Phase-dependent Effects of Beta Transcranial Alternating Current Stimulation on Corticospinal Excitability

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Summary

The following work constitutes the result of my Ph.D. project, carried out at the Division of Functional and Restorative Neurosurgery of the University of Tübingen. The work I have done is documented in three manuscripts I have completed during this period of time. The project focused on validating the ability of transcranial alternating current stimulation (tACS) to entrain beta sensorimotor rhythms. In the introduction, I will provide the background necessary to understand the impact of my work and the scientific questions that drove my research. I will thus describe the framework proposed to explain the functional role of brain oscillations and the research tools necessary to test its predictions. I will talk about tACS, and the putative mechanisms of interaction with these brain rhythms. Finally, I will describe the evidences of entrainment on sensory and motor systems. I will try to make my point that true evidence of entrainment is still sparse, and in the context of the motor system, insufficient. Thus, I will describe the three studies that composed my work, explaining how they are connected and how they contribute to the final goal. I will then conclude this work by including the three manuscripts I have produced with the support of my supervisor and other members of the team, as evidence of the work I have accomplished during my Ph.D.

Introduction

Brain oscillations and their role in cognition

Magneto/electroencephalographic techniques (M/EEG) provide non-invasive recordings of the local changes in brain electrical activity through electrodes positioned on the scalp. Signals recorded with these techniques reflect the sum of inhibitory and excitatory post-synaptic potentials of large neural populations activated synchronously [1]. Despite the remarkable temporal resolution offered by these techniques, one of the main limiting factors of M/EEG recordings is represented by the spreading of electrical currents through the biological tissue that results in a mixed activity coming from different sources reaching the same recording channel. As a consequence, raw signals collected by these techniques often lack a clear relationship to other behavioral indexes. Yet, by decomposing M/EEG recordings in a finite number of sinusoidal components, spectral analysis techniques are able to disentangle the activity of the underlying networks. This is possible due to the tendency of a large group of interconnected neurons to act as harmonic oscillators [2]. These neural oscillations are thought to be generated from the interplay of cellular and network mechanisms inducing brief alternating periods of depolarization and hyperpolarization in the cells membrane potentials. Specifically, the number of neurons synchronized to the same oscillation determines the amplitude (or spectral power) of the signal at that particular oscillating frequency, while the phase of the oscillation encodes the excitability state of the network [3]. The ubiquity of these oscillations in the central nervous system, and their reactivity to various tasks and pathological states, seem to suggest that they might constitute a general mechanism of neural computation. As a consequence, a strong effort has been made to understand the functional role that these rhythms play in regulating cognitive

processes. While some theories focused on specific cortical rhythms and try to explain them separately ([4–6], others tried to provide an integrated framework to explain all the oscillatory phenomena as different aspects of a unique mechanism [7]. An appealing hypothesis is that neural oscillations might be a convenient way to orchestrate complex and widely independent computational modules, promoting synchronization and communication within and between different areas of the nervous system by parceling the information packages into discrete time windows [8]. Thus, rhythmic brain activity would be responsible for local computation as well as communication across different areas [9], supervising the whole brain's cognitive apparatus. Due to the interest catalyzed by these phenomena, the study of oscillatory potentials assumed a dual importance for brain research: from one side, the centrality of the role they seem to play in regulating brain activity requires a systematic and careful testing of all the assumptions used to formulate this hypothesis. Additionally, brain oscillations constitute a novel way to analyze and quantify brain activity, which is largely independent, but complementary to other known explanatory mechanisms (e.g. neural coding).

A suitable research tool for brain oscillations

The hypothesized role that brain oscillations play in the organization of cognitive functions provides an enormous amount of predictions that can be tested. It is, therefore, crucial that these predictions find confirmation from experimental studies in order to circumscribe the real links that connects brain oscillations and behavior. Representing a new explanatory layer to understand brain activity, the study of neural oscillations also requires a new set of tools capable of inducing specific and targeted changes in the spectral profile of brain activity, and thus correlates it to their

effects on behavior. Despite the longstanding tradition in neuroscience of linking electrophysiological recordings to specific tasks or pathological states, this approach tends to provide data supporting the opposite line of evidence: indeed, by using the execution of the task as the independent variable and recording the induced changes in the brain activity patterns, these studies address the question of how the brain responds to the execution of a certain task. Nevertheless, this does not allow distinguishing which activity patterns are strictly responsible for the execution of the task. Because of this, evidences obtained by recordings are vitiated by several confounding factors that limit the conclusions that can be made on the effects of brain activity on behavior. Generally speaking, passive recordings display the activity that is correlated, but not casually linked to the behavior itself [10]. This might include activity patterns that are not directly involved in the task performance, but they are co-activated by secondary processes (i.e. attentional or sensory components). For this reason, the recorded activity might reflect a byproduct of neural computation which lacks any direct causal effect on the behavioral outcome. Thus, to assess the functional role of brain oscillations, a different approach is required.

The virtual lesion approach in neuroscience

Traditionally, brain stimulation techniques represent an answer to this problem, allowing scientists to directly interfere with brain activity and read the changes in behavior that result from this manipulation. A classic example is the virtual lesion approach used in transcranial magnetic stimulation (TMS) studies. By delivering a quick pulse of current on the scalp, TMS is capable of destroying ongoing neural computation in the cortical areas below the stimulation coil. The virtual lesion technique has been used in parallel to magnetic resonance (fMRI) studies to successfully link specific brain areas to their functions. By

scanning the subject's brain during a specific task, fMRI returns a topography of brain structures that increase their metabolic intake (and therefore their activity level) during the performance of a task. The results of the recordings are used to identify a subset of areas as possible candidates for being directly responsible for the task execution. By inducing a strong perturbation in these areas when the subject is performing the same task, and concurrently measuring changes in the performance, it is possible to reveal a direct involvement of the target area in the cognitive processes necessary to perform the task. Thus, despite some limitations [11], a combined 2-steps approach, that uses the recordings to guide brain stimulation techniques, is potentially capable of revealing the causal links connecting cognitive processes with the specific activity patterns generating them. Nevertheless, to apply the same concept to the study of brain oscillations necessitates a paradigm shift from the classic virtual lesion approach: rather than "shutting down" a specific area, the stimulation should promote ongoing brain activity oscillating with specific spectral characteristics, while leaving other computational processes virtually unchanged. With this goal in mind, new stimulation techniques have been developed and applied in different experimental settings to modulate the spectral characteristics of brain activity and measure the behavioral effects of the intervention.

Brain stimulation techniques: unspecific stimulation protocols

If brain oscillations represent a general mechanism of neuronal computation, virtually any electrical field applied to the brain can modulate its spectral profile. Nevertheless, in relation to the study of brain oscillations, we can distinguish between specific and unspecific stimulation protocols. Unspecific protocols have been

developed to test different aspects of brain physiology and then subsequently applied to the study of brain oscillations. They are characterized by currents lacking a well-defined spectral profile, such as random noise (tRNS), pulsed (TMS/TES), or constant currents (tDCS) stimulation. Therefore, rather than targeting a specific oscillation, these protocols tend to interact with brain oscillations in an unspecific way, exploiting the spectral characteristics of the target area. As an example, single TMS pulses are defined by a very complex and wide-band spectral profile; however, when applied to a cortical area, TMS induces a very specific spectral response that is both area and state dependent [12,13]. When applied on the visual system, TMS induces an increase in alpha oscillations at the site of the stimulation, but rather than being a direct response to the stimulation pulse, these oscillations are the result of a phase reset of ongoing alpha oscillators. A similar increase in cortical power is achieved by long lasting polarization of the visual cortex by means of tDCS currents [14,15]. Yet, the direct current of tDCS is not modulated over time, thus it does not have a specific frequency profile. For this reason, the connection between the stimulation and the brain oscillatory response seems to be due to an unspecific increase in excitability induced by the polarization of brain tissue. Therefore, despite several different stimulation protocols are able to elicit a spectral response in the brain, the causal chain leading to these effects is not clear. Given the loose link between the spectral profiles characterizing stimulation and brain response, it is possible that a second mechanism is responsible for the change in the brain spectral profile. This possibility represents a strong limitation for the inferences that can be made on the relationship between brain oscillations and behavior.

Not all stimulation protocols are equally susceptible to this risk: indeed, stimulation approaches directly linked to specific spectral characteristics may intuitively reduce the probability that the

brain spectral response (and the elicited behavior) is possibly due to an independent physiological mechanism. Specific stimulation protocols are characterized by a periodicity of stimulation that makes them suitable to interact with specific spectral characteristics of the brain. By applying a more targeted stimulation, these protocols might provide a clearer interpretation of the stimulation effects and their relationship with behavior. The present work focuses on one of these techniques, that is transcranial alternating current stimulation (tACS).

Transcranial alternating current stimulation

TACS is a variation of tDCS protocols, developed specifically to target directly brain oscillatory potentials in a selective way. As it happens with tDCS, tACS is delivered by two electrodes positioned on the scalp that are charged with currents of equal amplitude, but inverted polarity. Yet, during tACS, the amount of current in each electrode is modulated sinusoidally over time, and the polarity of the electrodes is inverted between the positive and negative cycles of the sinus. Thus, the spectrum of the sinusoidal tACS currents is characterized by a single frequency. Another difference between TMS and tACS is the amount of current used, which in the case of tACS is delivered with much lower amplitude. This is not only due to the painful skin sensations elicited by the stimulation, but also coherent with the desired physiological mechanisms of tACS. As previously described, TMS pulses can have a destructive effect on ongoing brain activity: the strong currents delivered to the target area induce a synchronous and unspecific firing of the targeted neurons, thus producing a phase reset that disrupts ongoing oscillatory activity [16]. On the other hand, tACS aims to promote these oscillations rather than destroying them. By effect of the periodicity of the stimulation currents, neurons are induced to synchronize their membrane

fluctuations to the tACS cycle, thus augmenting the number of neurons participating to the network oscillating at the stimulation frequency [17]. Indeed, in this sense tACS currents have been compared to a metronome dictating the time for several musical instruments to allow them to play as one. This mechanism takes the name of 1:1 frequency locking, or neural entrainment. Nevertheless, entrainment of neural oscillations by tACS is a hypothesis that still needs extensive testing; although it represents an appealing mechanism to explain the effects of tACS on brain activity, direct evidences of this mechanism are still sparse.

Entrainment hypothesis of tACS effects

Entrainment of brain oscillations has been proposed as the primary mechanism to explain behavioral and physiological changes happening in response to tACS. By its definition, entrainment is characterized by a robust phase-locking between the stimulation currents and the ongoing brain rhythms [17]. As consequence of this locking, neural oscillations synchronize an increasing number of neurons to the stimulation currents, resulting in an increase in spectral power at the frequency of stimulation. However, despite the phase-locking is a defining criterion for neural entrainment, as discussed in the previous chapter, an increase in spectral power can be the result of other brain stimulation mechanisms. Therefore, to support the entrainment hypothesis of tACS, scientists must provide evidences of an effective phase-locking of brain oscillations to the tACS waveform. Direct EEG recordings can be used to assess phase-locking and thus support the tACS entrainment effects. Alternatively, behavioral outcomes that are linked to specific features of brain oscillations can be used to infer the response of the brain to the stimulation. Additionally, the stimulation effects can be tested either on-line or offline (i.e. aftereffects) to the

stimulation, depending on the rationale of the study, and on methodological issues. Not requiring additional inferences, direct recordings are often preferred to behavioral outcome when trying to show entrainment effects. Nevertheless, the presence of stimulation artifacts [18], and stimulation electrodes covering a part of the scalp complicates the recordings. For this reason, when direct EEG recordings are used to test the effects of tACS, they either heavily rely on artifact rejection algorithms that might distort the EEG signals, or are often restricted to the pre- and post-stimulation periods which are not contaminated by the stimulation artifacts [19]. The pre-post approach raises some additional problems that should not be overlooked: First, the phase of brain oscillations has always been a very elusive phenomenon, and it is conceivable that any phase-locking, which is the primary index of entrainment, won´t survive for long after the end of the entraining stimulus. Secondly, it has been suggested that the after effects outlasting the stimulation might reflect a different mechanism from the online entrainment effects [20]. Thus, when assessing the ability of tACS to entrain brain oscillations, an online approach is highly preferable. This holds true despite the use of behavioral indexes to show the stimulation effects. By exploiting established links between specific brain activity patterns and behavior, it is possible to provide detailed information on the effects of the stimulation, without resorting to direct recordings. Here I will review the results obtained by stimulation of sensory alpha rhythms where the most convincing results in favor of the entrainment hypothesis have been obtained, and by stimulation of the sensorimotor system, which is also the focus of the present work.

tACS entrainment of sensory systems

Most of the evidence in support of an entrainment effect of tACS has been acquired from the visual system. The primary visual

cortex (V1) responsible for processing visual information is located in the occipital cortex. Oscillations in the alpha range (7 – 14 Hz) have been detected over these areas when subjects maintained their eyes closed, almost ninety years ago [21], and since then, occipital alpha rhythms became the focus of many studies. It is understandable that tACS research exploited the large amount of preexisting research on occipital alpha rhythm to probe its ability to entrain brain rhythms. The first study showing entrainment of alpha rhythms was published in 2010, by Zahele and colleagues [22]. In this study, the authors stimulated the occipital areas at the individual alpha peak for 10 minutes and recorded the pre-post changes in EEG activity, showing an increase in endogenous alpha rhythms after the cessation of the stimulation. Gamma oscillations have been linked to visual processing**,** and they are generally co-modulated with alpha activity [23,24]; thus Laczo et al. [25] stimulated the visual cortex with three different gamma frequencies (40, 60 and 80 Hz) showing an increase in a visual discrimination tasks presented during the stimulation. A similar effect, but for a different visual task, has also been found during very brief trains of 10 Hz stimulation [26]. The increase in cortical power after alpha tACS found in the early study of Zahele and colleagues [22] has been replicated by a second study [27], which tested whether in an intermittent stimulation protocol, the relative phase shift between two stimulation periods would modulate the tACS aftereffects. Surprisingly, the study revealed that despite tACS was capable of increasing the endogenous alpha power, the phase consistency of the stimulation was not one of the factors determining this effect. Entrainment requires the phase-locking between brain oscillations and the stimulation. This requires a stimulation protocol characterized by a strong phase consistency. Thus, the authors concluded that the increase in power induced by the stimulation must have been determined by alternative mechanisms (i.e. plastic changes), not related to brain oscillations. Nevertheless, a second study applied sophisticated artifact

rejection methods to clean the EEG traces and assess the phase and power of alpha oscillations during and after 10 Hz stimulation [28]. The authors have shown an increase in power and phase-locking to the stimulation currents both during and beyond the stimulation period. Additionally, they reported a modulation of the detection of visual stimuli dependent on the phase of the tACS current, providing the most comprehensive result in support of tACS entrainment. A similar entraining effect of tACS on M/EEG recordings, obtained by a different signal processing approach, has been reported by Runhau and colleagues [29], which also described a state-dependency of this effect, selective for the eyes open condition. Nevertheless, in this study a control condition characterized by a very low intensity level (0.05 mA) has been shown to be effective in modulating cortical power, thus casting some doubts on the preprocessing technique used to remove the stimulation artifact. Despite the amount of data available on the visual system, results on other sensory systems are still very limited: on the somatosensory system, Feurra and colleagues [30], showed that 5 seconds of tACS were able of eliciting tactile sensations over the contralateral hand in a frequency-dependent way (alpha, beta and gamma, but not delta, theta and low-gamma). Notably, a recent study [31] explored the phase-dependent effects of somatosensory tACS on the individual alpha peak, showing a phase-locking between the detection of near-threshold stimulation of afferent fibers and the tACS waveform. This last result has also been confirmed for the auditory system [32], where the phase of 10 Hz stimulation was linked to sinusoidal fluctuations of the auditory threshold. These results, although not including direct EEG recordings, are indicative of an underlying phase entrainment of oscillatory rhythms that modulate the behavioral performance.

Overall, although still limited, tACS research on sensory systems seems to converge toward an entrainment effect of alpha rhythms

by tACS currents. These effects have also been shown to have direct repercussions on several aspects of perception. Thus, research in these areas is slowly shifting from probing the effects of tACS on brain oscillations to showing the role of the latter in shaping behavior. Nevertheless, these results do not directly translate to the stimulation of the motor system, where evidences of tACS entrainment are still very inconsistent.

Stimulation of the motor system

Together with the visual system, the motor system is the other most extensively studied functional module of brain physiology. This is probably the result of a conjunction of factors, including the ability of the motor system to provide clear and measurable behavioral indexes reflecting its physiological state. The primary motor cortex (M1) contains the cell bodies of more than 40% of the projections to the motor neurons innervating skeletal muscles [33]; therefore, there is a strict correspondence between the physiology of M1 and the motor output. These characteristics of the motor system come with great advantages when testing the effects of tACS on cortical physiology. Nevertheless, this did not translate into stronge evidence for entrainment effects of tACS which are still very limited: In 2008, Antal and colleagues stimulated the primary motor cortex at different frequencies for 5 minutes [34]. The stimulation, characterized by a sinusoidal superimposed to a positive offset, was run at small intensities to avoid direct retinal stimulation. No effects on cortical excitability, measured as the size of TMS-induced motor evoked potentials (MEP), and no increase in cortical power were found following stimulation. Nevertheless, in a second experiment they found improvement in an implicit motor learning task executed during 10 Hz stimulation. TACS was applied during slow wave sleep with a stimulation frequency matching the frequency of the slow oscillations dominating the EEG traces in this state. Despite the

stimulation was delivered to the subjects for a relative short time (<30 seconds), the authors recorded a net increase of MEPs elicited during the stimulation, but no modulation of this effect by the phase of tACS was found [35]. In addition, this result was confounded by the application of an offset; together with the tACS oscillation that may have played a role in the MEP increase. The same group replicated this effect and showed that the MEP directionality of the modulation was indeed dependent on the polarity of the offset [36]. In line with the hypothesized role of sensorimotor beta rhythms [37], 20 Hz tACS over M1 has been shown to decrease movement speed and entrain oscillations in the peripheral muscles after stimulation periods of only 10 seconds [38]. Additionally, gamma tACS has been found to induce the opposite effect on movement speed [39], reflecting a well-known dissociation between sensorimotor beta and gamma rhythms [40]. M/EEG recordings after 15 minutes of 20 Hz stimulation in patients affected by Parkinson's disease evidenced a decrease in beta coherence between M1 and periphery, accompanied with a decrease in movement variability during a finger tapping task [41]. 15 Hz tACS has been found to also decrease corticospinal excitability [42], while 20 Hz stimulation increased the size of the MEPs recorded during <2 minutes of stimulation at rest [43,44]; and 5 Hz stimulation had a similar effect during a motor imagery task [44]. When TMS was triggered at specific points of the tACS waveform to assess the phase dependent effects of 10 Hz tACS on corticospinal excitably, Goldsworthy and colleagues showed that MEP were depressed when they were elicited at the trough of the tACS cycle. yet there was no effect with the level of alpha power post stimulation [45]. In Parkinson's patients, tACS delivered at the patients' tremor frequency was shown to be able to modulate the amplitude of the tremor. Crucially, the modulation was a function of the relative phase shift between tremor and tACS, indicating a causal relationship between cortical oscillations and tremor [46].

