiable human data (e.g., from MRI), need to be preapproved by the ethics committee (Institutional Review Board).

1.2.2. Ask the subject to fill out the TMS and MRI safety screening questionnaires. Acquire written informed consent for participation in the study and the planned use of data.

1.2.3. Acquire demographic data.

1.2.4. Assess subject handedness using standard inventories (e.g., the Edinburgh Handedness Inventory)[23].

1.2.5. Introduce the subject to the setup and stimulation procedure. Ensure that each participant is familiarized with the sensation of TMS and tolerates it well.

1.2.6. Acquire MRI for each participant prior to the TMS experimental sessions. Whole-head anatomical MR images are required, including the top of the scalp and anatomical landmarks (i.e., the tragus of both ears), as these will serve as fiducial points for neuronavigation in subsequent steps of this protocol.

1.2.7. Schedule the experimental sessions according to the specifications of the study protocol (i.e., take into account "washout periods" between the experiments).

NOTE: Ideally, subjects should come at the same time and on the same day of the week in protocols comparing different conditions in multiple sessions.

1.2.8. Instruct the participants to refrain from consuming alcohol, nicotine, or caffeine before the scheduled experimental sessions. The subjects should also have had their regular sleep on the night before the experiment and not be unusually tired.

2. Setup preparation

2.1. Real-time data stream-capable EEG system

2.1.1. Use a TMS compatible EEG/EMG amplifier that can handle the voltage spikes induced by the TMS pulse.

NOTE: The amplifier system needs to make a raw data stream available at a constant low latency (<5 ms) for subsequent processing by a real-time processor. In this experiment, a 24-bit 80-channel biosignal amplifier was used for EEG and EMG recordings.

2.1.2. Configure the EEG/EMG amplifier system to low-pass filter (e.g., 0.16 Hz cut-off), and down-sample the biosignal data to 5 kHz from the sampling rate at the amplifier head stage.

2.1.3. Ensure that the amplifier system sends data packets containing the relevant channels via a real-time user datagram protocol (UDP) to the real-time processor at regular constant intervals ≤1ms. Use a high sampling frequency (e.g., 5 kHz) to capture the EMG responses and to minimize filter delay of the EEG data.

2.2. EEG-compatible TMS stimulator

2.2.1. Use a TMS device that can be triggered externally with a fixed and minimal delay and which minimizes artifacts in the simultaneous EEG recording (e.g., line noise in the EEG through the TMS coil cable, recharging artifacts after the pulse).

2.2.2. Ensure that the distance between the TMS stimulator (including the coil and coil cable) and the EEG recording system is maximized to reduce electrical interference (at least 1 m). Where possible, turn off sources of electromagnetic interference such as fans and motors. Furthermore,

ensure that the EEG and EMG recording leads are positioned and aligned such that common interference cancels out.

2.3. Real-time EEG data processing system

NOTE: The real-time EEG data stream is acquired and analyzed using a real-time digital signal processing system, which then triggers the TMS device when a predetermined condition is met. Such a system has been custom-developed in our laboratory[11] to implement a phase detection algorithm similar to the approach by Chen et al.[24] and consists of the following steps.

2.3.1. Analyze a sliding window of data, 500 ms long (**Figure 1a**), to estimate the instantaneous phase of the target brain oscillation to phase-specifically trigger the TMS stimulator.

2.3.2. Perform bandpass filtering of the window for the frequencies of interest (e.g., between 9 and 14 Hz for the sensorimotor μ-alpha rhythm; **Figure 1b**). Consider adjusting the filter parameters to the individual peak frequency of the target oscillation.

2.3.3. Remove any data distorted by the filtering edge effects. Note that there is a trade-off in that stronger filters have larger edge effects.

2.3.4. Use an autoregressive model to forward predict the signal (Yule–Walker, order 30; **Figure 1c**).

2.3.5. Apply a Hilbert transform of the resulting window of data to yield the analytic signal, from which the instantaneous phase of the signal is determined by taking the angle of the complex number at the relevant time-point.

2.3.6. Estimate the EEG power spectrum from the sliding window of data in the frequency bins of interest (e.g., 9–14 Hz) using a short-time Hann-windowed FFT.

2.3.7. When both phase and power meet a predetermined criterion (e.g., a negative peak, the minimum power threshold), generate a digital output (TTL) pulse with the real-time system to trigger the TMS device.

2.4. Neuronavigation system

2.4.1. To monitor coil position and achieve accurate and consistent TMS targeting within and across sessions, use a neuronavigation system.

NOTE: A stereo infrared camera system is used to precisely locate reflective trackers in three-dimensional space, which are mounted on the subject's head and the stimulation coil, enabling precise relative positioning of the coil with respect to the individual's brain anatomy after calibration and MRI registration. For single-session studies and when planning to analyze only the EMG and not the EEG responses to TMS, navigation based on a standard brain instead of an

individual MRI is sufficient.

2.4.2. Load the individual structural MRI data into the navigation system software prior to starting the experiment for each participant.

2.5. Experimental control computer

2.5.1. Use an experimental control computer that is connected to the EEG system, the TMS stimulator, the real-time device, and the neuronavigation system.

NOTE: The EEG software controls the EEG amplifier system, sets parameters, and starts and stops EEG data archiving. The TMS stimulator can be remote-controlled to change stimulation parameters (intensity, current direction, etc.) with a remote-control toolbox[25].

2.5.2. Remotely control the real-time device to set the desired trigger conditions.

NOTE: The neuronavigation system can be remote-controlled, for example to target different coil locations.

2.5.3. Combine all of the above in an experimental control script to enable the automation of the experimental conditions and control flow.

2.6. EEG recording electrodes

2.6.1. Ensure that TMS-compatible EEG recording caps with the desired electrode layout are available in different sizes. Measure the subject's head circumference and prepare the appropriately sized cap.

2.6.2. Keep the required materials for EEG preparation handy (e.g., abrasive and conductive gels, syringes with sterile blunt needles, etc.).

2.7. EMG recording electrodes

2.7.1. Keep the surface EMG electrodes, leads, and required materials for skin preparation ready.

3. Conducting the experiment

3.1. Preliminaries

3.1.1. Ensure that the required paperwork is in order (study consent form is signed) and that the participant has had no adverse effects since the previous session.