Yet, despite the presence of such phase-dependent effects for very slow tACS stimulation, there is a fundamental lack of results showing these effects at faster sensorimotor rhythms. Although an increase in corticospinal excitability has been found during 20 Hz stimulation, it is important to repeat that this was not sufficient evidence for entrainment of sensorimotor rhythms. Since the stimulation might induce the same effects by different mechanisms without interfering with ongoing brain oscillations, or even induce the same non-specific beta increase found following TMS stimulation [47]. The frequency specificity of this effect certainly reinforces the possibility of effective entrainment, but it can easily significate that the mechanism responsible for the stimulation effect is sensitive to beta periodicity, which would be unsurprising considering the importance of these rhythms for the motor system. Thus, it is impossible to over-stress the importance of effects that are modulated by the tACS phase, or a direct phase-locking to the stimulation, to indicate the presence of entrainment. Very few studies address this point in the context of the motor system [38,46]. Here, we want to fill this gap by showing a modulation of the increase of cortical excitability by effects of beta tACS found in the literature [43,44], based on the phase of the stimulation cycle.

Description of the project

Rationale

As I discussed in the introduction, phase-locking effects are the primary characteristics of entrained brain oscillations. Thus, any study that wants to show the success of the intervention must show a modulation of the effects depending on the stimulation phase. This has been clearly shown for the visual [28], somatosensory [31], and auditory system [32]. When tACS was

applied over the cortical areas responsible for these sensory modules, perception was modulated by the phase of the stimulation waveform. This is a very strong evidence of an underlying entrainment effect. Nevertheless, the phase-dependent effects of the stimulation have not been addressed yet in the context of the motor system. This is the case despite the well documented and largely reproduced results of beta tACS over the sensorimotor system. In the present work, we reported the first evidence of clear phase dependent effects of 20 Hz stimulation over the sensorimotor system. In order to provide these results, we proceeded by steps: first by documenting the undesired effects of the stimulation that could hamper the blinding of the intervention (first study), secondly by developing a dedicated protocol that allowed us to robustly target the tACS waveform at a desired phase (second study), and last, by testing this protocol in a larger group of subjects by probing the level of corticospinal excitability at the instantaneous phase of the tACS waveform (third study).

Understanding the side-effects of tACS

In one of its first application, tACS has been found to induce strong visual sensations when applied over the occipital areas, in a frequency- and state-dependent fashion [48]. This result was welcomed with initial optimism, as clear index of a direct entrainment of oscillatory activity by the stimulation currents. For a short time, tACS entrainment mechanisms seemed very straight forward. Nevertheless, these results have been questioned very early, and the visual sensations revealed to be due to direct stimulation of the retinal photoreceptors [49]. Due to the spread of the stimulation currents through the scalp, tACS was indeed capable of eliciting a direct response in the retinal cells, thus inducing flickering sensations. From being an early success of tACS research, it turned out to be one of the major

issues of electrical stimulation protocols: the alternating currents applied on the scalp seemed to directly interact with the sensory organs adjacent the brain tissue, thus eliciting detectable sensations in the subjects. This represents a twofold problem, when analyzing the effects of the stimulation: first, the elicited sensations might inform the subjects on the kind of stimulation, thus not blinding the intervention and, therefore, hampering the inferential power of the results. Secondly, the sensations might directly interfere with the performance of the subjects, by reducing the attention to the task or by directly hampering its execution. It is easy to predict that a phase-locked stimulation of the retina, inducing visual sensations oscillating at the frequency of stimulation might result in a consequent modulation of the subject's performance in a visual discrimination task. Thus, it is crucial to understand the real extent of these effects, and evaluate the conditions that increase their occurrence. In our first study we evaluated the neuro-sensory effects elicited in different stimulation protocols. We asked the subjects to report on specific sensations that were triggered by the stimulation (flickering, dizziness, pressure, and skin sensations); thereby, finding stimulation intensity, frequency, and montage effects for most of the sensations analyzed. Thus we provided an overview of the risks associated to different stimulation parameters, providing a useful tool for effectively blinding the stimulation. Additionally, we used the results of this study to carefully blind the subjects to the stimulation parameters used in the next studies.

Synchronizing the hardware for phase calculations

One of the reasons why few studies addressed the phase-dependent effect of tACS is the high time-resolution required to reveal such effects. Indeed, the phase of brain oscillations act in a much faster time-scale compared to their amplitude, thus studies investigating the role of phase need to come up with a protocol

capable of matching this speed; additionally, the faster the frequency of interest, the faster the phase changes. This might be an additional reason why the phase-dependent results reported so far were limited to alpha or lower stimulation frequencies. In our study, we aim to probe the phase-dependent effects of tACS on the motor system, thus we faced several problems that have not been addressed by previous studies. First, we had to handle frequencies that oscillate at twice the speed of alpha oscillations, thus requiring an experimental setup characterized by very high temporal precision. Additionally, we faced the problem of the slower time-scale of the motor processes' that are characterized by high inter-trial variability that exceeds the fast phase dynamics. Thus we needed to identify a read-out reflecting the instantaneous state of the system, and build a stimulation protocol capable of sampling the data with a high temporal precision. We therefore decided to use a passive index of cortical excitability, namely MEPs, rather than relying on overt motor control, at the expenses of an increased complexity of the experimental setup. Indeed, MEPs allow for a passive sampling of the instantaneous excitability level of the corticospinal system. They have been already found to be sensitive to beta tACS in previous studies [43,44]; thus they represent an optimal read-out for the phase-dependent effect of the stimulation. Nevertheless, the assessment of the modulation of MEPs by the tACS phase requires an additional TMS setup to be implemented together with tACS, and a careful synchronization of the two stimulators. In order to time the TMS pulse to specific phases of the tACS waveform, several challenges need to be faced connected to the delay and jitter of the hardware involved in the setup. The second study of my project describes how I solved the synchronization problems and the precision I achieved with the combined setup. The same approach has then been used in the third and last study to evaluate the effects of the stimulation.

Phase dependent entrainment of corticospinal excitability

In the third and last study of the present project we tested our protocol for assessing the phase-dependent effects of sensorimotor beta tACS on the corticospinal excitability level of 18 healthy subjects. The subjects were blinded to the stimulation based on the results of the first study, and the stimulation protocol was validated by an independent publication described in the second study. Thus we were able to isolate the specific effect of tACS phase on the corticospinal response to single TMS pulses. With the hypothesis of a close relationship between tACS phase and MEP size, we sampled the tACS waveform by using four equidistant targets: the peak, the trough, the falling and the rising flank of the sinus characterizing the stimulation. We then modeled the MEP response in the parameters of intercept and slope using a linear regression. These parameters represent the initial response and the cumulative effect of the stimulation on the MEP sizes. We then tested our hypothesis of a sinusoidal modulation of the parameters and showed the temporal dynamics of this effect over the course of the stimulation. Additionally, we explored different TMS intensity and found such effects only for a specific intensity (110% of resting motor threshold). Based on these results we suggested that tACS might act on the inhibitory interneurons present in the motor cortex and sensible to this intensity range [50]. We thus provided a detailed characterization of the already documented effects of beta tACS on corticospinal excitability; reproducing the phase-dependent effects of tACS on cortical excitability found in the sensory domain and suggesting a specific substrate responsible for the tACS effects.

Bibliography

[1] Davidson, Richard J; Jackson, Daren C; Larson CL. Human electroencephalography.pdf. Handb. Psychophysiol. 2, 2000, p. 27–52.

[2] Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. Science 2004;304:1926–9. doi:10.1126/science.1099745.

[3] Busch N, Dubois J, VanRullen R. The phase of ongoing EEG oscillations predicts visual perception. J Neurosci 2009.

[4] Klimesch W. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Res Rev 1999.

[5] Aumann TD, Prut Y. Do sensorimotor ??-oscillations maintain muscle synergy representations in primary motor cortex? Trends Neurosci 2015;38:77–85. doi:10.1016/j.tins.2014.12.002.

[6] Jensen O, Mazaheri A. Shaping functional architecture by oscillatory alpha activity: gating by inhibition. Front Hum Neurosci 2010.

[7] Donner TH, Siegel M. A framework for local cortical oscillation patterns. Trends Cogn Sci 2011;15:191–9. doi:10.1016/j.tics.2011.03.007.

[8] Fries P. A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 2005;9:474–80. doi:10.1016/j.tics.2005.08.011.

[9] Fries P. Rhythms for Cognition: Communication through Coherence. Neuron 2015;88:220–35. doi:10.1016/j.neuron.2015.09.034.

[10] Silvanto J, Pascual-Leone A. Why the assessment of causality in brain–behavior relations requires brain stimulation. J Cogn Neurosci 2012.

[11] Weber MJ, Thompson-Schill SL. Functional neuroimaging can support causal claims about brain function. J Cogn Neurosci 2010;22:2415–6. doi:10.1162/jocn.2010.21461.

[12] Herring JD, Thut G, Jensen O, Bergmann TO. Attention Modulates TMS-Locked Alpha Oscillations in the Visual Cortex. J Neurosci 2015;35:14435–47. doi:10.1523/JNEUROSCI.1833-15.2015.

[13] Rosanova M, Casali A, Bellina V, Resta F. Natural frequencies of human corticothalamic circuits. J 2009.

[14] Hanley CJ, Singh KD, McGonigle DJ. Transcranial modulation of brain oscillatory responses: A concurrent tDCS-MEG investigation. Neuroimage 2015. doi:10.1016/j.neuroimage.2015.12.021.

[15] Antal A, Varga ET, Kincses TZ, Nitsche MA, Paulus W. Oscillatory brain activity and transcranial direct current stimulation in humans. Neuroreport 2004;15:1307–10. doi:10.1097/01.wnr.0000127460.08361.84.

[16] Van Der Werf YD, Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. Exp Brain Res 2006;175:231–45. doi:10.1007/s00221-006-0551-2.

[17] Thut G, Schyns P, Gross J. Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. Front Psychol 2011.

[18] Noury N, Hipp JF, Siegel M. Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. Neuroimage 2016.

[19] Veniero D, Vossen A, Gross J, Thut G. Lasting EEG/MEG Aftereffects of Rhythmic Transcranial Brain Stimulation: Level of Control Over Oscillatory Network Activity. Front Cell Neurosci 2015;9:477. doi:10.3389/fncel.2015.00477.

[20] Vossen A, Gross J, Thut G. Alpha power increase after transcranial alternating current stimulation at alpha-frequency (α-tACS) reflects

plastic changes rather than entrainment. Brain Stimul 2014;8:499–508. doi:10.1016/j.brs.2014.12.004.

[21] Berger H. Über das elektrenkephalogramm des menschen. Eur Arch Psychiatry Clin 1929.

[22] Zaehle T, Rach S, Herrmann CS. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One 2010;5:e13766. doi:10.1371/journal.pone.0013766.

[23] Spaak E, Bonnefond M, Maier A, Leopold DA, Jensen O, Maier A, et al. Layer-Specific Entrainment of Gamma-Band Neural Activity by the Alpha Rhythm in Monkey Visual Cortex. Curr Biol 2012;22:2313–8. doi:10.1016/j.cub.2012.10.020.

[24] Voytek B, Canolty RT, Shestyuk A, Crone N, Parvizi J, Knight RT. Shifts in gamma phase–amplitude coupling frequency from theta to alpha over posterior cortex during visual tasks. Front Hum Neurosci 2010;4:191. doi:10.3389/fnhum.2010.00191.

[25] Laczó B, Antal A, Niebergall R, Treue S, Paulus W. Transcranial alternating stimulation in a high gamma frequency range applied over V1 improves contrast perception but does not modulate spatial attention. Brain Stimul 2012;5:484–91. doi:10.1016/j.brs.2011.08.008.

[26] Kar K, Krekelberg B. Transcranial alternating current stimulation attenuates visual motion adaptation. J Neurosci 2014;34:7334–40. doi:10.1523/JNEUROSCI.5248-13.2014.

[27] Vossen A, Gross J, Thut G. Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (α-tACS) Reflects Plastic Changes Rather Than Entrainment. Brain Stimul 2015;8:499–508. doi:10.1016/j.brs.2014.12.004.

[28] Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK,

Herrmann CS. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr Biol 2014;24:333–9. doi:10.1016/j.cub.2013.12.041.

[29] Ruhnau P, Neuling T, Fuscá M, Herrmann CS, Demarchi G, Weisz N. Eyes wide shut: Transcranial alternating current stimulation drives alpha rhythm in a state dependent manner. Sci Rep 2016;6:27138. doi:10.1038/srep27138.

[30] Feurra M, Paulus W, Walsh V, Kanai R. Frequency Specific Modulation of Human Somatosensory Cortex. Front Psychol 2011;2:1–6. doi:10.3389/fpsyg.2011.00013.

[31] Gundlach C, Müller MM, Nierhaus T, Villringer A, Sehm B. Phasic Modulation of Human Somatosensory Perception by Transcranially Applied Oscillating Currents. Brain Stimul 2016. doi:10.1016/j.brs.2016.04.014.

[32] Neuling T, Rach S, Wagner S, Wolters CH, Herrmann CS. Good vibrations: Oscillatory phase shapes perception. Neuroimage 2012;63:771–8. doi:10.1016/j.neuroimage.2012.07.024.

[33] Rothwell J. Chapter 1 – Motor control. Phys. Manag. Neurol. Rehabil., 2004, p. 3–19. doi:10.1016/B978-072343285-2.50005-X.

[34] Antal A, Boros K, Poreisz C, Chaieb L, Terney D, Paulus W. Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimul 2008;1:97–105. doi:10.1016/j.brs.2007.10.001.

[35] Bergmann TO, Groppa S, Seeger M, Mölle M, Marshall L, Siebner HR. Acute changes in motor cortical excitability during slow oscillatory and constant anodal transcranial direct current stimulation. J Neurophysiol 2009;102:2303–11. doi:10.1152/jn.00437.2009.

[36] Groppa S, Bergmann TO, Siems C, M??lle M, Marshall L, Siebner HR.

Slow-oscillatory transcranial direct current stimulation can induce bidirectional shifts in motor cortical excitability in awake humans. Neuroscience 2010;166:1219–25. doi:10.1016/j.neuroscience.2010.01.019.

[37] Engel AK, Fries P. Beta-band oscillations--signalling the status quo? Curr Opin Neurobiol 2010;20:156–65. doi:10.1016/j.conb.2010.02.015.

[38] Pogosyan A, Gaynor LD, Eusebio A, Brown P. Boosting cortical activity at Beta-band frequencies slows movement in humans. Curr Biol 2009;19:1637–41. doi:10.1016/j.cub.2009.07.074.

[39] Joundi R a, Jenkinson N, Brittain J-S, Aziz TZ, Brown P. Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol 2012;22:403–7. doi:10.1016/j.cub.2012.01.024.

[40] Pfurtscheller G, Graimann B, Huggins JE, Levine SP, Schuh LA. Spatiotemporal patterns of beta desynchronization and gamma synchronization in corticographic data during self-paced movement. Clin Neurophysiol 2003;114:1226–36. doi:10.1016/S1388-2457(03)00067-1.

[41] Krause V, Wach C, Südmeyer M, Ferrea S, Schnitzler A, Pollok B. Cortico-muscular coupling and motor performance are modulated by 20 Hz transcranial alternating current stimulation (tACS) in Parkinson's disease. Front Hum Neurosci 2013;7:928. doi:10.3389/fnhum.2013.00928.

[42] Zaghi S, de Freitas Rezende L, de Oliveira LM, El-Nazer R, Menning S, Tadini L, et al. Inhibition of motor cortex excitability with 15Hz transcranial alternating current stimulation (tACS). Neurosci Lett 2010;479:211–4. doi:10.1016/j.neulet.2010.05.060.

[43] Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S.

Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J Neurosci 2011;31:12165–70. doi:10.1523/JNEUROSCI.0978-11.2011.

[44] Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi a., Rossi S. State-Dependent Effects of Transcranial Oscillatory Currents on the Motor System: What You Think Matters. J Neurosci 2013;33:17483–9. doi:10.1523/JNEUROSCI.1414-13.2013.

[45] Goldsworthy MR, Vallence AM, Yang R, Pitcher JB, Ridding MC. Combined transcranial alternating current stimulation and continuous theta burst stimulation: A novel approach for neuroplasticity induction. Eur J Neurosci 2016;43:572–9. doi:10.1111/ejn.13142.

[46] Brittain J, Probert-smith P, Aziz TZ. Report Tremor Suppression by Rhythmic Transcranial Current Stimulation 2013:436–40.

[47] Van Der Werf YD, Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. Exp Brain Res 2006;175:231–45. doi:10.1007/s00221-006-0551-2.

[48] Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. Curr Biol 2008;18:1839–43. doi:10.1016/j.cub.2008.10.027.

[49] Schutter DJLG, Hortensius R. Retinal origin of phosphenes to transcranial alternating current stimulation. Clin Neurophysiol 2010;121:1080–4. doi:10.1016/j.clinph.2009.10.038.

[50] Garry MI, Thomson RHS. The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. Exp Brain Res 2009;193:267–74. doi:10.1007/s00221-008-1620-5.

Statement of contributions

Study n° 1:

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We performed the study in the functional and restaurative neurosurgery department. Raco V. designed, implemented and performed the measurements of the study. Analyzed the data, and wrote the manuscript. Bauer R. designed the study, supervised the analysis of the data and corrected the manuscript. Olenik M. and Brkic D. collected the data. Gharabaghi A. Supervised the study and corrected the manuscript.

Study n° 2:

Raco V, Bauer R, Tharsan S, Gharabaghi A. Combining TMS and tACS for Closed-Loop Phase-Dependent Modulation of Corticospinal Excitability: A Feasibility Study. Frontiers in Cellular Neuroscience. 10: 143. PMID 27252625 DOI: 10.3389/fncel.2016.00143

We performed the study in the functional and restaurative neurosurgery department. Raco V. designed, implemented and supervised the measurements of the study. Analyzed the data, and wrote the manuscript. Bauer R. Designed the study, supervised the analysis of the data and corrected the manuscript. Tharsan S. Performed the measurements. Gharabaghi A. Supervised the study and corrected the manuscript.

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We performed the study in the Functional and Restaurative Neurosurgery Department. Raco V. designed, implemented and supervised the measurements of the study. Analyzed the data, and wrote the manuscript. Bauer R. Designed the study, supervised the analysis of the data and corrected the manuscript. Tharsan S. Performed the measurements. Gharabaghi A. Supervised the study and corrected the manuscript.

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Neurosensory Effects of Transcranial Alternating Current Stimulation

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ABSTRACT

Background: Electrical brain stimulation can elicit neurosensory side effects that are unrelated to the intended stimulation effects. This presents a challenge when designing studies with blinded control conditions.
Objective: The aim of this research was to investigate the role of different transcranial alternating current stimulation (tACS) parameters, i.e. intensity, frequency, and electrode montage, on the probability, duration and intensity of elicited neurosensory side effects.
Methods: In a first study, we examined the influence of tACS on sensations of phosphenes, dizziness, pressure, and skin sensation in fifteen healthy subjects, during 8 s of stimulation with different amplitudes (1500 μA, 1000 μA, 500 μA, 250 μA), frequencies (2 Hz, 4 Hz, 8 Hz, 16 Hz, 32 Hz, 64 Hz), and montages (F3/F4, F3/C4, F3/P4, P3/F4, P3/C4, P3/P4). In a second study, ten healthy subjects were exposed to 60 s of tACS (1000 μA, 2 Hz versus 16 Hz, F3/F4 versus P3/P4) and were asked to rate the intensity of sensations every 12 s.
Results: The first study showed that all stimulation parameters had an influence on the probability and intensity of sensations. Phosphenes were most likely and strongest for frontal montages and higher frequencies. Dizziness was most likely and strongest for parietal montages and at stimulation frequency of 4 Hz. Skin sensations and pressure was more likely when stimulation was performed across central regions and at posterior montages, respectively. The second study also revealed that the probability and the intensity of sensations were neither modified during more extended periods of stimulation nor affected by carry-over effects.
Conclusion: We demonstrated that the strength and the likelihood of sensations elicited by tACS were specifically modulated by the stimulation parameters. The present work may therefore be instrumental in establishing effective blinding conditions for studies with tACS.

Introduction

Electrical brain stimulation can elicit side effects that are unrelated to the intended stimulation effects. For transcranial direct current stimulation (tDCS), the feasibility of blinding the participants with regard to the experimental conditions has been successfully introduced, particularly for stimulation amplitudes below 2 mA [1]. There is conclusive evidence that sensations elicited by tDCS fade with stimulation time [2]. Study designs based on a fade-in/stimulation/fade-out approach have thus been proven effective in blinding subjects [3]. In many clinical trials, subjects are therefore not able to distinguish between active and sham tDCS [4,5].

However, designing studies with blinded control conditions for transcranial alternating current stimulation (tACS), still poses a challenge. This emerging technique modulates the ongoing oscillatory brain activity, rendering it a valuable tool for investigating brain function in healthy and diseased conditions [6–9]. For tACS, entrainment effects have been found to be linked to ongoing

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oscillatory activity [10,11], and strongly depend on the current state of the central nervous system [12,13]. Furthermore, tACS might affect switching between states [14], rendering it particularly suitable for closed-loop stimulation protocols which detect specific brain states and trigger short-term stimulation to specifically modulate neurophysiological activity [15,16]. However, tACS may elicit sensations [6] that are unrelated to the stimulation effects, thus possibly inducing unwanted side effects. For closed-loop applications of tACS, effective blinding of short-term stimulation will therefore be important. Consequently, this research direction cannot rely on the established fade-in/stimulation/fade-out approach [3], but will require a better understanding of the neurosensory effects induced by short-term tAC stimulation, e.g. for several seconds up to 1 min. The contribution of different stimulation parameters, such as stimulation amplitude, frequency, montage and duration on the induction of neurosensory side effects therefore requires further research.

During earlier studies, three primary sensations were shown to be elicited by non-invasive electrical brain stimulation. These were phosphenes (i.e. flickering perception of a light) during tACS [6], skin sensations (e.g. perception of itching or tingling) during transcranial direct current stimulation (tDCS) [17], and sensations of dizziness during alternating current galvanic vestibular stimulation (AC-GVS) [18]. Earlier studies indicated that the strongest phosphenes were perceived when stimulation was applied in the low beta band [16,19], and the sensitivity to phosphenes decreased as the distance between the stimulation electrodes and the retina increased [20]. With regard to skin sensations, studies on the electrical stimulation of skin revealed a linear dependency between the sensation ratings and stimulation intensity and frequency [21,22]. In other words, high stimulation intensities/frequencies resulted in more perceptible skin sensations. When it came to sensations of dizziness, Stephan et al. [18] showed a frequency-dependency in an AC-GVS study, with an inverse relation between the strength of the sensations and the stimulation frequency, indicating that the strongest sensations for stimulation frequencies lie in the low frequency range (<4 Hz).

The aim of this research project was to provide a deeper insight into the neurosensory side effects of tACS. In a first study, the acute effects of different stimulation parameters were explored to identify those settings resulting in the highest likelihood and intensity of sensations. The second study was based on those settings with the highest probability for side effects and explored the influence of stimulation duration.

Methods

Subjects

We recruited eighteen healthy subjects (mean age 23.9 years, SD 2.25, eleven females) for the first study, and ten healthy subjects (mean age 26.2 years, SD 2.28, five females) for the second study. Subjects had no history of psychiatric or neurological conditions, and participated after giving written informed consent. The study was approved by the local ethics committee. For the first study, three subjects could not be included in the analysis, as one subject reported a persistent headache, while two others were not following the instructions adequately.

Experimental setup

Experiments were conducted using a multi-channel transcranial AC stimulator (NeuroConn, Ilmenau, Germany). We used rubber electrodes (size of reference electrode 5×7 cm^2 and size of stimulation electrode 4×4 cm^2) inserted into sponges soaked with tap water. Please note that tap water has been shown to smoothen the current density distribution [10]. Whenever montages were switched, the wetness of the electrodes was inspected and if necessary, additional tap water was added. The electrodes were attached to the scalp by a non-conductive elastic band and a cap which is usually used for electroencephalography. Stimulation and reference electrodes were positioned based on the international 10–20 EEG system, with stimulation electrodes always being placed on the right hemisphere (F4, C4 or P4) and reference electrode on the left hemisphere (F3 or P3). Impedance was checked at the beginning of each stimulation block and kept below 17 kΩ. Stimulation waveform was sinusoidal and without DC offset. Subjects were seated on a comfortable chair with their eyes open in a dimly lit room, facing a dark wall or a computer screen in the first and the second study, respectively. Subjects were kept blinded toward the stimulation condition.

Sensation rating system

We had initially planned to instruct subjects to pay special attention to three specific sensations, but as the additional sensation of pressure was reported by the first subject in the first study, we decided to include it in the list of possible sensations for all subsequent measurements. Subjects were therefore informed before the task that four sensations were most likely: phosphenes, pressure, dizziness and skin sensations. In the first study, subjects were instructed to pay attention to any feelings induced by the stimulation and to provide a qualitative description of any sensation. In the second study, subjects were instructed to concentrate on only one sensation which was indicated by a text presented on a computer screen. Following their qualitative description, subjects rated the intensity of the perceived sensation on a 6-point scale, with 0 and 6 indicating no sensations and strong sensations, respectively. The probability of a sensation was later calculated by comparing intensity ratings to zero, resulting in binary values.

$$\delta_i = \begin{cases} 0 & if\ i = 0 \\ 1 & if\ i \geq 1 \end{cases}$$

All reports of visual sensations, such as flashes, lights, flickering, foggy and blurred vision, were grouped together as phosphenes. All reports of increased weight or pressure, either focal at the electrodes sites or generalized across the whole head were grouped together as pressure. All reports of changes in spatial perception, such as vertigo or being off-balance as well as lightheadedness, were grouped together as dizziness. All reports of tingling, itching or heat causing a desire to scratch or withdraw were grouped together as skin sensations.

First study

In a three-factorial design, we researched the effect of amplitude, frequency and montage on probability and intensity of sensations. We explored six different bipolar montages (F3–F4, F3–C4, F3–P4, P3–F4, P3–C4, P3–P4). For each electrode montage, six stimulation frequencies (2 Hz, 4 Hz, 8 Hz, 16 Hz, 32 Hz, and 64 Hz) at four different stimulation intensities (1500 μA, 1000 μA, 500 μA, and 250 μA) were explored, resulting in 24 trials per montage. At the start of each trial, stimulation was ramped up for 3 s; subsequently stimulation was kept constant for 4 s and followed by 1 s of ramping down. In the following interval without stimulation, subjects were asked to describe and rate any sensation experienced. After 4 s, the next trial started. The approximate duration of the whole experiment was 90 min for each subject.

Second study

In a three-factorial design, we examined the effect of duration, frequency and montage on probability and intensity of sensations. Stimulation intensity was kept constant at 1000 μA while we explored two different bipolar montages (F3–F4, P3–P4) and two stimulation frequencies (2 Hz versus 16 Hz). Every possible combination was explored four times; on each occasion, the subject was instructed to focus on one specific sensation (phosphenes, pressure, dizziness or skin sensations), thus resulting in 16 trials. To avoid potential carry-over effects, the inter-stimulation break was extended to 60 s in this study. Prior to each trial, the subject was informed about the sensation of interest within this trial. At the onset of each trial, stimulation was ramped up for 1 s; subsequently stimulation was kept constant for 58 s, followed by 1 s of ramping down. At the onset of the trial and every 12 s from then on, an auditory cue was given, and subjects rated the intensity of the sensation of interest by pressing the respective number on a keyboard (0–6). The approximate duration of the whole experiment was 50 min for each subject.

Data analysis

The statistical analysis of the results was performed using *R* and Matlab. Given the factors amplitude, montage and frequency for the first study and time, montage and frequency for the second study, we performed a 3-way repeated measures ANOVA on the probability of a sensation being experienced. We included only cases in which a sensation was perceived, and subsequently performed a 3-way repeated measures ANOVA on the intensity of each sensation. For the second study, we also performed a 4-way repeated measures ANOVA with the factors sensation, stimulation duration, frequency and montage on the response time of ratings and the probability of a sensation.

Results

First study

Phosphenes were reported by all fifteen subjects. The probability of experiencing phosphenes depended on the frequency ($F(5, 2132) = 69.1$, $P < 0.001$), amplitude ($F(3, 2132) = 64.3$, $P < 0.001$), and montage ($F(5, 2132) = 18.5$, $P < 0.001$). There was clear evidence for a direct relationship between phosphenes and amplitude as well as between frontal and parietal montages (see Fig. 1A). What is more, the effect was most likely when stimulation was performed at 16 Hz or higher. The strength of phosphenes depended on frequency ($F(5, 436) = 5.45$, $P < 0.001$), amplitude ($F(3, 436) = 22.7$, $P < 0.001$), and montage ($F(5, 436) = 8.1$, $P < 0.001$). Phosphenes were rated as stronger when stimulation frequency was at 16 Hz or higher. They were stronger for frontal than for parietal montages and there was a clear relationship to amplitude, with strength of sensation increasing with stimulation amplitude (see Fig. 2A).

Dizziness was reported by fourteen subjects. Two subjects reported simultaneous presence of black spots in their visual fields. The probability of experiencing dizziness depended on frequency ($F(5, 2132) = 2.3$, $P = 0.039$) and montage ($F(5, 2132) = 2.68$, $P < 0.02$). The probability of dizziness was highest when stimulating with 4 Hz and increased from frontal to parietal montages (see Fig. 1B). The strength of dizziness depended on amplitude ($F(3, 384) = 14.2$, $P < 0.001$), and montage ($F(5, 384) = 3.8$, $P = 0.003$). There was a clear relationship to amplitude, with the strength of sensation increasing with stimulation amplitude, and a trend for frontal to parietal montage (see Fig. 2B).

Pressure was reported by twelve subjects. The probability of experiencing pressure depended only on montage ($F(5, 2132) = 8.49$, $P < 0.001$). Although the pattern was less evident, it still resembled an increase from frontal to parietal montages (see Fig. 1C). The strength of pressure depended on amplitude ($F(3, 308) = 6.7$, $P < 0.001$), and montage ($F(5, 308) = 8.1$, $P < 0.001$). There was a clear relationship to amplitude, with the strength of sensation increasing with stimulation amplitude, and with the parietal montage eliciting the strongest sensations (see Fig. 2C).

Skin sensations were reported by thirteen subjects. The probability of experiencing skin sensations depended on amplitude ($F(3, 2132) = 137.1$, $P < 0.001$) and montage ($F(5, 2132) = 8.49$, $P < 0.001$). There was clear evidence for a direct relationship to amplitude, and central montages were more liable to cause skin sensations than frontal or parietal montages (see Fig. 1D). The strength of skin sensations depended on amplitude ($F(3, 463) = 56.3$, $P < 0.001$), and montage ($F(5, 463) = 9.9$, $P < 0.001$). There was a clear relationship to amplitude, with the strength of sensation increasing with stimulation amplitude, and a trend for frontal and central rather than for parietal montages (see Fig. 2D).

Second study

The probability ($F(4, 184) < 1.2$, $P > 0.31$) or the intensity ($P > 0.29$) of any sensation was not affected by the stimulation duration, indicating that the sensations remained stable over time (see Figs. 3 and 4). Subjects perceived the respective sensations immediately at the onset of the stimulation and maintained that perception during the course of stimulation.

The findings from the first study could be replicated. The probability of phosphenes was higher for 16 Hz versus 2 Hz stimulation ($F(1, 184) = 95.1$, $P < 0.001$) and lower for parietal versus frontal montage ($F(1, 184) = 27.57$, $P < 0.001$). The probability of dizziness was higher for 2 Hz versus 16 Hz stimulation ($F(1, 184) = 13.5$, $P < 0.001$) (see Fig. 3). The intensity of flickering was higher for 16 Hz ($F(1, 64) = 11.1$, $P = 0.0015$) and frontal montage ($F(1, 64) = 88.9$, $P < 0.001$) and dizziness was perceived more intensely at parietal montage ($F(1, 74) = 5.9$, $P = 0.017$) (see Fig. 4). In addition, we found that the probability of skin sensations increased for parietal versus frontal montage ($F(1, 184) = 10.7$, $P = 0.0013$), while the intensity was increased for 16 Hz stimulation ($F(1, 96) = 6.1$, $P = 0.015$). The intensity of pressure was higher for parietal montage ($F(1, 55) = 6.9$, $P < 0.011$).

Having compared the sensations with each other, we found that frequency ($F(1, 781) = 0.73$, $P > 0.39$) or montage ($F(1, 781) = 1.6$, $P > 0.46$) had no effect on the reaction time for ratings, whereas stimulation duration ($F(4, 781) = 47.4$, $P > 0.001$) and type of sensation ($F(3, 781) = 51.2$, $P < 0.001$) did (see Fig. 5). With longer stimulation period, reaction time decreased from 5.4 s to saturate at around 3.2 s (see Fig. 5B). What is more, the reaction time for rating dizziness had increased significantly (see Fig. 5D). Regardless of the type of sensation, the probability was not affected by stimulation duration ($F(4, 781) = 0.2$, $P > 0.96$) (see Fig. 6B), but increased for 16 Hz versus 2 Hz ($F(1, 781) = 5.5$, $P = 0.019$) (see Fig. 6A) and decreased for parietal versus frontal montage ($F(1, 781) = 15.8$, $P > 0.001$) (see Fig. 6C). We also observed an increased probability of skin sensations, and the lowest probability for pressure ($F(3, 781) = 9.2$, $P < 0.001$) (see Fig. 6D).

Discussion

The goal of this study was to investigate neurosensory side effects of tACS elicited by different stimulation set-ups to inform the design of placebo-controlled studies. As well as phosphenes and skin sensations, we studied pressure and dizziness. To the best

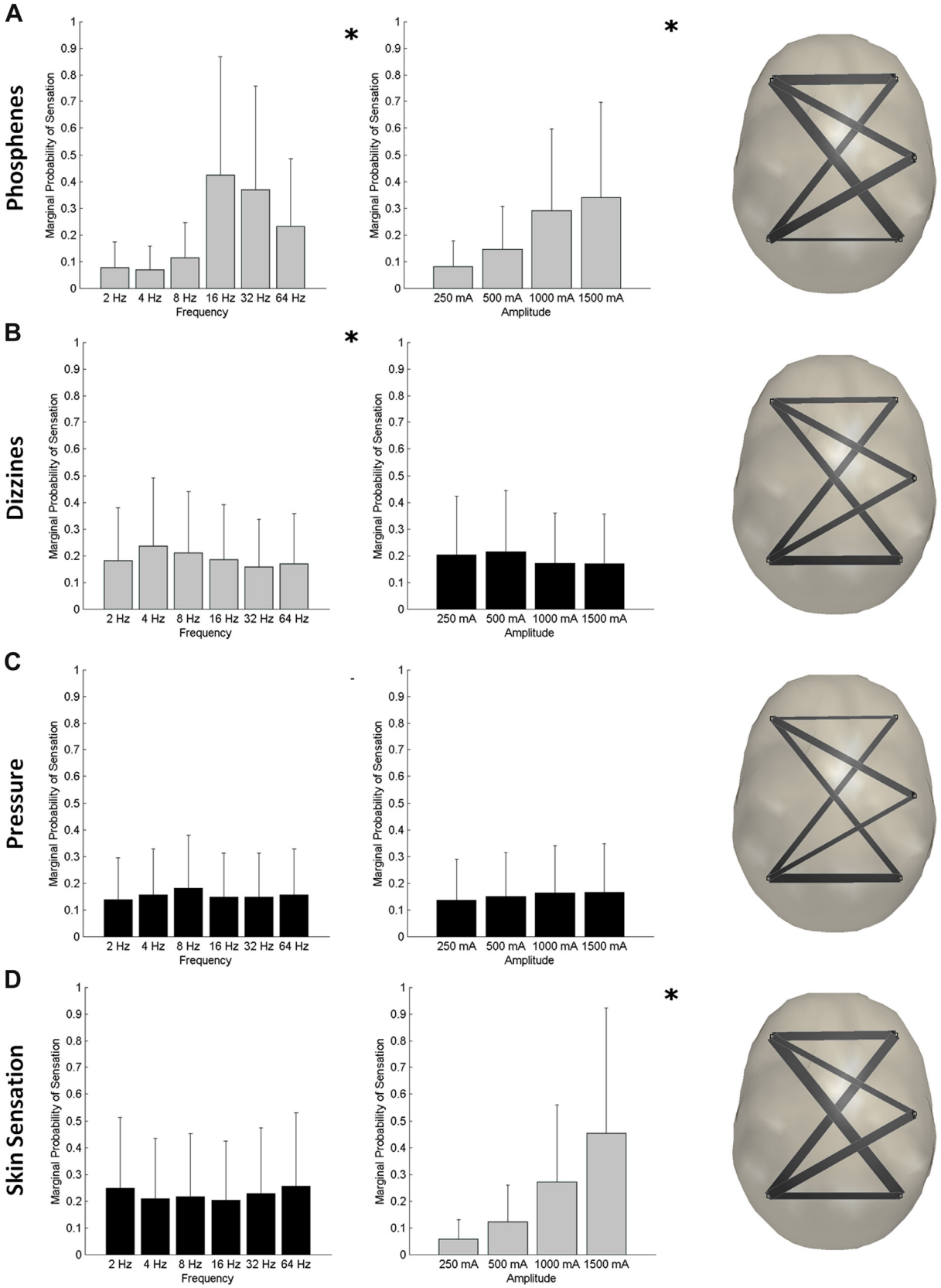

Figure 1. Probability of neurosensory effects for different factors. A to D indicate Phospenes (A), Dizziness (B), Pressure (C) and Skin Sensation (D). Factors with a significant influence according to a 3-way repeated measures ANOVA on the sensation are colored light grey. Error bars indicate standard deviance. Probabilities for montages are indicated by thickness of line between stimulation electrodes (F4, C4, P4) to reference electrodes (F3, P3). Asterisk indicates significant findings.