3.1.2. Seat the subject in a comfortable reclining position to minimize movement of the head during the experiment. A vacuum pillow wrapped around the neck and lower head can help to

support the participant's head without causing additional muscle tension (e.g., like a chin rest would do).

3.2. EEG and EMG preparation

3.2.1. Place the appropriately sized EEG cap on the subject's head and position the cap correctly. Avoid excessive tension below the chin to reduce cranial and neck muscle activity that could contaminate the EEG[26].

3.2.2. Register the subject in the EEG recording software.

3.2.3. Prepare the EEG electrodes according to the lab-specific protocol (e.g., apply abrasive gel followed by conductive gel).

3.2.4. Check that EEG electrode impedances are below 5 kΩ.

3.2.5. To keep the conducting gel from drying up or getting smeared to the adjacent electrodes by any movement of the TMS coil, cover the EEG cap with plastic wrap. Then, fit a net cap above the plastic wrap to keep the cables in a fixed position to reduce EEG artifact variability, and apply adhesive tape to increase the stability of the multiple layers.

3.2.6. Attach the surface EMG electrodes over the target muscles after having cleaned and lightly abraded the skin (e.g., use a bipolar recording from the right abductor pollicis brevis hand muscle in belly-tendon montage).

NOTE: Here, a bipolar recording from the right abductor pollicis brevis hand muscle in belly-tendon montage was used. The placement of EMG electrodes is important as surface electrodes generally record activity from multiple underlying muscles.

3.2.7. Verify the correct matching between the actual EEG sensors on the head and the traces recorded in the EEG system by tapping on a few EEG electrodes to cause artifacts. As a sanity check, verify that occipital alpha increases when the participant closes their eyes.

3.2.8. Visually inspect the ongoing EEG and EMG signal for artifacts (e.g., line noise, muscle activity) or bad electrodes.

3.2.9. Ensure that the participant remains awake and keeps their eyes open throughout the experiment to avoid occipital alpha oscillations contaminating the signal.

3.3. Preparation of the neuronavigation

3.3.1. Attach the reflective head tracker to the participant's head with sufficient adhesive tape to ensure stability throughout the experiment.

3.3.2. Use the pointer tool to coregister the head model with the relevant anatomical landmarks (e.g. the nasion, the tragi of both ears, the corners of the eyes).

3.3.3. Attach a coil tracker to the stimulation coil and calibrate the coil.

3.3.4. Place the pointer at different points on the head surface and verify the correctness of the displayed position on the monitor of the neuronavigation system.

3.3.5. Pinpoint the EEG sensor locations for coregistration with the individual MRI.

3.4. Baseline EEG

3.4.1. Demonstrate typical EEG artifacts to the subject (e.g., swallowing, chewing, eye blinks) and instruct the subject to avoid them throughout the experiment. Also, ask them to avoid jaw clenching, yawning, or talking.

3.4.2. Ask the subject to fixate on a point with the eyes open and perform a short recording of resting-state EEG with the eyes open.

3.4.3. If required for the computation of real-time filters, record additional EEG activity during tasks.

3.5. Finding the motor "hotspot" and determination of the resting motor threshold

3.5.1. Find the motor "hotspot" (i.e. the stimulation location over which the single-pulse TMS elicits well-shaped MEPs of a comparably consistent amplitude across trials) and save the corresponding coil position (including coil orientation and angulation) in the neuronavigation system.

3.5.2. Find the resting motor threshold (RMT) by applying single TMS pulses over the motor cortex at gradually increasing stimulation intensities until the elicited MEPs have peak-to-peak amplitudes greater than 50 µV in more than 50% of the trials[21].

3.5.3. If available, use an automated script for parameter estimation by sequential testing (PEST), for instance, following a maximum likelihood strategy[27] which also provides an online estimate of the confidence interval of RMT based on the observed variability of single responses and which typically requires ca. 30 test pulses of adaptively varying intensity to obtain a robust RMT estimate.

3.5.4. If this is not the first experimental session, compare the coil position with the previous position and compare the obtained RMT with the previous RMT to validate consistency.

3.5.5. If required, determine stimulation intensities for the active motor threshold (AMT) or for the 1 mV peak-to-peak MEP amplitude using standard procedures[21].

3.6. Final participant preparation

3.6.1. Optionally, immobilize the head of the subject using a vacuum pillow.

3.6.2. Optionally, deliver a masking noise through earplugs (when planning to analyze TMS-evoked EEG potentials). Otherwise, provide the subject with earplugs and headphones for hearing protection.

3.6.3. Optionally, align and fix the coil at the desired position using a mechanical arm.

3.7. Pre-experiment data quality validation

3.7.1. Check that the real-time processor is receiving data from the EEG system.

3.7.2. Check the signal obtained from the desired EEG spatial filter (e.g., C3-centered Laplacian montage) for obvious artifacts.

3.7.3. Visually confirm the EEG signal quality, check for bad electrodes, excessive line noise, and muscle artifacts, and adjust the time window and amplitude scaling on the EEG system software for ongoing visual inspection during the experiment.

3.8. Main experimental session

3.8.1. Unless the stimulator intensity is remote-controlled in the experimental script, manually set the stimulation intensity to the desired value (e.g., 110% of the RMT).

3.8.2. Start the experimental script to apply pulses at different phases of the target oscillation in a randomized order.

3.8.3. During the experiment, monitor the trigger condition thresholds (artifact detection threshold, pre-innervation threshold, minimum power, etc.).

NOTE: Stimuli will be triggered at irregular intervals, as the real-time processor is waiting for the trigger conditions to occur. However, the conditions should be set such that most stimuli occur within a predictable interval (e.g., 2–3 s after the previous pulse), and long pauses (e.g., in this case, >5 s) are avoided as these would lead to larger evoked responses due to novelty.

3.8.3.1. Alternatively, use post hoc stratification to remove trials following overly long intervals.

3.8.4. To achieve sufficient statistical power to differentiate phase-specific stimulation effects, acquire a sufficient number of trials

NOTE: We typically chose 80–120 interleaved trials per condition[20].