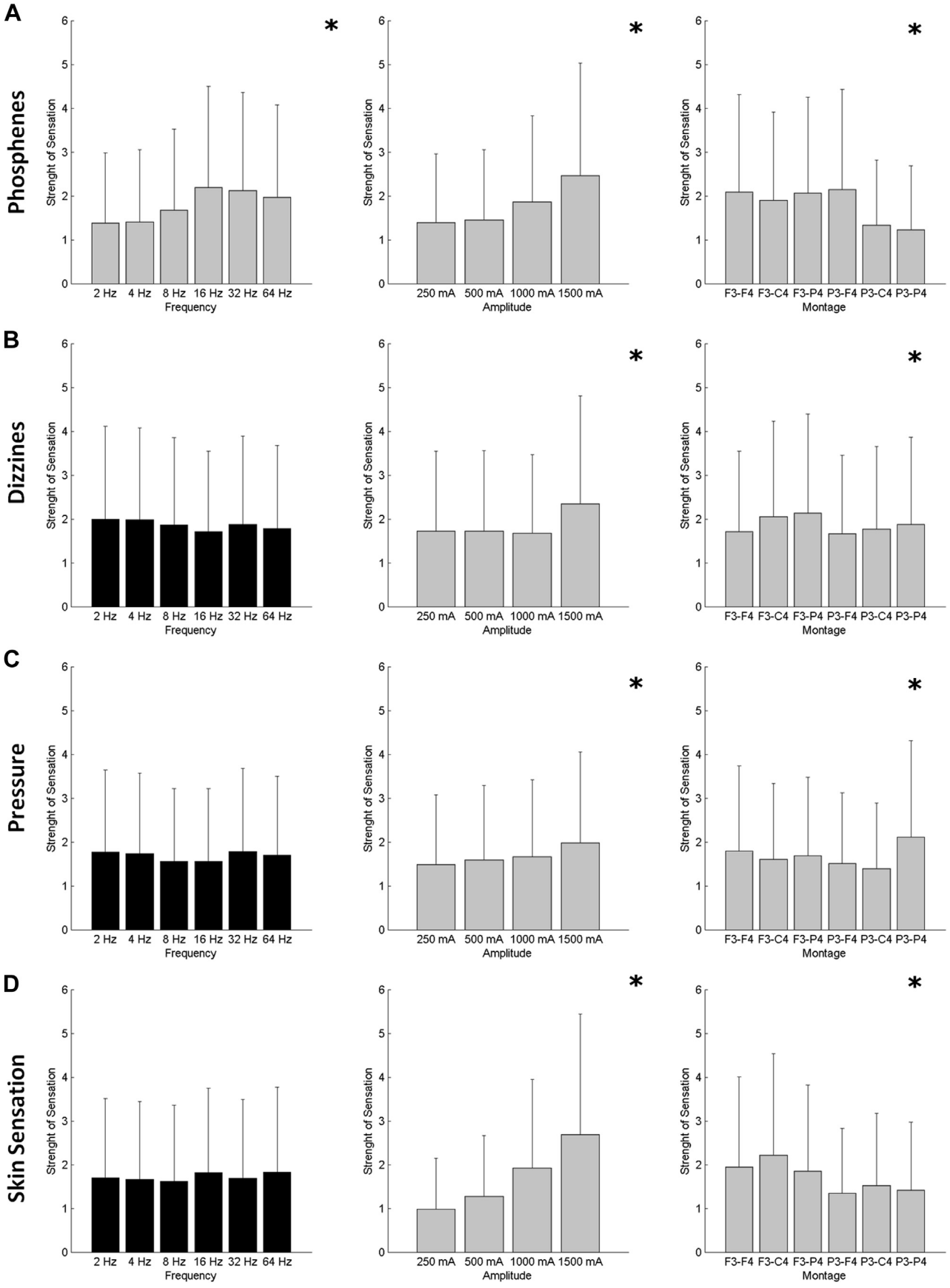

Figure 2. Intensity of neurosensory effects for different factors. A to D indicate Phospenes (A), Dizziness (B), Pressure (C) and Skin Sensation (D). Factors with a significant influence according to a 3-way repeated measures ANOVA on the sensation are colored light grey. Error bars indicate standard deviance. Asterisk indicates significant findings.

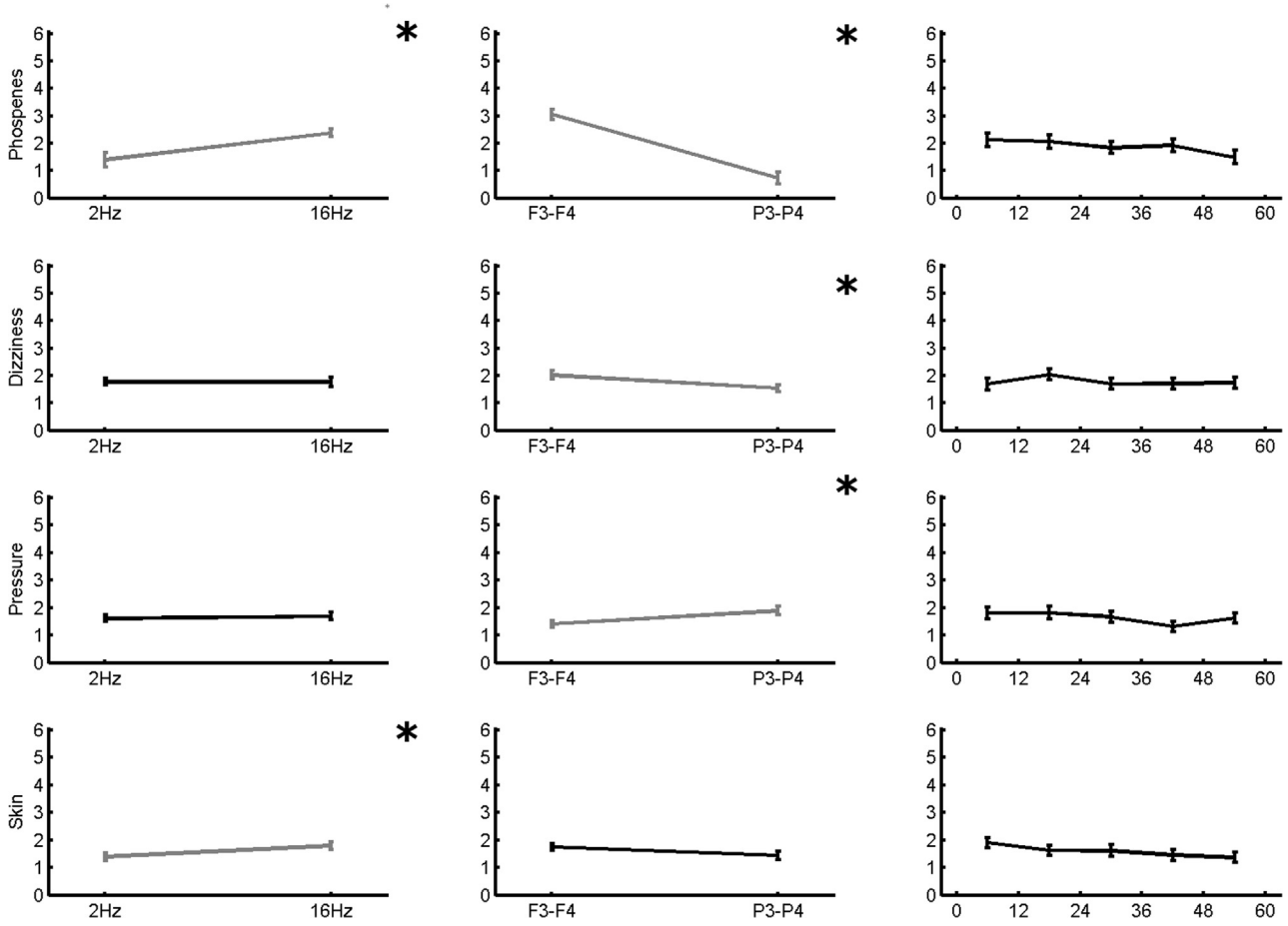

Figure 3. Probability of sensation for different stimulation parameters. Light grey lines indicate significant differences according to ANOVA. Factors included in the model were frequency (first column), montage (second column) and stimulation duration (third column). Asterisk indicates significant findings.

of our knowledge, this is the first time that these two sensations have been reported during tACS.

In both the studies presented here, we were able to replicate earlier findings [6,20,23] on the induction of phosphenes, showing that stronger phosphenes were elicited when tACS was applied in higher frequency bands and when electrode montages were closer to the retina. At even higher frequencies in the ripple range (not tested in our study) some of these effects have been shown to be reversed [23]. Phosphenes are already a widely researched sensation and their induction by ACS is currently also being explored for therapeutic interventions in the context of retinal disorders [24,25]. In support of electrical field modeling studies [26], we provide further evidence that the flickering following ACS is of retinal, not cortical origin [19].

On the basis of previous findings in AC-GVS experiments [18], and because of the possible involvement of the vestibular nerve [27], we anticipated that the strongest sensations of dizziness would occur when stimulating at low frequencies and posterior montages. Consistent with this hypothesis, our results showed the strongest dizziness ratings for the parietal montage and the lower frequencies condition. There are several possible explanations as to why there are no reports of dizziness in previous tACS studies. Low stimulation frequencies inducing dizziness have rarely been explored in the past. Moreover, in previous studies, a sagittal alignment of stimulation and reference electrodes [1] was often used, inducing a current flow orthogonal to the vestibular nerve. By contrast, the parietal electrode montage in the present study induced a current flow parallel to the nerve. It should also be noted that dizziness showed the longest reaction time of all sensations. This could be explained by the wavelength of low frequency stimulation, making it necessary to evaluate this sensation over a longer period of time.

The sensation of pressure was not included in the original list of sensations, but was added after being reported by the first subject. We were surprised to find a significant modulation by montage in the first study, which was replicated in the second study. Although the origin of this sensation is unclear, we tend to assume the possible involvement of muscle proprioceptors close to the scalp, namely those of the occipitalis muscle. The sensation of muscle fiber stretching is probably perceived as increased head weight as if pressure would be applied externally. The absence of this effect in other tACS studies might be related to a different electrode positioning than the parietal electrode montage that caused the more pronounced effects in this study.

We observed a modulation of skin sensations by amplitude and montage in the first study. We additionally replicated earlier studies indicating an effect of stimulation frequency [21,23] and showed in the second study that the probability was increased for the frontal montage. The effect of stimulation amplitude can be explained by increased current density, while findings about the impact of electrode montage and stimulation frequency are complex and still lack conclusive interpretation. One possible explanation might be related to the regionally specific sensitivity of tactile nerves in the skin [28].

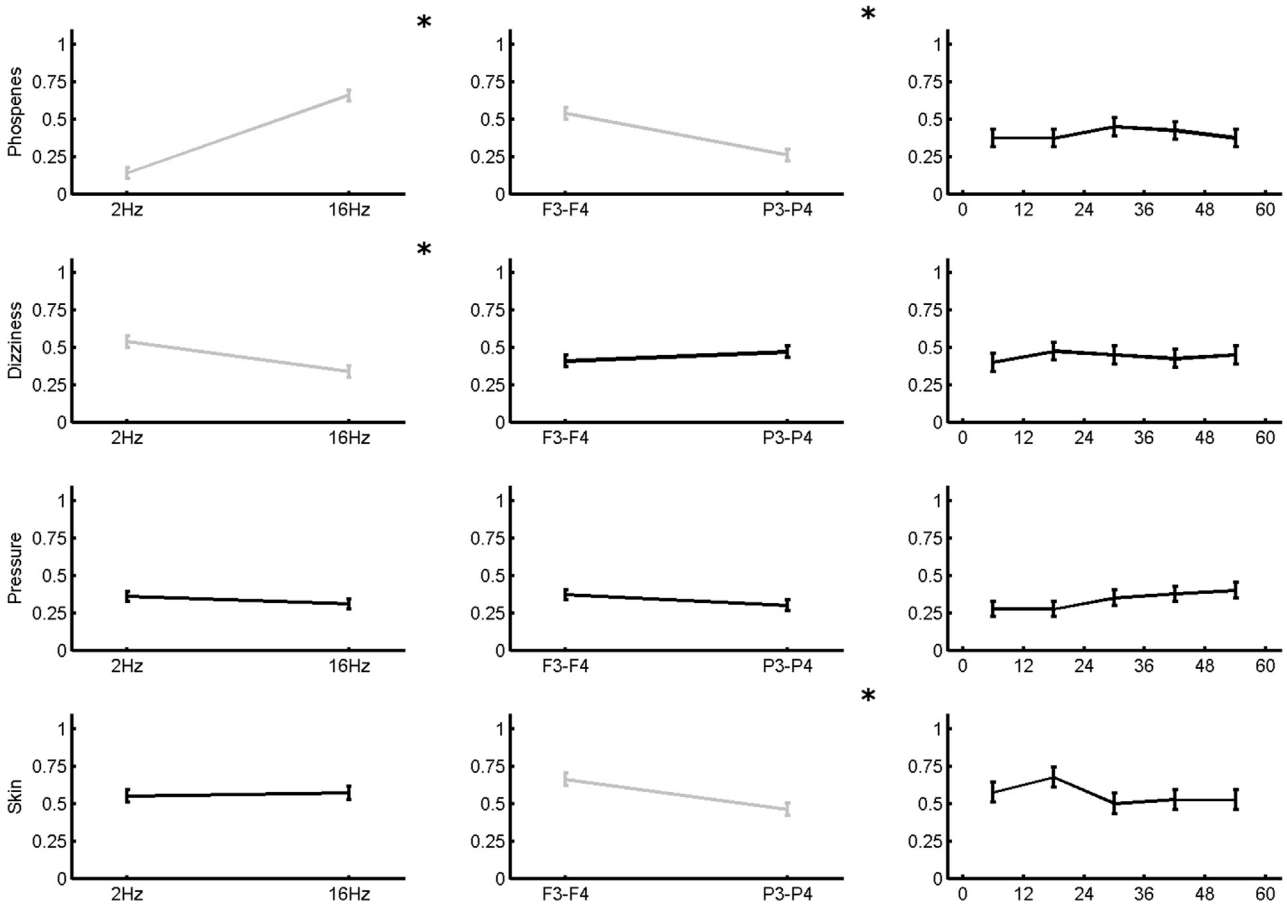

Figure 4. Intensity of sensation for different stimulation parameters. Light grey lines indicate significant differences according to ANOVA. Factors included in the model were frequency (first column), montage (second column) and stimulation duration (third column). Asterisk indicates significant findings.

When it comes to the design of placebo-controlled non-invasive brain stimulation studies, two different strategies have been proposed [29]. The first, known as sham controlled stimulation (SCS), applies low current stimulation or no stimulation whatsoever to ensure that subjects are unaware of the experimental conditions [2]. Thus, this approach intends to avoid sensory effects in the

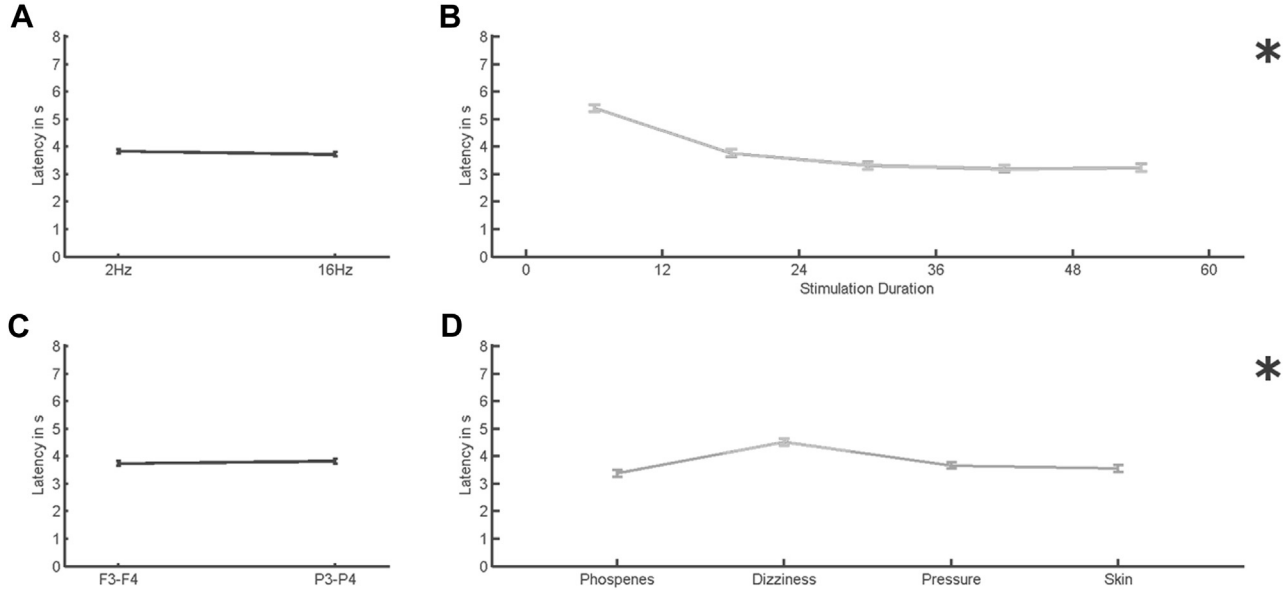

Figure 5. Latency of ratings for different factors. Light grey lines indicate significant differences according to ANOVA. Factors included in the model were frequency (A), stimulation duration (B), montage (C) and type of sensation (D). Asterisk indicates significant findings.