3.8.5. Document the start and end times of the various sessions and keep a record of any unusual occurrences.

REPRESENTATIVE RESULTS:

The utilization of the real-time EEG-triggered TMS in the primary human motor cortex reveals differential corticospinal excitability and plasticity effects. Using the protocol described above, real-time EEG-TMS was applied, synchronizing TMS with the ongoing EEG oscillatory phase of the endogenous sensorimotor μ-rhythm in three trigger conditions (positive peak, negative peak, and random phase) in randomized order. A Laplacian EEG montage was used to extract the sensorimotor μ-rhythm by referencing the EEG electrode C3 to the average of four surrounding electrodes (FC1, FC5, CP1, and CP5). **Figure 2a** shows the average prestimulus EEG signal in the 400 ms before the TMS pulse for the three predefined conditions. The average elicited MEPs recorded from the right-hand muscles are depicted in **Figure 2b.** These results show that the negative EEG deflection of the μ-rhythm corresponds to a higher cortical excitability state (leading to larger MEP amplitudes) as compared to the positive EEG deflection, with low intertrial variability of the noted corticospinal excitability effects, presented in **Figure 2c**.

FIGURE LEGENDS:

Figure 1: Brain state-dependent EEG-phase-triggered TMS. Scalp EEG raw data derived from a five-channel Laplacian montage centered on the C3 electrode over the left sensorimotor cortex was acquired sample-by-sample by a real-time digital signal processing system. (**a**) A 500 ms sliding window of EEG data was processed by the algorithm every 2 ms. (**b**) The signal after band-pass filtering and removal of the edge artifacts. (**c**) The forward-predicted signal (red trace) based on an autoregressive forecasting model that was calculated from the window of data. The phase at time zero ("right now") was estimated using a Hilbert transform, the spectral power was estimated from the window of data. The TMS stimulator was triggered when a predefined phase and spectral amplitude condition were met. TMS over the left primary motor cortex resulted in MEPs in right-hand muscles recorded with surface EMG.

Figure 2: Data from one exemplary subject who received real-time EEG-triggered TMS over the left M1, targeting the phase of the 10 Hz sensorimotor μ-rhythm. A hundred stimuli each were applied according to three phase trigger conditions (positive peak, negative peak, and random phase) in combination with a constant minimum 10 Hz spectral power threshold condition, in randomized order, with an intertrial interval of approximately 3 s. A Laplacian EEG montage was used to extract the sensorimotor μ-rhythm by referencing the EEG electrode C3 to the average of four surrounding electrodes (FC1, FC5, CP1, and CP5). (**a**) Average prestimulus EEG signal in the 400 ms before the TMS pulse for the three conditions. (**b**) Average EMG trace of the motor evoked potential (MEP) recorded from the right abductor pollicis brevis muscle for each condition. (**c**) Peak-to-peak MEP amplitude (in microvolts) of each trial over time, per trigger condition. Note that the MEPs are largest in the negative peak condition, smallest in the positive peak condition, and intermediate in the random phase condition. (**d**) The mean MEP amplitude in each condition is shown with error bars illustrating the standard error of the mean. Note that

a participant with a particularly clear effect has been selected for illustration purposes and that this effect size is not representative for the group average.

DISCUSSION:

Brain state-dependent EEG-triggered TMS is a novel method with unique perspectives with respect to effectiveness and consistency of the ensuing brain stimulation effects[8,9,31]. The main advantage of the method is that a functionally relevant endogenous brain state may be specifically targeted to trigger the TMS pulse, inducing potentially less variable and longer-lasting brain responses[11]. Real-time EEG-triggered repetitive TMS in the negative phase of the sensorimotor μ-rhythm of human M1 (i.e., the state of increased corticospinal excitability, **Figure 2**) induced significantly stronger LTP-like plasticity (a long-term increase of MEP amplitude) compared to brain-state independent TMS[11,20]. In addition to its scientific utility, the application of real-time EEG-TMS to cortical areas, such as the dorsolateral prefrontal cortex (DLPFC), has the potential to increase the effectiveness of current therapeutic brain stimulation protocols.

In this manuscript, we presented the methodological steps for the implementation of real-time EEG. Fundamental requirements for the conduction of experiments with this method are, first, the use of a TMS-compatible EEG system with a real-time digital out option and, second, the use of real-time signal processing with the implementation of a phase-detection algorithm[24], which extracts the desired brain rhythm (e.g., sensorimotor μ-rhythm) from the recorded EEG signal using spatial filters (e.g., C3-centered Laplacian filter) and applies stimulation when preselected conditions (i.e., phase and power of the targeted brain rhythm) are met. The performance and accuracy of the algorithm depend strongly on the SNR of the EEG recording[20]. Thus, the EEG preparation steps of the protocol are crucial to achieve a high SNR and ensure accurate triggering of the TMS, and a preselection of participants may need to be considered if the respective target oscillation is not sufficiently observable with EEG in every individual. Furthermore, the use of mechanical support arms for the coils and vacuum pillows to immobilize the participant's head is advisable, in order to minimize artifacts due to the varying pressure of the coil on the electrodes.

Regarding the application of the real-time EEG-TMS method in experimental paradigms, the selection of the brain rhythm of interest may vary. Thus, adjustments of the filtering are advisable to facilitate the identification of the targeted brain activity. Recently, several spatial filtering methods have been proposed to optimally extract a functionally relevant brain state (e.g., in channel space[19], with current source density[13], with local spatial filters[11,28], and with individualized filters using, for example, spatial-spectral decomposition[29]). Yet, so far, no unequivocal method exists to extract from surface EEG signals (sensor space) the real brain-oscillation phase (source space). Future studies that assess the correspondence of surface and source space signals are warranted to improve the precision of real-time EEG algorithms.

Whereas in this protocol we have focused on the 8–14 Hz sensorimotor μ-rhythm to demonstrate the influence of the instantaneous phase of this oscillation on corticospinal excitability, other oscillations (e.g., beta, theta, or infraslow oscillations) may also play a role. This method can, in principle, be used to target the phase for any oscillation that can be isolated with a sufficient SNR, including multiple superimposed oscillations (e.g., a negative cycle of alpha and a

simultaneous positive peak of gamma).