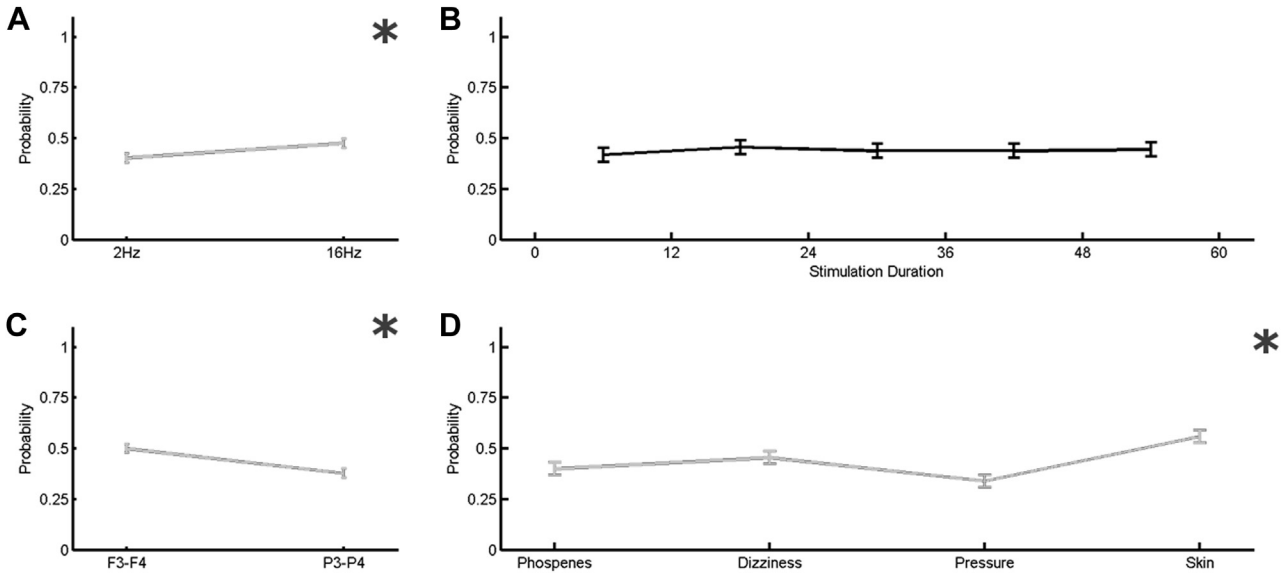

Figure 6. Probability of sensation for different factors. Light grey lines indicate significant differences according to ANOVA. Factors included in the model were frequency (A), stimulation duration (B), montage (C) and type of sensation (D). Asterisk indicates significant findings.

experiment. The second strategy, known as off-target active stimulation (OAS), aims to mimic the neurosensory effects of the experimental condition without affecting the nervous system. For both approaches, it is essential to accurately define any stimulation intensities, frequencies and locations that cannot be distinguished by the subject. In this vein, we were able to demonstrate that all three studied factors, i.e. stimulation amplitude, frequency and electrode montage, determined the probability of sensations perceived by the subject. In the second study, we were also able to show that a longer stimulation period did not modulate the probability or the intensity of side effects but decreased the reaction time for reporting the side effects. We interpret this decrease in reaction time to be foremost a training effect.

The reaction time has already been reported to be influenced by the stimulation frequency when two sensations were rated *simultaneously* [23]. When we explored the response time for different sensations *separately*, we found no effect of the stimulation frequency. These findings of our second study therefore support the notion that split-attention processes are relevant for the subject's perception of neurosensory side effects. We postulate that sensory perception is partially caused by different resonance frequencies for various sensations, and that the subsequent longer time-constant reflects the sensory integration. Here, it should be noted that dizziness presented the longest reaction times. Effects resonating more strongly at higher versus lower frequencies might be related to more sensory events over a shorter time-scale and might therefore be detected more quickly and more reliably. In this context, split-attention processes would result in a simultaneous inhibition of perceiving sensations resonating at lower frequencies. This sensory integration perspective is supported by the exponential decrease in reaction time over stimulation duration. Future studies should address this hypothesis more specifically and might even include more elaborate mathematical models predicting perception of tACS-induced neurosensory effects.

Stimulation amplitude was the most versatile parameter to modulate the probability and the intensity of neurosensory effects: the amplitude determined the intensity of all sensations, but influenced the probability for phosphenes and skin sensations only, not the probability for dizziness and pressure (see Figs. 1 and 2).

As regards stimulation frequencies, those above 16 Hz induced the highest probability and strength of perception for the most common neurosensory effect, i.e. phosphenes. This is of particular significance, since higher frequencies are often used for tACS paradigms. However, these side effects could be reduced by choosing low stimulation intensities and electrode montages further away from the retina, e.g. the P3–P4 montage.

Our findings have implications for both SCS and OAS approaches. For SCS, it will be imperative to define an accurate threshold of stimulation intensities that do not induce neurosensory side effects. Special attention should therefore be paid to the diversity of possible sensations, particularly the less common ones, to detect them adequately. OAS, both experimental and control conditions might necessitate stimulation settings that induce the same neurosensory effects. However, these specific neurosensory effects might not always be experienced by subjects in the same way. Even if the experimental condition were to induce similar effects in different subjects, the control condition mimicking these still might need to be individually adapted. The introspective discrimination – both qualitative and quantitative – of neurosensory side effects would pose a relevant challenge in any case, requiring full concentration for the complex task. What is more, the reported sensations are prone to misinterpretation or even to random guesses. This might apply to the first study as well, since the participating subjects had to name all the sensations at once that they had experienced during a stimulation session. This might have introduced a bias toward the strongest sensation. Interestingly enough, the main findings of the first study were replicated in the second study, where subjects had to focus on one specific sensation at a time only. However, it should be borne in mind that this might have biased the perception to higher probabilities.

In both studies, we identified the specific influence of different stimulation parameters and electrode montages on various neurosensory side effects. However, the particular mechanism influencing the sensory organs will require further investigation. Our results indicated that subjects could easily distinguish the actual experimental condition from control conditions based on the probability and intensity of different neurosensory side effects, making the design of placebo-controlled stimulation studies a challenging task. Thus, the set-up and stimulation paradigms for

tACS experiments have to be chosen with care, e.g. by selecting those stimulation parameters that do not elicit sensations or selecting stimulation parameters for the sham condition that are able to mimic the neurosensory effects of the verum condition effectively.

References

[1] O'Connell NE, Cossar J, Marston L, et al. Rethinking clinical trials of transcranial direct current Stimulation: Participant and assessor blinding is inadequate at intensities of 2 mA. PLoS One 2012;7:e47514.

[2] Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol 2006;117:845–50.

[3] Ambrus GG, Al-Moyed H, Chaieb L, Sarp L, Antal A, Paulus W. The fade-in–short stimulation–fade out approach to sham tDCS–reliable at 1 mA for naïve and experienced subjects, but not investigators. Brain Stimul 2012;5:499–504.

[4] Boggio PS, Rigonatti SP, Ribeiro RB, et al. A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. Int J Neuropsychopharmacol 2008;11:249–54.

[5] Palm U, Reisinger E, Keeser D, et al. Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. Brain Stimul 2013;6:690–5.

[6] Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. Curr Biol 2008;18:1839–43.

[7] Zaehle T, Sandmann P, Thorne JD, Jäncke L, Herrmann CS. Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: combined behavioural and electrophysiological evidence. BMC Neurosci 2011;12:2.

[8] Joundi RA, Jenkinson N, Brittain J-S, Aziz TZ, Brown P. Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol 2012;22:403–7.

[9] Polanía R, Nitsche MA, Korman C, Batsikadze G, Paulus W. The importance of timing in segregated theta phase-coupling for cognitive performance. Curr Biol 2012;22:1314–8.

[10] Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr Biol 2014;24(3):333–9.

[11] Brittain J-S, Probert-Smith P, Aziz TZ, Brown P. Tremor Suppression by Rhythmic transcranial current stimulation. Curr Biol 2014;23(5):436–40.

[12] Neuling T, Rach S, Herrmann CS. Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. Front Hum Neurosci 2013;7:161.

[13] Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi A, Rossi S. State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. J Neurosci 2013;33:17483–9.

[14] Kutchko KM, Fröhlich F. Emergence of metastable state dynamics in interconnected cortical networks with propagation delays. PLoS Comput Biol 2013;9:e1003304.

[15] Walter A, Ramos Murguialday A, Spüler M, et al. Coupling BCI and cortical stimulation for brain-state-dependent stimulation: methods for spectral estimation in the presence of stimulation after-effects. Front Neural Circuits 2012;6:87.

[16] Gharabaghi A, Kraus D, Leão MT, et al. Coupling brain-machine interfaces with cortical stimulation for brain-state dependent stimulation: enhancing motor cortex excitability for neurorehabilitation. Front Hum Neurosci 2014;8:122.

[17] Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull 2007;72:208–14.

[18] Stephan T, Deutschländer A, Nolte A, et al. Functional MRI of galvanic vestibular stimulation with alternating currents at different frequencies. NeuroImage 2005;26:721–32.

[19] Schwiedrzik CM. Retina or visual cortex? The site of phosphene induction by transcranial alternating current stimulation. Front Integr Neurosci 2009;3:6.

[20] Rohracher H. Über subjektive Lichterscheinungen bei Reizung mit Wechselströmen. Z Sinnesphysiol 1935:164–81.

[21] Tuckett RP. Itch evoked by electrical stimulation of the skin. J Invest Dermatol 1982;79:368–73.

[22] Minhas P, Datta A, Bikson M. Cutaneous perception during tDCS: role of electrode shape and sponge salinity. Clin Neurophysiol 2011;122:637–8.

[23] Turi Z, Ambrus GG, Janacsek K, et al. Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. Restor Neurol Neurosci 2013;31:275–85.

[24] Fedorov A, Jobke S, Bersnev V, et al. Restoration of vision after optic nerve lesions with noninvasive transorbital alternating current stimulation: a clinical observational study. Brain Stimul 2011;4:189–201.

[25] Sabel BA, Fedorov AB, Naue N, Borrmann A, Herrmann C, Gall C. Non-invasive alternating current stimulation improves vision in optic neuropathy. Restor Neurol Neurosci 2011;29:493–505.

[26] Laakso I, Hirata A. Computational analysis shows why transcranial alternating current stimulation induces retinal phosphenes. J Neural Eng 2013;10:046009.

[27] Goldberg JM, Smith CE, Fernández C. Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. J Neurophysiol 1984;51:1236–56.

[28] Gilman S. Joint position sense and vibration sense: anatomical organisation and assessment. J Neurol Neurosurg Psychiatry 2002;73:473–7.

[29] Davis NJ, Gold E, Pascual-Leone A, Bracewell RM. Challenges of proper placebo control for non-invasive brain stimulation in clinical and experimental applications. Eur J Neurosci 2013;38(7):2973–7.

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METHODS
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Combining TMS and tACS for Closed-Loop Phase-Dependent Modulation of Corticospinal Excitability: A Feasibility Study

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Background: The corticospinal excitability indexed by motor evoked potentials (MEPs) following transcranial magnetic stimulation (TMS) of the sensorimotor cortex is characterized by large variability. The instantaneous phase of cortical oscillations at the time of the stimulation has been suggested as a possible source of this variability. To explore this hypothesis, a specific phase needs to be targeted by TMS pulses with high temporal precision.

Objective: The aim of this feasibility study was to introduce a methodology capable of exploring the effects of phase-dependent stimulation by the concurrent application of alternating current stimulation (tACS) and TMS.

Method: We applied online calibration and closed-loop TMS to target four specific phases (0°, 90°, 180° and 270°) of simultaneous 20 Hz tACS over the primary motor cortex (M1) of seven healthy subjects.

Result: The integrated stimulation system was capable of hitting the target phase with high precision (SD ± 2.05 ms, i.e., ± 14.45°) inducing phase-dependent MEP modulation with a phase lag (CI95% = −40.37° to −99.61°) which was stable across subjects ($p = 0.001$).

Conclusion: The combination of different neuromodulation techniques facilitates highly specific brain state-dependent stimulation, and may constitute a valuable tool for exploring the physiological and therapeutic effect of phase-dependent stimulation, e.g., in the context of neurorehabilitation.

Keywords: brain state-dependent, phase-dependent, adaptive, targeted modulation, beta oscillations

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INTRODUCTION

Transcranial magnetic stimulation (TMS) is capable of probing corticospinal excitability, modulating brain activity and disrupting pathological patterns (Hallett and Chokroverty, 2005; Siebner and Ziemann, 2007; Chen et al., 2008). However, there is a physiological trial-to-trial variability in motor-evoked potential (MEP) amplitude following identical TMS pulses most likely related to the brain state at the time of stimulation (Kiers et al., 1993; Thickbroom et al., 1999; Darling et al., 2006). A solid understanding of the interplay of stimulation effects

with the underlying cortical physiology is crucial to the reliable implementation of this technology in a therapeutic setting. TMS has therefore been combined with electroencephalographic (EEG) recordings to explore this interaction. There is increasing evidence that the prestimulus cortical power (mainly in the alpha and beta range) has a significant influence on the MEP (Zarkowski et al., 2006; Lepage et al., 2008; Sauseng et al., 2009; Mäki and Ilmoniemi, 2010; Feurra et al., 2013; Takemi et al., 2013; Gharabaghi et al., 2014; Kraus et al., 2016a,b). In addition, recent studies have applied different methodologies to explore the influence of the prestimulus phase of cortical rhythms on the MEP (Ferreri et al., 2011; Keil et al., 2013; Schulz et al., 2014; Berger et al., 2014; Kundu et al., 2014). The estimation of phase-dependency is challenged by the necessity to acquire evenly distributed TMS pulses across the phase spectrum to reduce any bias due to unequal distribution of the sampled phases. Many studies therefore applied a time jitter between stimulation pulses (Ferreri et al., 2011; Keil et al., 2013; Schulz et al., 2014; Berger et al., 2014; Kundu et al., 2014) instead of fixed time-intervals (van Elswijk et al., 2010). However, to evaluate this data, different analysis methods such as Fourier (Mäki and Ilmoniemi, 2010; van Elswijk et al., 2010), Hilbert (Keil et al., 2013) or Wavelet transformation (Berger et al., 2014) were applied, making it difficult to draw direct comparisons between the different results.

One alternative to a *post hoc* analysis of the interaction of randomly applied stimuli and the corresponding brain state is to apply the pulses in a more controlled way, e.g., by triggering them on the basis of online detection of the current phase. By applying adaptive thresholding of the brain signal in the time-domain, for example, stimuli were directed towards the peak and trough of low frequency oscillations (0.16 and 2 Hz) during sleep (Bergmann et al., 2012). Zrenner et al. (2015a,b) recently proposed the use of dedicated real-time recording and analysis hardware for phase-locked stimulation in the alpha-range on the basis of forward projection of a sliding window Fourier-transformation approach. Since any triggering is subject to an inherent time lag and is based on noisy measurements in a dynamical system, phase-dependent stimulation faces several obstacles. On the basis of features of the measured data, a predictive model of the underlying brain activity has first to be developed (predictability problem). Secondly, the speed of the technical system, mainly determined by the delay of signal analysis and triggering, must be faster than the dynamics of the target feature (real-time problem). Finally, the timing of the whole system must be precise enough to successfully target the desired features, i.e., phase jitter must be low (precision problem). Phase-dependent stimulation is also affected by the issue of a methodological flexibility (albeit less than *post hoc* approaches) during estimation of the phase spectrum. While all transformation methods estimating the instantaneous phase may, in theory, provide equal results (Bruns, 2004), their flexibility with regard to the exact implementation may cause inferential problems (Gelman and Loken, 2014).

To overcome the above-mentioned problems, we propose the combination of two non-invasive brain stimulation methods to study the dependency of stimulation effects on the phase of cortical oscillations. Specifically, we used transcranial alternating current stimulation (tACS) to modulate the spontaneous oscillatory activity, thus addressing the predictability and real-time problem. Moreover, to deliver TMS at the desired phase of the tACS, calibration of the systematic time-lag was applied, thereby addressing the precision problem. The basic concept of combining tACS with TMS has already been applied, e.g., to assess pre-post changes in cortical excitability following repetitive stimuli (Goldsworthy et al., 2016). It has also been used at a very low tACS frequency (0.8 Hz) with a positive current offset (Bergmann et al., 2009). Here, we extend this line of research by implementing synchronous recording of the tACS signal and the TMS artifact to assess and calibrate the temporal precision of the applied single pulses in relation to oscillations at a higher frequency than has ever been studied before, i.e., in the beta band (20 Hz). As well as testing its methodological feasibility, we also aimed to exploit the temporal precision of this approach by studying phase specific modulation of corticospinal excitability.

MATERIALS AND METHODS

Subjects

Having given written informed consent, seven healthy subjects (mean age: 22 years, STD: 3 years; 5 males; all right handed) took part in this methodological feasibility study which is part of a larger ongoing study. None of the subjects had any history of neurological diseases or medication. The study protocol was approved by the local Ethical Committee of the medical faculty of the University of Tübingen and was carried out in accordance with the principles of the Declaration of Helsinki.

Preparation

Bipolar electromyography (EMG) recording of the first dorsal interosseous (FDI) muscle of the right hand was performed in belly-tendon montage with a sampling rate of 5 kHz (BrainAmp ExG, Brain Products, Munich, Germany). We determined the location of the FDI hotspot in the primary motor cortex (M1) as the spot that elicits the highest MEP with the lowest TMS intensity. TMS was delivered by an integrated neuro-navigated system (Nexstim, Helsinki, Finland) with a figure-8-shaped coil that induced a posterior-anterior current flow. Once the hotspot had been determined, a rubber ring electrode (internal diameter 2.5 cm, external diameter 5 cm) was positioned over the hotspot and a second rectangular electrode (5 × 6 cm) was positioned over Pz. Both electrodes were attached to a DC/AC stimulator (NeuroConn, Ilmenau, Germany) and electrolyte gel was used to keep the impedance below 10 KΩ. The electrodes were kept in place by a tight EEG cap that covered the scalp. In addition, a fraction of the tACS signal current was routed via current division (1 MΩ vs. 1 kΩ) and subsequently recorded using a bipolar amplifier with 5 KHz sampling rate. Since the amplifier's input resistance was 10 GΩ, the current lost to recording was negligible.

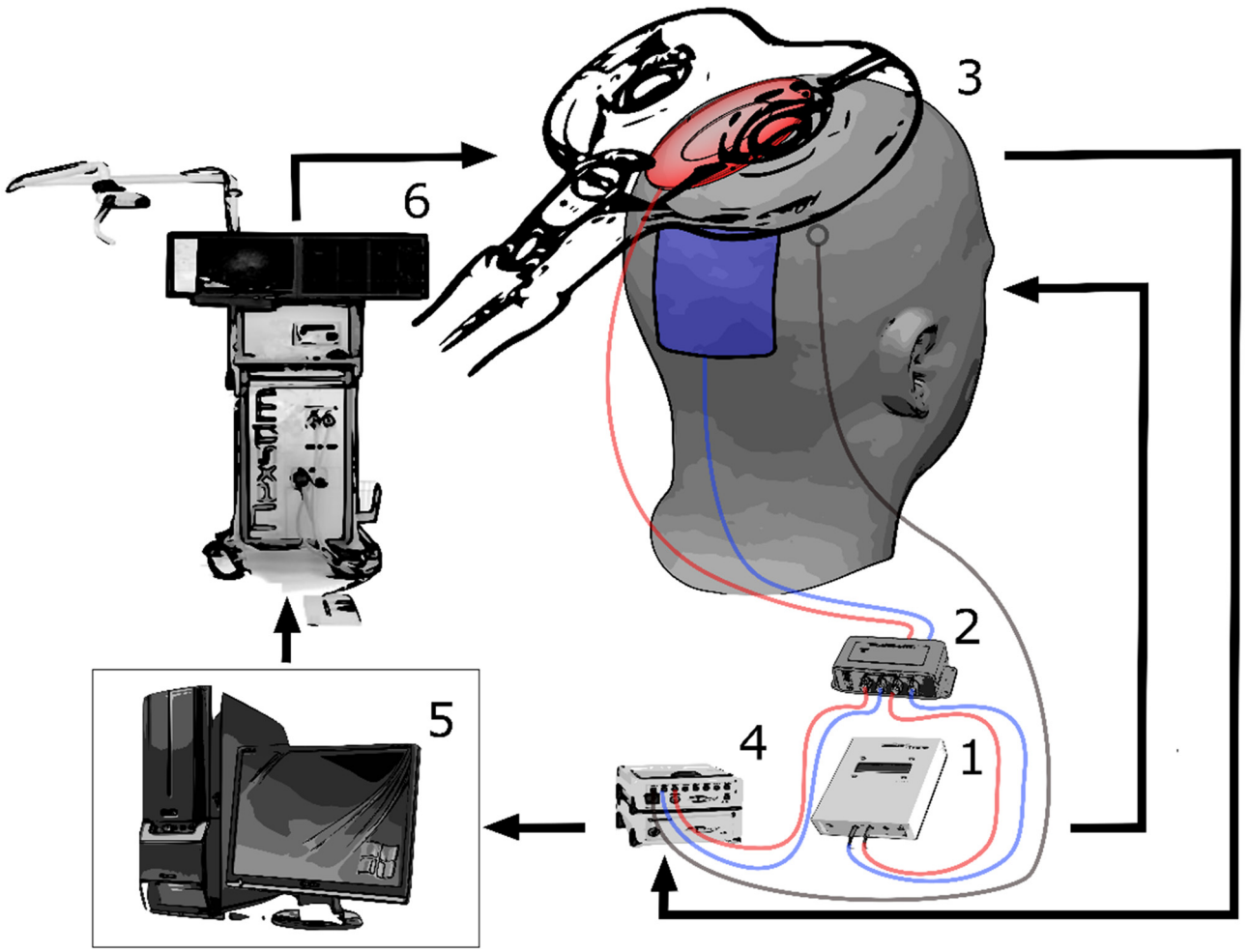

FIGURE 1 | The experimental setup is shown. The alternating current stimulation (tACS) stimulator (1) is connected to a current divider (2) that re-routes a part of the tACS signal directed to the subject (3) back to the electroencephalographic (EEG) amplifier (4) for recording. The recording computer (5) also triggers the transcranial magnetic stimulation (TMS) system (6). The stimulation artifact is recorded via an EEG electrode positioned on the subject's head. By converging the two stimulation artifacts to the controlling phase-consistency (PC), a precise synchronization of the whole system can be carried out after a test pulse. Thereafter, TMS pulses can be applied at specific phases of the tACS waveform.

Furthermore, we added two passive Ag/Ag-Cl-electrodes next to the hotspot position, i.e., directly under the TMS-coil, to detect any artifacts. Having positioned the stimulation electrodes, we used the neuro-navigated TMS system to keep coil position and orientation constant over the determined hotspot during the subsequent measurement and intervention. We assessed the resting motor threshold (RMT) of the FDI, using a staircase procedure to detect the TMS intensity inducing MEPs above 50 μV in 50% of the pulses. We calculated six stimulation intensities (SI) at 90%, 100%, 110%, 120%, 130% and 140% relative to the RMT for each subject. The setup is shown in **Figure 1**.

Technical Procedure

The intervention was performed in six runs, in each of which TMS was applied at a different SI. The order of the SI of each run was randomized across subjects. In the present methodological feasibility study, we report the findings during the SI of 110% only. Each run lasted around 3 min, with a 1-min break between runs. During each run, 200 s of tACS (20 Hz, 1 mA, 1 s ramp-up, 1 s ramp-down) were delivered to the subject, limiting the total stimulation duration of the study to 20 min (Nitsche and Paulus, 2007). In earlier research, we observed that 20 Hz tACS are liable to induce phosphene sensations (Raco et al., 2014). However, none of the subjects in this study reported neurosensory effects.

At the beginning of each run, we used a series of TMS test pulses to synchronize tACS phase and TMS stimulation timing. Following calibration (see below), TMS pulses were triggered at the run-specific intensity every 5 s (±500 ms predefined jitter) while targeting one of four specific tACS phases: peak, falling flank, trough, and rising flank (i.e., 0°, 90°, 180° and 270°) in random order. Each of these four phases was targeted at random 10 times during each run,

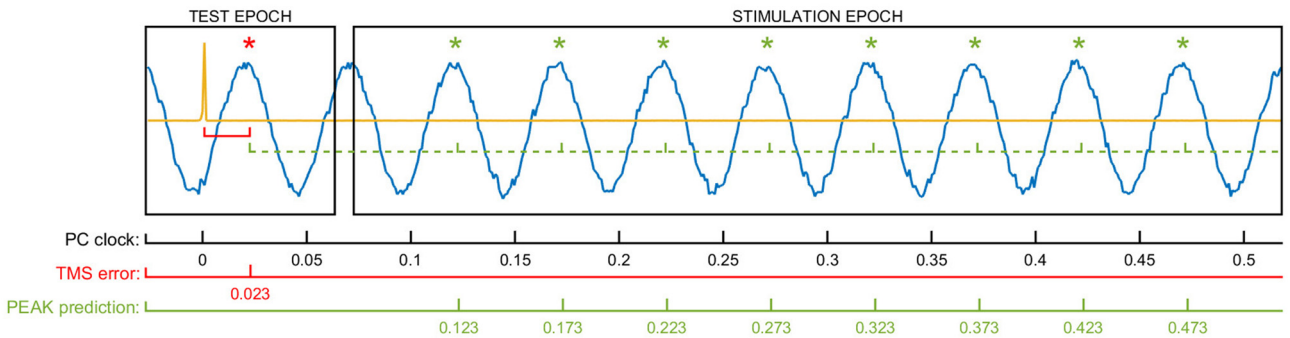

FIGURE 2 | The figure shows exemplary data used for the phase-specific stimulation algorithm and the respective variables involved in the calculations. The yellow signal represents the TMS artifact of the test pulse delivered randomly at the beginning of the epoch. The sinus line shows the recorded raw tACS waveform. The delay between the TMS pulse and the first target phase in the data (TMS error) is used to calculate the future time windows to trigger the TMS at the specific tACS phase. In the example shown here, the 23 ms TMS error is added to a multiple of the stimulation cycle time (50 ms) to detect the tACS peaks (PEAK prediction). By using this method, the delays connected to streaming of the data and the triggering of both TMS and tACS are implicitly considered in the calculation and don't need to be addressed separately.

resulting in a total of 40 stimulation pulses per run. To achieve the necessary precision, we synchronized the two stimulators using a closed-loop automatic calibration lasting for approximately 1 s at the beginning of each run. This procedure is specified in the code below. For this calculation, a random TMS pulse was briefly triggered at the onset of the tACS while the phase that was hit by this first TMS test pulse was analyzed. This enabled us to estimate the time/phase-lag of the stimulation system following the pseudo-code which illustrates the applied algorithm in detail, Moreover, exemplary signal fed to the algorithm is shown in **Figure 2**.

Pseudo-Code for Hardware Synchronization

%% TEST PULSE AND HARDWARE SYNCHRONIZATION
Start tACS
Start recording
Initialize clock
Deliver TMS test pulse
Determine tACS phase of TMS
for $n = 1$: number_of_trials
Wait for defined inter-trial-interval (plus jitter)
Determine current tACS phase based on clock
Select target phase from a (permuted) set of phases
Calculate shortest waiting time necessary to hit target phase with TMS
Wait for the waiting time
Trigger_TMS_pulse
end

Preprocessing and Analysis

The recorded EMG data was divided in epochs, with a time range of ±500 ms centered on the TMS artifact. The data was visually inspected, and trials contaminated by artifacts, and thus preventing the detection of MEPs, were removed (minimum number of trials removed per subject: 1, mean: 2.1, maximum: 4, total: 15, percentage of all trials: 1.5%). The peak-to-peak amplitude of the MEPs was measured as the range of the EMG trace from 10 to 50 ms following the TMS pulse. Within each subject, MEP amplitudes were normalized relative to the MEP amplitude at the 95th percentile of all measured MEPs. We averaged the MEPs over windows, i.e., for the first three and last three trains.

Please note that, although the stimuli were applied in random order, their distribution over the tACS waveform was even. Since they translate to a period length N of 4, we were subsequently able to apply discrete Fourier transformation to the MEP values to estimate magnitude and phase-lag of the interaction between tACS phase and TMS effect. The complex values could also be used to estimate the coherence of the phase-lag across subjects in a manner similar to that for inter-trial coherence (ITC). We began by transforming the phase of every subject to a vector on the unit circle according to the formula (1):

$$\hat{x} = e^{(1i*\theta(x))} \tag{1}$$

where $\hat{x}$ represents a unit-length complex value, e is the Euler's number and $\theta(\vec{x})$ represents the angle of the original complex value. Since we wished to test the phase-consistency (PC) across subjects, we took the absolute value of the mean of $\hat{x}$ across subjects using the following formula (2), where N is the number of subjects:

$$PC = \left| \frac{1}{N} \sum_{i=1}^{N} \hat{x}(n) \right| \tag{2}$$

PC is bound to the range between 0 (no coherence) and 1 (full coherence) and can be understood geometrically as the length of the mean vector. This length represents the stability of the phase-dependent MEP modulation across the subjects. To assess statistical significance, we permuted 1000 times the four MEP values for each subject and repeated the analysis. We considered the MEPs to be significantly modulated by the tACS phase

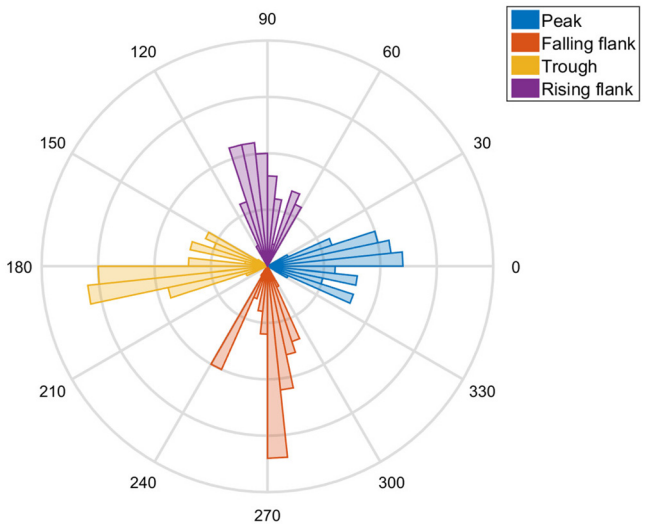

FIGURE 3 | A polar plot of the tACS phases hit by the TMS in all subjects is shown. Clear peaks at 0°, 90°, 180° and 270° are visible as evidence of the precision of the method.

when the actually measured phase consistency exceeded the 95th percentile of the distribution with permutation.

System Precision

To assess the precision of the system, we concatenated the trials of the seven subjects. We assessed the phase of the actual stimulation on the basis of a Fourier transformation of the 500 ms prior to the TMS pulse. The distribution is illustrated by a histogram (**Figure 3**). We then shifted the actual phase measured by the targeted phase of that trial (i.e., 0°, 90°, 180° and 270°) and used the CircStat toolbox (Berens, 2009) to assess the confidence intervals.

RESULTS

Phase and Temporal Precision

Visual inspection of the distribution revealed that the actual phase angle did indeed exhibit a distribution centered on the anticipated angle (**Figure 3**). The targeted phase was well within the confidence intervals of the distribution of the stimulated phases. The data of the seven subjects suggests that the phase lag was not significantly different from zero, indicating that there was no systematic bias (p = 0.65). The combined stimulation system was capable of hitting the target phase with high temporal precision (SD ± 2.05 ms), i.e., with ±14.72° standard deviation of the angle.

Phase-Dependent Modulation

The data shows a phase-dependent modulation of the MEPs at the end of the intervention (**Figure 4**). Statistical analysis (**Figure 5**) reveals no evidence of a phase-dependent modulation of the first MEPs (p = 0.082). The PC was well within the distribution of the values obtained with the permutation. In contrast, the PC of the last three MEPs showed a significant and strong phase alignment across the seven subjects (p = 0.001). Please note that the individuals' phase lag in the final three trials was always negative and did not differ significantly from −90° (CI95% = −40.37° to −99.61°).

DISCUSSION

Phase and Temporal Precision

In the present work, we describe a method for investigating the phase-dependency of TMS. Phase-dependent approaches require considerably higher temporal precision than closed-loop TMS on the basis of cortical band-power (Takemi et al., 2013; Gharabaghi et al., 2014; Kraus et al., 2016b). A number of approaches has been employed, most of which are based on *post hoc* assessment of the oscillatory phase (van Elswijk et al., 2010; Ferreri et al., 2011; Keil et al., 2013; Schulz et al., 2014; Berger et al., 2014; Kundu et al., 2014). A smaller number of studies employed closed-loop stimulation, by online triggering of the stimulation at the desired phase of the EEG (Bergmann et al., 2012; Zrenner et al., 2015b) or by combining tACS with TMS to control the phase at which stimulation should take place (Bergmann et al., 2009; Goldsworthy et al., 2016). In earlier approaches using tACS-TMS, the exact method for achieving phase-precise stimulation remains ambiguous. Moreover, reports of the precision achieved are rare. One study reports 1 ms jitter by using dedicated real-time hardware (Zrenner et al., 2015a), which is comparable with the 2 ms precision achieved by applying regular clinical hardware in our approach.

Perfect temporal precision can obviously only be achieved if all components run in a fully deterministic environment. However, this is often not the case, and labs do not have full control or knowledge about the precision of stimulation and recording devices. Without calibrations, the actual timing of the full system is affected by the behavior of the non-deterministic components, which can, at worst, cause a systematic bias. Furthermore, if medical certification of the devices is necessary, the desired control over certified components or the purchasing of dedicated and costly real-time recording hardware might not be feasible. The control approach presented here addresses precision, predictability and speed of the closed-loop system in three ways: first, by calibrating the set-up with a test pulse, second, by shifting the stimulation in time when the phase-delay is too large and third, by validating the system using a synchronous measurement of the tACS signal and the TMS-pulse artifact. The whole system can be easily implemented even if different hardware components are employed. The calibration is deemed to be particularly advantageous, since it allows for variability in communication delay, e.g., when different recording PCs, TCS or TMS hardware are being used. Additionally, by shifting the stimulation by a fixed phase-lag ($2^*\pi$) the pulse can be triggered in an even more flexible real-time environment, e.g., when the desired phase cannot be hit because of the intrinsic delay of the system. Finally, the synchronous recording enables us to check individual trials and weigh or discard them according to the achieved precision.

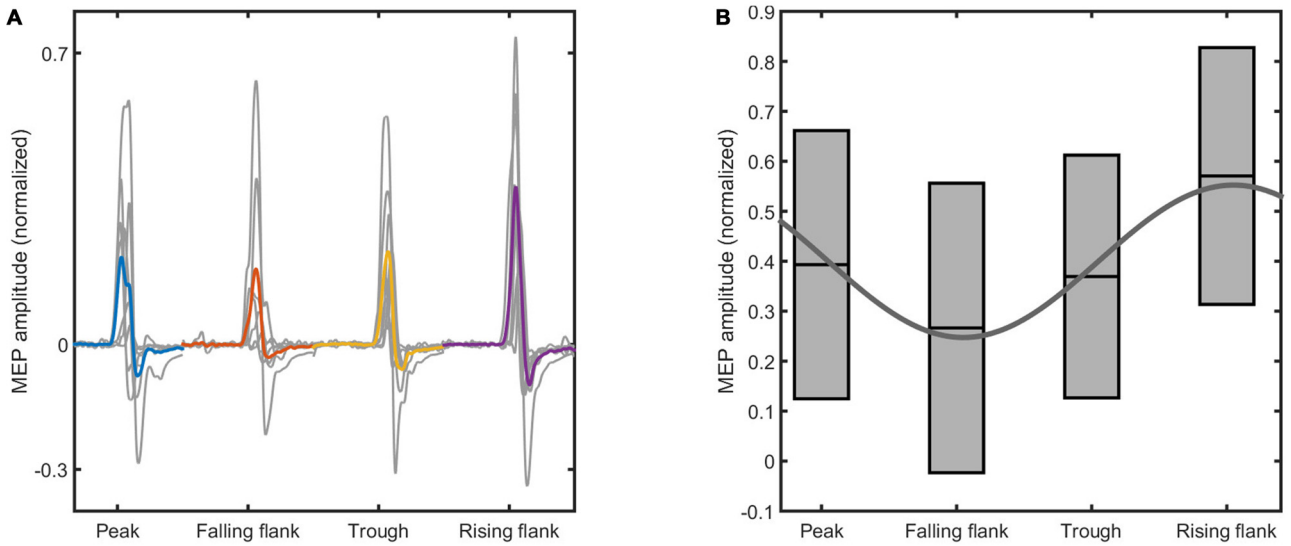

FIGURE 4 | The figure shows the raw motor evoked potential (MEP) data elicited at the end of the intervention. (A) Shows the mean MEPs for each subject elicited at different phases of the tACS waveform (in gray), and the average across all the subjects (color coded). **(B)** Shows the boxplots obtained from the mean and standard deviation of the MEPs across all the subjects. The sinus is the result of the fitting of the mean MEP amplitude across the four tACS phases. The phase conditions and the normalized MEP amplitudes are indicated on the *x*-axis and the *y*-axis of both figures, respectively.