One main limitation of the real-time EEG-TMS experiments is that the spatiotemporal resolution with respect to the brain sources is strongly dependent on artifact occurrence and consistency of the stimulation. Therefore, a critical prerequisite of the protocol is the monitoring of the performance of the algorithm (i.e., ensuring that stimulation occurs upon the detection of neuronal and not artifactual activity throughout the experiment). Furthermore, the utilization of neuronavigation for optimal and consistent positioning of the stimulation coil (especially in experimental paradigms using stimulation sites such as the DLPFC) is helpful for reducing response variability due to variability in coil position. Note also, as a further limitation, that specifically selected and configured EEG/EMG, TMS, and real-time processing devices are required, along with experience in preparing and conducting the experiments in such a way as to minimize external sources of response variability that may mask the effect of instantaneous brain-state.

In conclusion, we demonstrated a standard protocol for conducting real-time EEG-TMS experiments and introduced a novel method for utilizing the endogenous brain states of interest (i.e., preselected phases and power of a targeted endogenous brain oscillation) to trigger brain stimulation. Further research using the real-time EEG-TMS method will allow methodological improvements and facilitate the development of effective protocols for the study and modulation of human brain networks.

ACKNOWLEDGMENTS:

C.Z. acknowledges support from the Clinician Scientist Program of the Faculty of Medicine, University of Tübingen. U.Z. acknowledges support from the German Research Foundation (grant ZI 542/7-1). T.O.B. acknowledges support from the German Research Foundation (grant BE 6091/2-1). The authors acknowledge support by the Open Access Publishing Fund of the University of Tübingen.

DISCLOSURES:

C.Z. and P.C.G. are partially funded through an EXIST Transfer of Research grant by the German Federal Ministry for Economic Affairs and Energy (grant 03EFJBW169). C.Z. reports additional employment as a part-time employee of the not-for-profit medical innovation foundation (Stiftung für Medizininnovationen, Tübingen, Germany); a subsidiary of this foundation is producing the real-time processor used in this article (Medical Innovations Incubator GmbH, Tübingen, Germany).

REFERENCES:

1. Hallett, M. Transcranial magnetic stimulation: a primer. *Neuron.* **55** (2), 187-199 (2007).
2. Barker, A. T., Jalinous, R., Freeston, I. L. Non-invasive magnetic stimulation of human motor cortex. *Lancet.* **1** (8437), 1106-1107 (1985).
3. Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., Fernandez-Del-Olmo, M. Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimulation.* **7** (3), 372-380 (2014).

4. Muller-Dahlhaus, J. F., Orekhov, Y., Liu, Y., Ziemann, U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Experimental Brain Research.* **187** (3), 467-475 (2008).
5. Ziemann, U., Siebner, H. R. Inter-subject and Inter-session Variability of Plasticity Induction by Non-invasive Brain Stimulation: Boon or Bane? *Brain Stimulation.* **8** (3), 662-663 (2015).
6. Arieli, A., Sterkin, A., Grinvald, A., Aertsen, A. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science.* **273** (5283), 1868-1871 (1996).
7. Thut, G., Ives, J. R., Kampmann, F., Pastor, M. A., Pascual-Leone, A. A new device and protocol for combining TMS and online recordings of EEG and evoked potentials. *Journal of Neuroscience Methods.* **141** (2), 207-217 (2005).
8. Zrenner, C., Belardinelli, P., Muller-Dahlhaus, F., Ziemann, U. Closed-Loop Neuroscience and Non-Invasive Brain Stimulation: A Tale of Two Loops. *Frontiers in Cellular Neuroscience.* **10**, 92 (2016).
9. Bergmann, T. O. Brain State-Dependent Brain Stimulation. *Frontiers in Psychology.* **9**, 2108 (2018).
10. Matthews, P. B. The effect of firing on the excitability of a model motoneurone and its implications for cortical stimulation. *Journal of Physiology.* **518 (Pt 3)**, 867-882 (1999).
11. Zrenner, C., Desideri, D., Belardinelli, P., Ziemann, U. Real-time EEG-defined excitability states determine efficacy of TMS-induced plasticity in human motor cortex. *Brain Stimulation.* **11** (2), 374-389 (2018).
12. Stefanou, M. I., Desideri, D., Belardinelli, P., Zrenner, C., Ziemann, U. Phase Synchronicity of mu-Rhythm Determines Efficacy of Interhemispheric Communication Between Human Motor Cortices. *Journal of Neuroscience.* **38** (49), 10525-10534 (2018).
13. Berger, B., Minarik, T., Liuzzi, G., Hummel, F. C., Sauseng, P. EEG oscillatory phase-dependent markers of corticospinal excitability in the resting brain. *BioMed Research International.* **2014**, 936096 (2014).
14. Keil, J. et al. Cortical brain states and corticospinal synchronization influence TMS-evoked motor potentials. *Journal of Neurophysiology.* **111** (3), 513-519 (2014).
15. Rogasch, N. C. et al. Analysing concurrent transcranial magnetic stimulation and electroencephalographic data: A review and introduction to the open-source TESA software. *NeuroImage.* **147**, 934-951 (2017).
16. Herring, J. D., Thut, G., Jensen, O., Bergmann, T. O. Attention Modulates TMS-Locked Alpha Oscillations in the Visual Cortex. *Journal of Neuroscience.* **35** (43), 14435-14447 (2015).
17. Romei, V. et al. Spontaneous fluctuations in posterior alpha-band EEG activity reflect variability in excitability of human visual areas. *Cerebral Cortex.* **18** (9), 2010-2018 (2008).
18. Sauseng, P., Klimesch, W., Gerloff, C., Hummel, F. C. Spontaneous locally restricted EEG alpha activity determines cortical excitability in the motor cortex. *Neuropsychologia.* **47** (1), 284-288 (2009).
19. Bergmann, T. O. et al. EEG-guided transcranial magnetic stimulation reveals rapid shifts in motor cortical excitability during the human sleep slow oscillation. *Journal of Neuroscience.* **32** (1), 243-253 (2012).
20. Schaworonkow, N., Triesch, J., Ziemann, U., Zrenner, C. EEG-triggered TMS reveals stronger brain state-dependent modulation of motor evoked potentials at weaker stimulation intensities. *Brain Stimulation.* **12** (1), 110-118 (2019).

21. Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology.* **120** (12), 2008-2039 (2009).
22. Kanal, E. et al. ACR guidance document for safe MR practices: 2007. *American Journal of Roentgenology.* **188** (6), 1447-1474 (2007).
23. Oldfield, R. C. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia.* **9** (1), 97-113 (1971).
24. Chen, L. L., Madhavan, R., Rapoport, B. I., Anderson, W. S. Real-time brain oscillation detection and phase-locked stimulation using autoregressive spectral estimation and time-series forward prediction. *IEEE Transactions on Biomedical Engineering.* **60** (3), 753-762 (2013).
25. Habibollahi Saatlou, F. et al. MAGIC: An open-source MATLAB toolbox for external control of transcranial magnetic stimulation devices. *Brain Stimulation.* **11** (5), 1189-1191 (2018).
26. Lioumis, P., Zomorrodi, R., Hadas, I., Daskalakis, Z. J., Blumberger, D. M. Combined Transcranial Magnetic Stimulation and Electroencephalography of the Dorsolateral Prefrontal Cortex. *Journal of Visualized Experiments.* (138), e57983 (2018).
27. Mishory, A. et al. The maximum-likelihood strategy for determining transcranial magnetic stimulation motor threshold, using parameter estimation by sequential testing is faster than conventional methods with similar precision. *The Journal of Electroconvulsive Therapy (ECT).* **20** (3), 160-165 (2004).
28. Thies, M., Zrenner, C., Ziemann, U., Bergmann, T. O. Sensorimotor mu-alpha power is positively related to corticospinal excitability. *Brain Stimulation.* **11** (5), 1119-1122 (2018).
29. Schaworonkow, N. et al. mu-Rhythm Extracted With Personalized EEG Filters Correlates With Corticospinal Excitability in Real-Time Phase-Triggered EEG-TMS. *Frontiers in Neuroscience.* **12**, 954 (2018).
30. Hjorth, B. An on-line transformation of EEG scalp potentials into orthogonal source derivations. *Electroencephalography and Clinical Neurophysiology.* **39** (5), 526-530 (1975).
31. Bergmann, T. O., Karabanov, A., Hartwigsen, G., Thielscher, A., Siebner, H. R. Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: Current approaches and future perspectives. *NeuroImage.* **140**, 4-19 (2016).