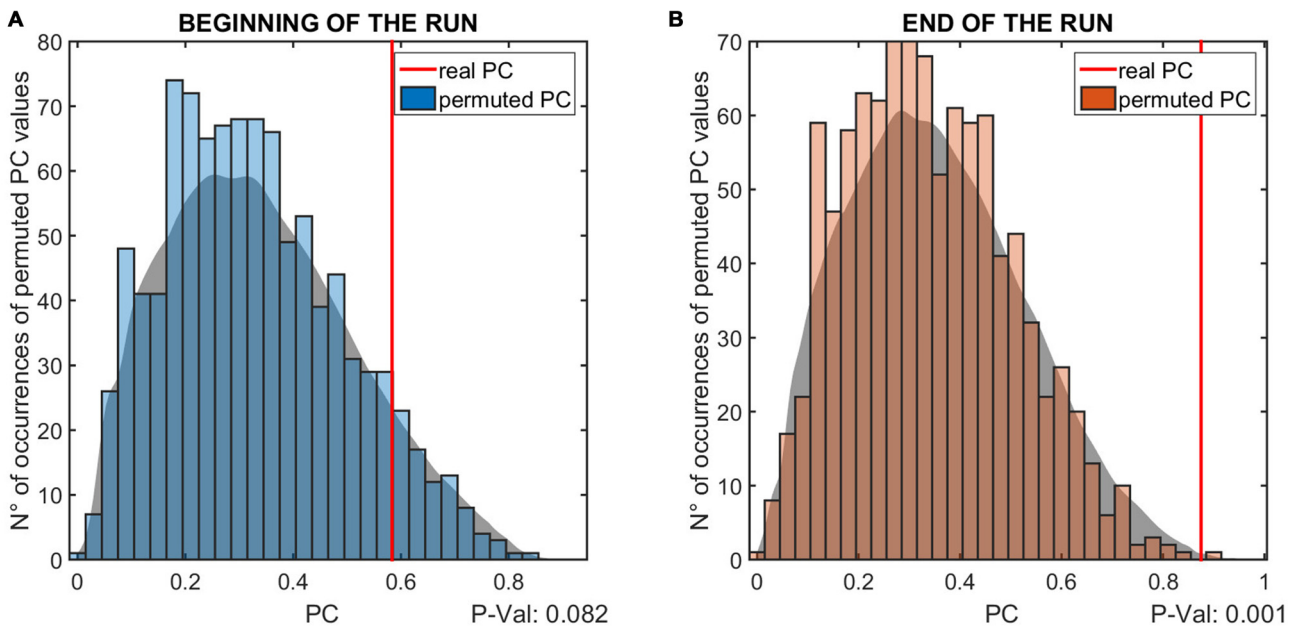

FIGURE 5 | The results of the permutation test for the phase coherence (PC) values of the MEP modulation is shown. The two panels show the results relative to the first **(A)** and last **(B)** three elicited MEPs. The vertical red lines indicate the PC value resulting from the real data, while the histogram shows the distribution of values obtained with the permutation test. The gray patch is a smoothed version of the histogram, to better highlight the distribution of PC values. The *P*-values below the panels indicate the probability that the PC values obtained from the analysis are lower than the permuted values, i.e., are due to measurement noise.

Phase-Dependent Modulation

Notably, when applied with 20 Hz tACS, the approach led to physiologically plausible results with regard to corticospinal excitability. Studies based on random stimulation found significant differences in the pre-stimulus beta-phase between high and low MEPs in occipital, but not in sensorimotor regions (Mäki and Ilmoniemi, 2010). Other studies reported significant angular-linear correlation between phase and MEP amplitude

over the sensorimotor region only (Keil et al., 2013). The phase of beta oscillations has been shown to be decisive for cortical and corticospinal computations and has also been linked with excitability of the corticospinal system (Miller et al., 2012; Aumann and Prut, 2015; Romei et al., 2016). Furthermore, 20 Hz tACS affects movement acceleration (Pogosyan et al., 2009), and unlike other frequencies, increases corticospinal excitability at rest (Feurra et al., 2013).

The physiological analysis in this study was exploratory and preliminary. However, the results suggest that phase-modulation occurs with the cumulative duration of the tACS. More specifically, we found no evidence for modulation during the first few TMS pulses, but a significant modulation during the last few pulses, with a distinct phase shift of approximately −90°. Please note that the current through a capacitor leads the voltage by 90° (Horowitz and Hill, 1989), which therefore suggest that the instantaneous current, and not the voltage, drives the cortical excitability during tACS.

Of course, the exploratory sample size used in this methodological feasibility study and the lack of direct cortical recordings do not permit us to draw too many far-reaching conclusions from these results. Nevertheless, the present findings validate the feasibility of the proposed approach, demonstrating that it is possible to apply phase-dependent stimulation with high precision.

Outlook

It is conceivable that the dot-product for the Fourier transformation could be calculated by taking the actual phases rather than the evenly spaced target phases. Depending on the noise level and its exact distribution in the estimation, this could reduce or increase the precision of the subsequent estimation of phase consistency and lag accordingly. Considering that the system has already achieved a good precision with regard to the targeted phases, we currently suggest that standard approaches to Fourier transformation be employed.

We are currently conducting a larger study, in which the interaction between phase and intensity of the TMS is being investigated. Many alternative research questions may be explored with this approach. For example, different phase lags could be explored for different frequencies to gain a better understanding of the response of the transcranial passage; or to ascertain whether there is a phase-alignment or a phase-drift over time thereby suggesting interactions with intrinsic frequencies.

CONCLUSION

We presented a combination of tACS and TMS that achieved high temporal and phase precision even when implemented with regular and (partially) non-deterministic hardware. We found preliminary evidence for phase-dependent effects of TMS leading at roughly 90° and therefore suggesting that effects are current driven rather than voltage driven. Future studies might explore these properties with regard to their entrainment, accumulation and interaction with stimulation intensity.

AUTHOR CONTRIBUTIONS

VR designed and performed research, analyzed data and wrote the article. RB analyzed data and wrote the article. ST performed research and edited the article. AG designed research and wrote the article.

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REFERENCES

Aumann, T. D., and Prut, Y. (2015). Do sensorimotor β-oscillations maintain muscle synergy representations in primary motor cortex? *Trends Neurosci.* 38, 77–85. doi: 10.1016/j.tins.2014.12.002

Berens, P. (2009). CircStat: a MATLAB toolbox for circular statistics. *J. Stat. Softw.* 31, 1–21. doi: 10.18637/jss.v031.i10

Berger, B., Minarik, T., Liuzzi, G., Hummel, F. C., and Sauseng, P. (2014). EEG oscillatory phase-dependent markers of corticospinal excitability in the resting brain. *Biomed Res. Int.* 2014:936096. doi: 10.1155/2014/936096

Bergmann, T. O., Groppa, S., Seeger, M., Mölle, M., Marshall, L., and Siebner, H. R. (2009). Acute changes in motor cortical excitability during slow oscillatory and constant anodal transcranial direct current stimulation. *J. Neurophysiol.* 102, 2303–2311. doi: 10.1152/jn.00437.2009

Bergmann, T. O., Mölle, M., Schmidt, M. A., Lindner, C., Marshall, L., Born, J., et al. (2012). EEG-guided transcranial magnetic stimulation reveals rapid shifts in motor cortical excitability during the human sleep slow oscillation. *J. Neurosci.* 32, 243–253. doi: 10.1523/JNEUROSCI.4792-11.2012

Bruns, A. (2004). Fourier-, Hilbert- and wavelet-based signal analysis: are they really different approaches? *J. Neurosci. Methods* 137, 321–332. doi: 10.1016/j.jneumeth.2004.03.002

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.-P., Magistris, M. R., et al. (2008). The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. *Clin. Neurophysiol.* 119, 504–532. doi: 10.1016/j.clinph.2007.10.014

Darling, W. G., Wolf, S. L., and Butler, A. J. (2006). Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Exp. Brain Res.* 174, 376–385. doi: 10.1007/s00221-006-0468-9

Ferreri, F., Pasqualetti, P., Määttä, S., Ponzo, D., Ferrarelli, F., Tononi, G., et al. (2011). Human brain connectivity during single and paired pulse transcranial magnetic stimulation. *Neuroimage* 54, 90–102. doi: 10.1016/j.neuroimage.2010.07.056

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., and Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J. Neurosci.* 33, 17483–17489. doi: 10.1523/JNEUROSCI.1414-13.2013

Gelman, A., and Loken, E. (2014). The statistical crisis in science. *Am. Sci.* 102:460. doi: 10.1511/2014.111.460

Gharabaghi, A., Kraus, D., Leão, M. T., Spüler, M., Walter, A., Bogdan, M., et al. (2014). Coupling brain-machine interfaces with cortical stimulation for brain-state dependent stimulation: enhancing motor cortex excitability for neurorehabilitation. *Front. Hum. Neurosci.* 8:122. doi: 10.3389/fnhum.2014.00122

Goldsworthy, M. R., Vallence, A.-M., Yang, R., Pitcher, J. B., and Ridding, M. C. (2016). Combined transcranial alternating current stimulation and cTBS: a novel approach for neuroplasticity induction. *Eur. J. Neurosci.* 43, 572–579. doi: 10.1111/ejn.13142

Hallett, M., and Chokroverty, S. (2005). *Magnetic Stimulation in Clinical Neurophysiology*, 2nd Edn. Philadelphia, PA: Elsevier Butterworth-Heinemann.

Horowitz, P., and Hill, W. (1989). *The Art of Electronics.* 2nd Edn. UK/USA: Cambridge University Press.

Keil, J., Timm, J., Sanmiguel, I., Schulz, H., Obleser, J., and Schöenwiesner, M. (2013). Cortical brain states and corticospinal synchronization influence TMS-evoked motor potentials. *J. Neurophysiol.* 111, 513–519. doi: 10.1152/jn.00387.2013

Kiers, L., Cros, D., Chiappa, K. H., and Fang, J. (1993). Variability of motor potentials evoked by transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol.* 89, 415–423. doi: 10.1016/0168-5597(93)90115-6

Kraus, D., Naros, G., Bauer, R., Leão, M. T., Ziemann, U., and Gharabaghi, A. (2016a). Brain-robot interface driven plasticity: distributed modulation of corticospinal excitability. *Neuroimage* 125, 522–532. doi: 10.1016/j.neuroimage.2015.09.074

Kraus, D., Naros, G., Bauer, R., Leão, M. T., Ziemann, U., and Gharabaghi, A. (2016b). Brain state-dependent transcranial magnetic closed-loop stimulation controlled by sensorimotor desynchronization induces robust increase of corticospinal excitability. *Brain Stimul.* doi: 10.1016/j.brs.2016.02.007 [Epub ahead of print].

Kundu, B., Johnson, J. S., and Postle, B. R. (2014). Pre-stimulation phase predicts the TMS-evoked response. *J. Neurophysiol.* 112, 1885–1893. doi: 10.1152/jn.00390.2013

Lepage, J.-F., Saint-Amour, D., and Théoret, H. (2008). EEG and neuronavigated single-pulse TMS in the study of the observation/execution matching system: are both techniques measuring the same process? *J. Neurosci. Methods* 175, 17–24. doi: 10.1016/j.jneumeth.2008.07.021

Mäki, H., and Ilmoniemi, R. J. (2010). EEG oscillations and magnetically evoked motor potentials reflect motor system excitability in overlapping neuronal populations. *Clin. Neurophysiol.* 121, 492–501. doi: 10.1016/j.clinph.2009.11.078

Miller, K. J., Hermes, D., Honey, C. J., Hebb, A. O., Ramsey, N. F., Knight, R. T., et al. (2012). Human motor cortical activity is selectively phase-entrained on underlying rhythms. *PLoS Comput. Biol.* 8:e1002655. doi: 10.1371/journal.pcbi.1002655

Nitsche, M. A., and Paulus, W. (2007). "Transkranielle Gleichstromstimulation," in *Das TMS-Buch. Handbuch der Transkraniellen Magnetstimulation*, eds H. R. Siebner and U. Ziemann (Berlin, Heidelberg: Springer), 533–542.

Pogosyan, A., Gaynor, L. D., Eusebio, A., and Brown, P. (2009). Boosting cortical activity at β-band frequencies slows movement in humans. *Curr. Biol.* 19, 1637–1641. doi: 10.1016/j.cub.2009.07.074

Raco, V., Bauer, R., Olenik, M., Brkic, D., and Gharabaghi, A. (2014). Neurosensory effects of transcranial alternating current stimulation. *Brain Stimul.* 7, 823–831. doi: 10.1016/j.brs.2014.08.005

Romei, V., Bauer, M., Brooks, J. L., Economides, M., Penny, W., Thut, G., et al. (2016). Causal evidence that intrinsic β-frequency is relevant for enhanced signal propagation in the motor system as shown through rhythmic TMS. *Neuroimage* 126, 120–130. doi: 10.1016/j.neuroimage.2015.11.020

Sauseng, P., Klimesch, W., Gerloff, C., and Hummel, F. C. (2009). Spontaneous locally restricted EEG alpha activity determines cortical excitability in the motor cortex. *Neuropsychologia* 47, 284–288. doi: 10.1016/j.neuropsychologia.2008.07.021

Schulz, H., Ubelacker, T., Keil, J., Muller, N., and Weisz, N. (2014). Now i am ready - now i am not: the influence of pre-TMS oscillations and corticomuscular coherence on motor-evoked potentials. *Cereb. Cortex* 24, 1708–1719. doi: 10.1093/cercor/bht024

Siebner, H. R., and Ziemann, U. (2007). *Das TMS-Buch. Handbuch der Transkraniellen Magnetstimulation.* Heidelberg: Springer Medizin.

Takemi, M., Masakado, Y., Liu, M., and Ushiba, J. (2013). Event-related desynchronization reflects downregulation of intracortical inhibition in human primary motor cortex. *J. Neurophysiol.* 110, 1158–1166. doi: 10.1152/jn.01092.2012

Thickbroom, G. W., Byrnes, M. L., and Mastaglia, F. L. (1999). A model of the effect of MEP amplitude variation on the accuracy of TMS mapping. *Clin. Neurophysiol.* 110, 941–943. doi: 10.1016/s1388-2457(98)00080-7

van Elswijk, G., Maij, F., Schoffelen, J.-M., Overeem, S., Stegeman, D. F., and Fries, P. (2010). Corticospinal β-band synchronization entails rhythmic gain modulation. *J. Neurosci.* 30, 4481–4488. doi: 10.1523/JNEUROSCI.2794-09.2010

Zarkowski, P., Shin, C. J., Dang, T., Russo, J., and Avery, D. (2006). EEG and the variance of motor evoked potential amplitude. *Clin. EEG Neurosci.* 37, 247–251. doi: 10.1177/155005940603700316

Zrenner, C., Tünnerhoff, J., Zipser, C., Müller-Dahlhaus, F., and Ziemann, U. (2015a). Brain-state dependent brain stimulation: real-time EEG alpha band analysis using sliding window FFT phase progression extrapolation to trigger an alpha phase locked TMS pulse with 1 millisecond accuracy. *Brain Stimul.* 8, 378–379. doi: 10.1016/j.brs.2015.01.213

Zrenner, C., Tünnerhoff, J., Zipser, C., Müller-Dahlhaus, F., and Ziemann, U. (2015b). V38. Brain-state dependent non-invasive brain stimulation using closed-loop real-time EEG signal analysis to trigger a TMS pulse with millisecond accuracy. *Clin. Neurophysiol.* 126:e85. doi: 10.1016/j.clinph.2015.04.116

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Title

Phase-dependent modulation of corticospinal excitability by sensorimotor beta-tACS

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Abstract

Background: Transcranial alternating current stimulation (tACS) has been shown to entrain occipital alpha-rhythms; a similar demonstration of phase-specific entrainment is lacking for sensorimotor beta-rhythms.

Objective: The aim of this study was to investigate phase-specific effects of beta-tACS on motor system output.

Methods: Single-pulse transcranial magnetic stimulation (TMS) was applied concurrent to 20 Hz tACS over the primary motor cortex of eighteen healthy subjects to probe the phase-specific modulation of corticospinal excitability indexed by motor evoked potentials (MEPs) at the contralateral first dorsal interosseous muscle. Four phases of the tACS waveform (0°, 90°, 180°, 270°) were targeted by closed-loop TMS, which was applied in a randomized blocked design at six different intensities (from 90% to 140% resting motor threshold, RMT). A sinusoidal fitting was performed on the regression parameters describing the MEP response elicited at different phases.

Results: Beta-tACS induced a sinusoidal modulation of MEPs with a phase shift of -120° and 60° for the slope and intercept, respectively. The phase-dependent modulation was specific for stimulation with 110% RMT and decreased gradually on both sides of this peak with lower and higher stimulation intensities. The pattern of the phase modulation changed over time showing a strong effect in the beginning of the intervention with a shift equal to the one found for the intercept; this early modulation decreased within the first minute of stimulation to then reappear after three minutes with inverted polarity.

Conclusion: This is the first evidence of phase-specific effects of tACS on corticospinal excitability.

Running title: Phase-dependent modulation of corticospinal excitability

Key words: sensorimotor rhythm, beta-band, phase-dependent stimulation, brain state-dependent stimulation, closed-loop stimulation

INTRODUCTION

Electrophysiological recordings of the brain in vivo reveal widespread oscillatory activity generated from subcortical and cortical structures [1]. The role of such rhythms in orchestrating different aspects of cognition and behavior has been researched in an increasing amount of studies and critical reviews [2–4]. To provide causal evidence for the functional significance of oscillatory brain activity, several stimulation protocols have been applied to interfere directly with these phenomena (for a review see: . Transcranial alternating current stimulation (tACS), for example, is a promising method to manipulate cortical rhythms by direct entrainment of oscillatory potentials [5]. The underlying concept of tACS entrainment predicts that ongoing cortical oscillations synchronize to the stimulation rhythms, thereby, augmenting the targeted spectral power and aligning the oscillatory phase to the stimulation cycle [6]. Most previous work on the efficacy of tACS in modulating the spectral characteristics of brain activity has been addressing the visual and motor systems (for a comprehensive review on tACS see: Veniero et al., 2015). Therefore, the respective stimulation protocols have mainly focused on the natural spectral signatures exhibited by these systems, namely sensory alpha- [8], and motor beta-rhythms [9] to probe the efficacy of this technique.