Figure 1

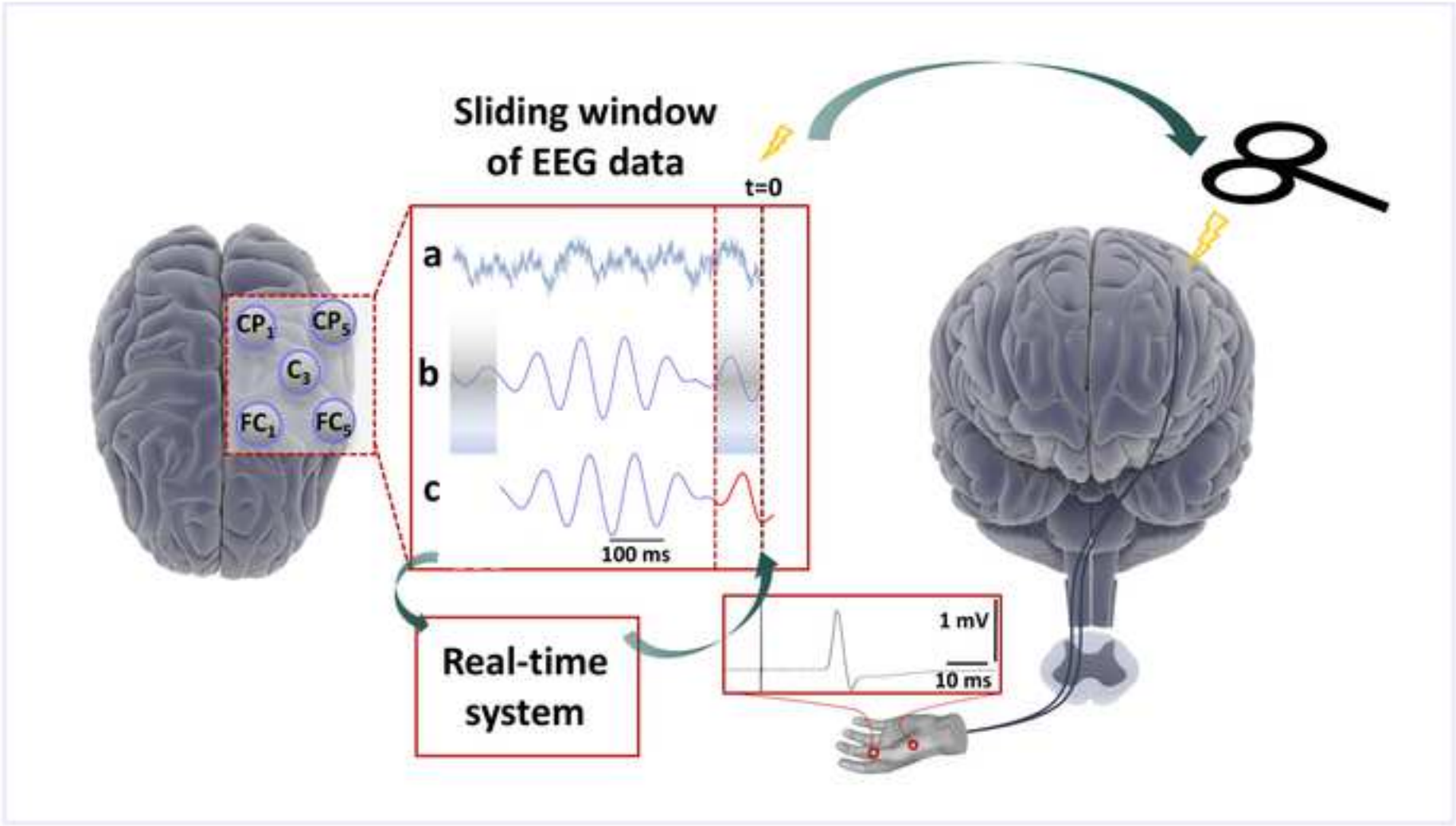


Figure 2

Click here to access/download;Figure;Figure2resubmission.tiff

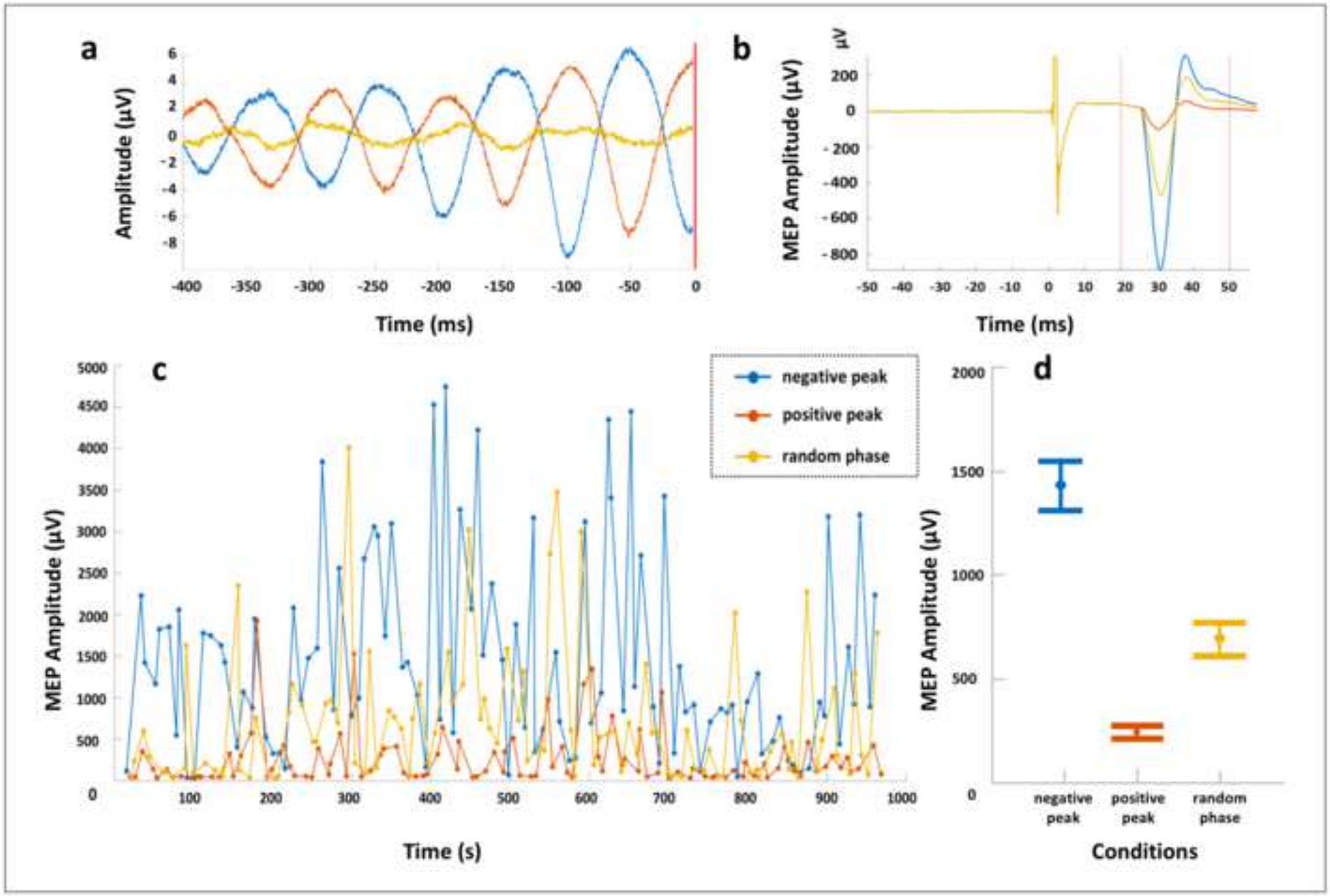


Table of Materials

Click here to access/download;Table of Materials;Table of materials.xlsx

Name	Company	Catalog No	Comments
EEG and EMG recording systems			
EEG/EMG amplifier	NeurOne with Real-time Digital Out, Bit		
TMS device	MAG & More Research 100, MAG & Mo		
Software	Mathworks Simulink Real-Time (Mathw		
Stereo infrared camera neuronavigation system including reflective head tracker, pointer tool,			
Experimental control PC that is connected to the EEG system, the TMS stimulator, the real-tim			
EEG electodes, EMG electrodes, syringes, abrasive and conductive gel			
Plastic wrap and adhesive tape			

tium Biosignals Ltd., Finland
re GmbH, Munich, Germany
orks Ltd, USA)
head tracker
e device and the neuronavigation system

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:	Brain state-dependent brain stimulation with real-time EEG-triggered TMS
Author(s):	Maria-Ioanna Stefanou, David Baur, Paolo Belardinelli, Til Ole Bergmann, Corinna Blum, Pedro Caldana Gordon, Brigitte Zrenner, Ulf Ziemann, Christoph Zrenner

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☒ Standard Access ☐ Open Access

Item 2: Please select one of the following items:

☒ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

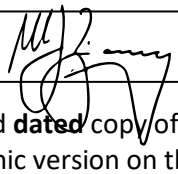
12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Prof. Ulf Ziemann
Department:	Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research
Institution:	University of Tübingen, Germany
Title:	Brain state-dependent brain stimulation with real-time EEG-triggered TMS
Signature:	
Date:	17.01.2019

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

Response to the Reviewer Comments

Brain state-dependent brain stimulation with real-time EEG-triggered TMS

Maria-Ioanna Stefanou, David Baur, Paolo Belardinelli, Til Ole Bergmann, Corinna Blum, Pedro Caldana Gordon, Brigitte Zrenner, Ulf Ziemann*, Christoph Zrenner

Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, University of Tübingen, Germany.

*Corresponding Author

We appreciate the time and effort of the editor and referees put into reviewing this manuscript. We have addressed all issues indicated in the review report. Here we examine the suggestions one by one and discuss how we accordingly revised the manuscript.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

The manuscript was checked for spelling and grammar.

2. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

We have rephrased accordingly the sections 2.4.2, 2.5.1 and 3.8.4 using imperative tense.

3. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

The discussion about the protocol was removed and one sentence about the ethical approval of the demonstrated experiments was moved up to the introduction.

4. Step 2.3 might be better suited for the Representative Results.

Thank you for this suggestion, but we respectfully disagree on this question, because Step 2.3. is a core component of the method and protocol and prerequisite for the performance of real-time EEG-triggered TMS. Therefore, we would not find it appropriate for the representative results section.

5. 3.2.6: How many electrodes are placed? Can you be more specific on the locations?
We have in the revised manuscript specified the specific montage used in our example. Also, in the section of representative results, we provide an example of EMG electrodes placed over the right-hand muscle (Abductor pollicis brevis), as depicted in Figure 2.

6. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
We have highlighted in light blue the parts of the protocol that should be included in the video.

7. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.
The highlighted steps provide a cohesive narrative and summary of the real-time EEG-triggered TMS method.

8. Please do not abbreviate journal titles.
All references were corrected to include the full journal titles.

Reviewers' comments:

Reviewer #1:

We thank the reviewer for their helpful comments.

Major Concerns:

No Major comments

Minor Concerns: There are some minor comments concerning the manuscript itself:

1. line 43: remove the word human. TMS is applied for this purpose both in healthy and diseased humans.
   We agree and have made the change accordingly.
2. Line 44: the spatial percision depends on navigation and intensity. Please rephrase accordingly.
   The sentence was revised referring to "high spatiotemporal precision" of TMS.
3. line 46: please remove "non-invasively using" and write:..can be recorded by EMG of the target ....
   The sentence was corrected as suggested.
4. line 47: replace TMS evoked EMG potentials with MEPs, or TMS-evoked MEPs

This was corrected to MEPs.

5. line 48: In this same paragraph introduce TMS-EEG. It comes a bit suddenly later on.
   We now introduce TMS-evoked EEG potentials in line 45.
6. line 58: I would definitely mention here a model of Matthews, although you need to generalize this sentence. P.B.C Matthews. The effect of firing on the excitability of a model motorneurone and its implications for cortical stimulation. The Journal of Physiology, 518:867-882, 1999.
   The suggested literature was added and the sentence was generalized to refer to optimization of the stimulus timing.
7. line 60: None of these references (7,10-12) have to do with is stated in lines 59-60. 10 is about tms-eeg methodoloy and the rest about tms-eeg amplifiers. Rephrase or use the correspong to the statement citations.
   Thank you for pointing this discrepancy in the references, the literature was accordingly revised.
8. line 118: A TMS-compatible EEG/EMG amplifier (references or suggestions of systems especially for the eeg)
   The following sentence was added " In this example, we use a 24-bit 80-channel biosignal amplifier for EEG and EMG recordings (NeurOne with Real-time Digital Out, Bittium Biosignals Ltd., Finland)".
9. paragraph 2.1.2 make it smaller and use shorter sentences. difficult to follow for non-experts
   This paragraph was revised as suggested.
10. line 132: please propose some references for such systems asd you describe.
    The following sentence was added " In this experiment, we use the MAG & More Research 100 (MAG & More GmbH, Munich, Germany) TMS device".
11. paragragraph 2.2.2: Give a suggestion, 1m 3m how much? Most importantly the cables of the eeg should be crossed so that electromagnetic interferences are cancelled out. Devices such as fans etc should be switched off etc.
    The sentence was revised as follows "Ensure that the distance between the TMS stimulator (incl. coil and coil cable) and the EEG recording system is maximized to reduce electrical interference (at least 1 m). Where possible, turn off sources of electromagnetic interference such as fans and motors. Furthermore, ensure that the EEG and EMG recording leads are positioned and aligned such that common interference cancels out."
12. paragraph 3.2: You can refer to reference 10 if you think and agree with the procedures, since those authors focused on these issues.
    Thank you for this suggestion, the reference was added.
13. paragraph 3.2.6: This can be an issue for EMG triggered TMS. The placing of the electrodes can be of big issue. Usually there is not a problem with FDI and ADM but APB is often confused with FPB. Maybe you should mention this here or in the discussion in the limitations paragraph.
    Thank you for this suggestion. We have revised the paragraph as follows: "Attach the surface EMG electrodes over the target muscles after having cleaned and lightly abraded the skin. In our experiment we use a bipolar recording from the right abductor pollicis brevis hand muscle in belly-tendon montage.

Note that the placing of EMG electrodes is important, as surface electrodes generally record activity from multiple underlying muscles".

14. line 3.2.8: Maybe you can add here to check eyes open eyes closed since it is important for this triggering protocol

    This is indeed a good point and was added as an extra step 3.2.9. "Ensure that the participant remains awake and keeps their eyes open throughout the experiment to avoid occipital alpha oscillations contaminating the signal".

15. 3.3.3 can be done at the end too. Preferably at the end

    This was corrected as suggested.

16. 3.6.1 b) I think this is very strict guideline. Most of patients have bigger than 60% especially if the stimulators are monophasic (many places use monophasic because of paired pulse dielivery and cortical inhibition befpre and after rTMS).

    We agree and have removed this criterion, which originated in previous experiments with different stimulators where overheating of the coil was an issue.

17. 3.6.3 If not white noise, earplugs and headphones together.

    We revised section 3.6. accordingly. In light of the above changes we have further revised section 3.6 changing the title to "Final participant preparation" and moving the alpha power criterion to section 1.1 Subject recruitment as 1.1.2.

18. 3.7.3 I would thing that at that point it could be suggested to check if the TMS is triggered when eyes are closed (elevated alpha, very easy task in order to check the cabling).

    This is a good suggestion, we have added this instruction in 3.2.7 in the revised manuscript "Verify that occipital alpha increases when the participant closes their eyes."

19. line 310: please add a reference there at the end of this line

    We added reference as suggested.

20. line 343: You could add here that this kind of experiments need high expertise, very specific and good/expensive devices and of course excellent lab designing. I would add also to take care the EMG electrode placing and the EEG placing/preparation.

    We agree with this point and we have added the following sentence to the revised manuscript "Note also as a further limitation, that specifically selected and configured EEG/EMG, TMS and real-time processing devices are required along with experience in preparing and conducting the experiments in such a way as to minimize external sources of response variability that may mask the effect of instantaneous brain-state.".

Reviewer #2:

I have a few minor suggestions and comments.

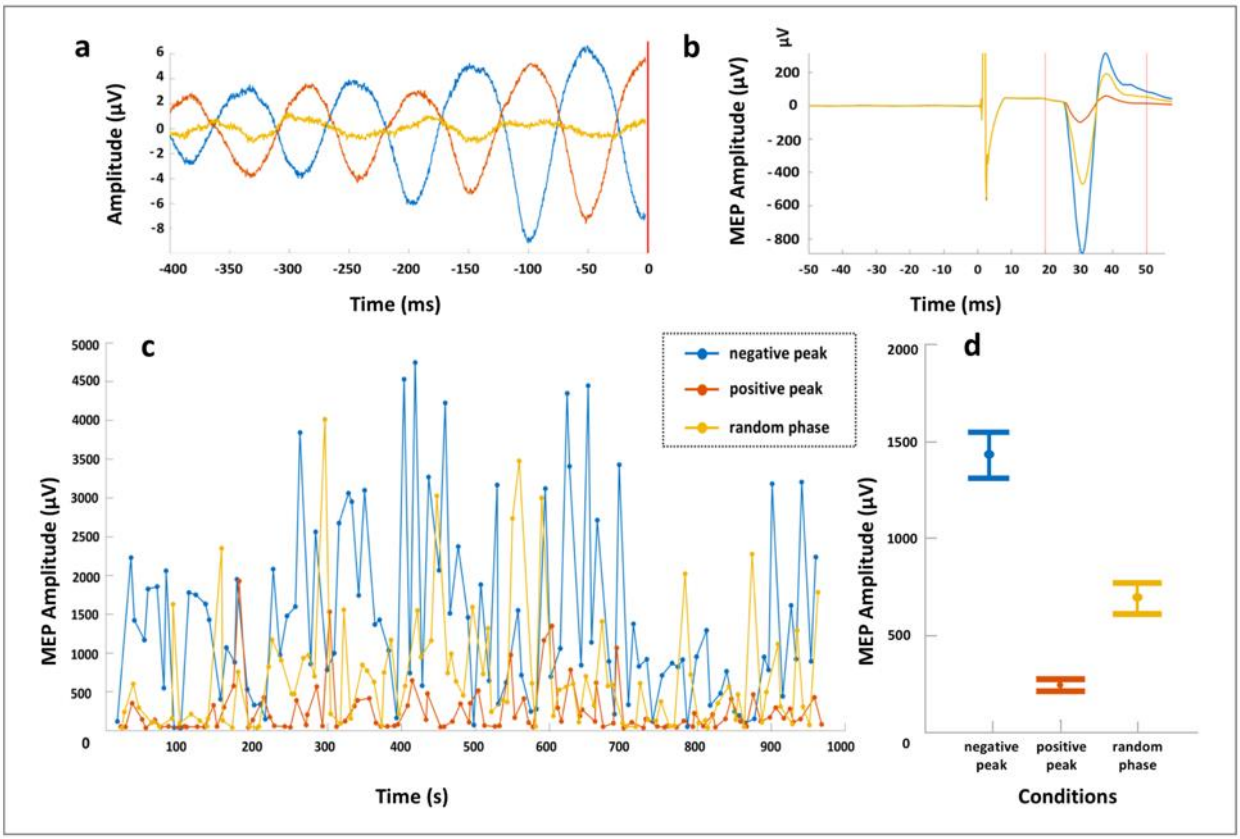
1. subsection 2.1.1. Authors suggest that to handle large voltage TMS spikes the the amplifier needs to be 24-bit, but this assumes a particular resolution (a 2 bit amplifier can also 'handle' large voltage spikes

setting the range to -500 to +500 mV, but this would preclude reliable measurement of the EEG). Please correct.

We agree with this comment. We corrected the subsection 2.1.1, which now reads as follows "A TMS compatible EEG/EMG amplifier is required that can handle the voltage spikes induced by the TMS pulse".

2. In figure 2, subpanel C, showing the raw data, I would suggest to add (e.g. an additional subplot) the mean and sd values for each of the three experimental conditions.

We like this suggestion. We have added this subpanel in Figure 2 as subpanel D, indicating the mean and standard error of the mean. We have chosen the standard error over the standard deviation in order to illustrate the confidence of the true mean since the variability of the data can be discerned from the raw data in panel C. We have accordingly updated the figure legend.



3. Authors may discuss the potential phase dependency of the response for other frequencies (e.g. theta or infraslow activity), and if this would complicate the real-time phase extraction for the protocol.

We agree that this is a relevant point and have added the following sentence to the discussion: “Whereas in this protocol we have focused on the 8-14 Hz sensorimotor μ-rhythm to demonstrate the influence of instantaneous phase of this oscillation on corticospinal excitability, other oscillations (e.g. beta, theta, or

infraslow oscillations) may also play a role. This method can in principle be used to target the phase for any oscillation that can be isolated with sufficient signal-to-noise ratio, including multiple superimposed oscillations (e.g. negative cycle of alpha and a simultaneous positive peak of gamma).", we have also rephrased the following paragraph for clarity.

4. As 'the RMT' is not constant - in part a function of the phase of the mu-rhythm- how reliable is this estimate and or can authors provide a rule as to how many trials are needed to estimate the *mean* RMT?

This is a good point, and part of the motivation for using a maximum likelihood strategy when estimating RMT. We have added the following to protocol 3.5.2. reading "which also provides an online estimate of the confidence interval of RMT based on the observed variability of single responses and which typically requires ca. 30 test pulses of adaptively varying intensity to obtain a robust RMT estimate."

We appreciate the comments of the reviewers and hope that the revised version of the manuscript can meet the journal publication requirements.