In the visual system, this approach has provided convincing evidence for entrainment of occipital alpha-rhythms by tACS [10–13], thereby, producing relevant insights into the functional role of alpha-oscillations during visual processing. Regarding the sensorimotor system, behavioral findings following beta-tACS have been reported showing an increase in muscle stiffness, slowing of movement and decrease in movement readiness [14–16]. Physiological effects of beta-tACS have been demonstrated by using measures of corticospinal excitability (CSE) such as motor evoked potentials (MEPs) following transcranial magnetic stimulation (TMS). Specifically, brief trains of beta-tACS have been shown to increase the CSE levels during [17,18] and right after [19] sensorimotor cortex stimulation. However, none of these studies addressed phase-specific effects of beta-tACS, unlike the findings during occipital alpha-stimulation [13]**.**

In this study, we intended to close this gap and contribute to the characterization of 20 Hz sensorimotor tACS by addressing its phase-specific effects on corticospinal excitability. The rationale for this study was based on two assumptions: (i) The phase of the entrained beta-band oscillations aligns to the phase of the extrinsic stimulation

similar to the findings with lower stimulation frequencies [20,21]. (ii) The phase of the pre-TMS sensorimotor beta-oscillations encodes the excitability level of the corticospinal system similar to the observations during spontaneous oscillations, i.e. without concurrent tACS [22,23]. We, therefore, predicted that 20 Hz tACS would induce a sinusoidal modulation of MEPs, phase-locked to the stimulation waveform. Such an effect would, furthermore, provide causal evidence for an effective synchronization between extrinsic stimulation and intrinsic sensorimotor oscillatory activity in the beta-band.

MATERIAL AND METHODS

Subjects: Eighteen healthy subjects (mean age: 24 (19-28) years, all right handed, 13 males) participated in this study after providing written informed consent. The study protocol was approved by the local ethical committee of the medical faculty of the University of Tübingen and was carried out in accordance with the principles of the Declaration of Helsinki.

Preparation: Electromyography (EMG) activity was recorded from the first dorsal interosseous (FDI) muscle of the right hand with a bipolar montage. Data was acquired with a 5 kHz sampling rate (BrainAmp ExG, Brain Products, Munich, Germany). Stimulation pulses were delivered by a navigated TMS system (Nextim eXimia NBS, Helsinki, Finland) with a figure-8-shaped coil inducing a monophasic current flow in posterior-anterior direction. The TMS hotspot for the recorded muscle was determined as the cortical location in the left hemisphere robustly eliciting MEPs with the lowest stimulation intensity. The hotspot search procedure started at a random location on the scalp overlaying the left parietal bone, with a coil orientation perpendicular to the scalp and oriented in the posterior-anterior direction. The initial TMS amplitude was set at 40% of the stimulator output, the stimulation was manually triggered while the coil was gradually moved around the initial position. If the search didn´t elicit any detectable MEPs, the intensity was increased in 5% steps and the search performed again. Once the location robustly eliciting the highest MEPs was found, the stimulator intensity was reduced using a staircase approach to diminish the current spread of the stimulation, hence restricting the hotspot area eliciting MEPs.

A rubber ring electrode (internal diameter 2.5 cm, external diameter 5 cm) was positioned over the individual hotspot of each subject and connected to a battery

driven DC/AC stimulator (NeuroConn DC stimulator, Ilmenau, Germany). A second rectangular electrode (5 cm x 6 cm) was located over Pz in accordance with the 10-20 system. The impedance of the two tACS electrodes was kept below 10 KΩ using a dedicated electrolyte gel (EASYCAP GmbH). A small fraction of the current passing through the electrodes was routed to the amplifier by using a custom-made splitter box connecting the electrodes and the DC stimulator. Two additional bipolarized Ag/Ag-Cl electroencephalography (EEG) electrodes were positioned next to the hotspot to record the artifact induced by the TMS pulses. An EEG cap (EasyCAP, Brain Products, Munich, Germany) secured the position of the tACS and recording electrodes on the scalp. The navigation system was used to keep the TMS coil position and orientation stable over the hotspot during the whole measurement. We then evaluated the resting motor threshold (RMT) of the subject using a staircase procedure to find the stimulation intensity inducing MEPs higher than 50μV in at least 5 of 10 consecutive pulses. Six individualized stimulation intensities (SI) were then calculated at 90%, 100% 110% 120% 130% and 140% of the RMT. The experimental setup is shown in Figure 1a.

Experimental Procedure:

We applied online calibration and closed-loop TMS to target four specific phases (0°, 90°, 180°, 270°) of simultaneous 20 Hz tACS over the primary motor cortex (M1); for this purpose, the TMS system was synchronized to the phase of the tACS waveform, and delivered stimuli so that they were selectively targeting peak, trough, falling, and rising flank of the tACS cycle, thereby, applying a method that we have described in detail elsewhere [24]. We tested our protocol over six predefined TMS stimulation intensities (see above) to identify parameters that captured modulations of CSE without disrupting the phase of the ongoing brain oscillations.

The experiment consisted of six runs per subject lasting approximately three minutes each, with one-minute breaks in between, and characterized by simultaneous TMS-tACS. During each run single TMS pulses were applied at one of the six TMS intensities (90%, 100% 110% 120% 130% or 140%). The order of stimulation intensities was randomized across subjects (Figure 1b). The tACS train started at the beginning of each run (20 Hz, 1mA, 1s ramp-up, 1s ramp-down) and lasted for the whole duration of the run. The total stimulation time was about 20 minutes in

accordance with the tACS safety guidelines [25]. The subjects were instructed to report the occurrence of phosphene sensations commonly elicited by setups similar to the one used in the present study [26]. However, none of the subjects reported the presence of such sensations.

Each run started with a TMS test pulse used to synchronize the computer clock with the tACS phase. Following the synchronization, TMS pulses were delivered to the hotspot every 5 seconds (± 500ms jitter), targeting one of four pre-selected tACS phases: peak, falling flank, trough, and rising flank (i.e. 0°, 90°, 180° and 270°) in randomized order. Each phase was targeted 10 times through each run, resulting in a total of 40 pulses per run (Figure 1c). We have previously shown that this integrated stimulation system was capable of hitting the target phase with high precision (SD ± 2.05 ms, i.e. ± 14.45°; Raco et al., 2016).

Preprocessing and Analysis: The recorded EMG data was divided into epochs centered on the TMS artifact. The data was visually inspected, and trials contaminated by artifacts were removed. The peak-to-peak amplitude of the MEPs was measured as the range of the EMG trace from 10 to 50 milliseconds following the TMS pulse. Within each subject, MEP amplitudes were normalized relative to the MEP amplitude at the 95th percentile of all measured MEPs.

Phase-dependent MEP modulation: To test whether the phase of tACS would induce a modulation of the MEP sizes depending on the time of stimulation we performed a linear regression on the MEP values with regard to the number of TMS pulse. The regression analysis was performed for each subject, TMS intensity and tACS phase separately. Thereby, we obtained for each condition the intercept and slope values of the regression line, which represented the initial offset and the evolution of the MEPs during the different tACS phases, respectively. Under the assumption of a linear relationship between the tACS phase and MEP modulation over time, a sinusoidal function was fit to the subjects' regression parameters per TMS intensity. In sum, we acquired for each subject the intercept and slope values of the MEP regression for four different tACS phases at six TMS intensities each. These data were normalized between -1 and 1 before fitting a sinus for the different phases and intensities. The fitting resulted in two parameters: the amplitude and the phase of the sinus fitted to the data. While the phase of this sinus indicated the mean

phase of the modulation, the amplitude revealed the extent of the modulation's phase consistency across subjects. Since the parameters space of the fitting was unconstrained, some fitting solutions converged to negative amplitude values, these are mathematically equivalent to a sinus of positive amplitude shifted of 180°. Thus, to correctly assess amplitude and phase of the modulation we took the absolute value of the amplitude parameter and shifted the angles relative to negative amplitudes of 180°. Thus, the resulting amplitude parameter was bounded between 0 (representing a truly random phase relationship between the tACS waveform and the intrinsic oscillations) and 1 (indicating absolute phase locking of tACS and intrinsic oscillations across subjects).

To assess whether the resulting phase consistency values reflected a real sinusoidal modulation of the regression parameters and not just fluctuations of noise, we performed a non-parametric permutation test. Specifically, the phase order was randomly scrambled for each subject before repeating the fitting for 1000 times. We, thereby, obtained for each stimulation intensity a distribution of permuted fitting parameters with the assumption of a random phase relationship between the different subjects. We considered the data to be significantly modulated by a sinus function when the sinus absolute amplitude obtained by the fitting exceeded the 95th percentile (uncorrected) of the distribution of values obtained from the permutation algorithm. Significance levels were adjusted for the multiple comparisons problem relative to the repeated testing of six different intensities, thereby, reducing the significance of p-values to values of less than 0.008 after Bonferroni correction.

Time-course of tACS entrainment: To characterize the time-course of the phase-dependent effects reflected on the modulation of slope and intercept parameters we repeated the fitting procedure at specific time intervals. On the basis of the slope and intercept values of each subject recalculated the values of the regressed MEPs every 20 seconds within the 3 minutes epoch. We thus repeated the fitting and permutation tests described above for each time point, showing the tACS modulation effects over the whole epoch.

RESULTS

Phase-dependent modulation:

The fitting process converged successfully for all intensities, indicating the use of an adequate model to fit the data. The fitting of the slope and intercept parameters returned an estimate of both phase, and robustness of the sinusoidal modulation. The permutation test revealed a significant modulation of both parameters (Slope: $p = 0.0012$, Intercept: $p = 0.0062$) for the 110% RMT intensity. Table 1 reports the values of phase-shift and amplitude of the sinusoidal modulation, as well as the significance level for all intensities. The stimulus-response curve for this effect evidences a clear peak at 110% intensity condition for both parameters, gradually decreasing across intensities (figure 2). The exact values of phase of the modulation for the 110% Intensity show a phase shift of roughly 180° (Slope: $\theta = -120°$, Intercept: $\theta = 60°$), indicating an inverse effect of the stimulation on the regression parameters (figure 3a-b).

Time-course of the effect:

To assess how the modulation changes over the whole epoch, we recalculated the fitting for the regression values at different points in time, for the significant 110% RMT intensity condition (figure 3c). The values of the regression line for the four phase condition was recalculated every seconds for the whole epoch. The figure shows a clear modulation in the first minute of stimulation, as expected from the results of the intercept. Due to the 180° shift between slope and intercept, this initial modulation decreases, gradually flattening to the zero line, indicating a lack of modulation and the absence of phase dependent effects. Subsequently, the modulation gradually reappears with inverted polarity reaching significant levels again toward the end of the epoch. The presence of an effective phase-modulation is confirmed by the permutation testing, whose p-values are reported below the figure.

DISCUSSION

In the present study we used TMS to assess the phase dependent effects of sensorimotor beta tACS on corticospinal excitabilty. We found that the phase of tACS has a direct effect on the parameters used to model the subjects' MEP response to the stimulation (Phase-dependent effects). Our analysis reveals an early modulation of the response, phase locked to the tACS phase, which lasts for some seconds to reappear at the end of the epoch (Time-Dependent effects). This effect

was found selectively for the 110% RMT condition, and it decreased with varying the TMS intensity (Intensity-Dependent effects).

Phase-dependent effects of tACS:

The physiological mechanisms through which tACS interferes with ongoing brain activity have been discussed in numerous reviews [5–7,27–29]**.** One of the hypotheses that received the most attention is the direct entrainment of ongoing brain oscillatory potentials. Evidence for phase-locking effects has been provided in the context of the sensory systems showing a modulation of physiological or behavioral indexes correlated with the phase of the tACS current [13,30,31]. Yet, such effects have not yet been shown in case of sensorimotor beta-tACS. In the present study used a combination of tACS and TMS to sample the response of the corticospinal system to specific phases of the expected sensorimotor beta-oscillations. Based on the definition of entrainment, we postulate that evidence of a modulation of the response by the instantaneous phase of the tACS waveform would suggest entrainment of oscillating brain activity [6]. We thus modeled the subjects' individual responses to the four experimental condition using a linear regression model. We argue that the assumption of linearity of the MEPs, necessary for the regression model, is a realistic constrain for relatively short epochs, and previously used in literature [32]. The regression allowed us to reduce the dimensionality of the data to the two regression parameters: intercept and slope, and successfully disentangle what is the initial response, and the cumulative effects of the stimulation. The results of the intercept analysis indicate that at the beginning of the epoch, the phase of tACS effectively entrains corticospinal excitability, resulting in a sinusoidal modulation of the initial MEPs. By effects of the phase shift of this entrainment (~60°), the strongest MEPs of this early response to the stimulation are elicited at the falling flank of the tACS waveform, while when the stimulation phase is at its rising flank the excitability level is minimum. The analysis of the slope parameter shows how this initial modulation changes over time. As we can see the phase shift between the intercept and the slope modulation is characterized by a ~180° angle. This indicates that through the whole epoch, MEPs invert their original tendency shown by the intercept. Thus, the MEPs that were the strongest at the beginning of the epoch (e.g. falling flank), are the ones that decrease the most over time, while

the weakest response (e.g. rising flank) increases over the course of the stimulation period.

Time-Dependent effects of tACS:

While the regression parameters represent global measures of the MEP response to the stimulation, they lack a clear time dimension. Thus, to understand the temporal dynamics of the effect evidenced by the analysis of the regression parameters we projected our model back to specific time points. In this way we were able to compare the response to the four phase conditions at any given point in time, independently of when the single MEPs were elicited. This process was necessary since each subject was tested only once per TMS intensity, thus the time-dependent response to the different tACS phases could not be assessed using single MEPs.

As suggested by the analysis of the regression parameters, the phase-dependent effects of tACS are characterized by an early modulation of corticospinal excitability that decreases over time, to rise again with inverted polarity. The existence of an - albeit short - initial phase locking between stimulation and MEPs is in line with the idea that tACS is capable of entraining the phase of cortical rhythms. Nevertheless, rather than being sustained over the whole stimulation length, this initial effect appears to be inconsistent and modulated over time.

Although puzzling, this result can be easily interpreted as an interference effect of the stimulation on ongoing brain rhythms. In fact, as discussed previously, entrainment consists in a robust phase-relationship between ongoing brain oscillations and the stimulation current. This is possible if the oscillatory periods (e.g. the inverse of the frequency) characterizing the two signals have a similar duration. In cases where the stimulation frequency is adapted to closely match the frequency of the subjects' individual brain rhythms, or where the difference between the two signals is sufficiently small to allow for a frequency shift in of the endogenous brain rhythms by effect of the tACS current, this is indeed the case. Nevertheless, in stimulation protocols where the stimulation frequency is fixed for all the subjects, the subjects' individual frequency peaks might differ substantially from the predefined stimulation frequency. These differences are reflected in the period length of the two signals, thereby inducing a small but constant increase in the phase shift between the two signals. This effect accumulates over several cycles forcing the phases of the two oscillations to drift apart. The relative phase shift of the two signals would

periodically span the whole cycle and return to its original angle resulting in transient periods of constructive and destructive interferences. By effect of the time-varying angle between the two signals, ongoing oscillations get facilitated (i.e. When the phase shift is close to 0°), or disrupted (i.e. When the phase shift is close to 180°) by the instantaneous polarity of the stimulation currents, thus resulting in a cyclic amplitude modulation as the one evidenced in our study. Additionally, such interference pattern would exhibit a time varying phase-relationship, characterized by in- and out-of-phase periods with the two original oscillations, similar to the inverted phase shown by the late modulation. A similar result was reported in a simulation study testing the effects of stimulation frequency on the resulting entrainment patterns [21]. In this study, the authors showed how a robust and long lasting entrainment effect would become strongly disturbed, showing a bursting behavior, in case of a mismatch between the oscillatory frequency of the simulated network, and the frequency of the stimulation. The use of fixed stimulation frequency, and a general variability of the subjects' individual beta peaks can therefore account for the pattern of entrainment found in our study. Nevertheless, we would expect to find a modulation repeating at a period which reflects the frequency difference between the two signals (e.g. the inverse of the absolute difference between the two frequencies). In our study the period of the modulation is roughly 3 minutes, thus this would reflect a very small difference between the subjects' individual frequencies (f1) and the exogenous tACS oscillation (f2). Thus we speculate that additionally to the phase-locking shown in our analysis, tACS entrains also the subjects' individual frequencies, pulling them toward the stimulation frequency. This frequency pulling effect of tACS induces an artificially close match between stimulation frequency and endogenous frequencies, resulting in a slow temporal modulation of the phase-locking effects.

Additionally, it is possible that the temporal modulation of the tACS effects is caused by a complex interaction with the TMS currents. TMS pulses are known to induce a phase reset of ongoing cortical oscillations detectable for two or three cycles after the stimulation [33]. Nevertheless, despite this the TMS-induced phase reset might be responsible of destroying the phase-locking between ongoing beta oscillation and tACS after every TMS pulse, it is constant through the number of TMS pulses delivered [34]. Thus we conclude that despite a TMS induced phase-resetting effect

might be present in our protocol, this is hardly responsible for the slow modulation of the tACS effect we find in our study.

Intensity-Dependent effects of tACS:

TMS pulses are known to target different populations of neurons depending on the stimulation intensity, as evidenced by direct recordings of the spinal responses to the stimulation [35]. While low TMS intensities induce MEPs via recruitment of early I-waves, with increasing stimulation amplitude, later I-waves gradually contribute to the propagation of the motor signal. These waves are thought to be generated by cortico-cortical circuitry [36] projecting to the corticospinal neurons. Further increasing stimulation intensity results in the direct activation of the axons of the corticospinal neurons, recorded as a D-wave. Short Intra Cortical Inhibition (SICI), a phenomenon linked to specific GABAb receptors [37] has shown to be induced by a narrow range of TMS intensities (110-120% RMT; Garry and Thomson, 2009) similar to the ones found in our study. The authors referred to the results of Di Lazzaro to conclude that at this specific intensities TMS pulses target mainly the neural substrate responsible for late I-waves.

In light of this, we tested our protocol over a range of TMS intensities. By analyzing the intensity dependent effect of our concurrent tACS-TMS stimulation, we aimed to gain insight on the neural substrate modulated by tACS currents. The results show a clear peak in the magnitude of the phase modulation, centered at the 110% RMT condition. Based on the existing literature on TMS elicited spinal volleys and SICI, we interpret this as evidence for a direct involvement of late I-wave circuits on the tACS effects. Specifically, the same neural substrate responsible for intracortical inhibition during SICI protocols. For the same argument, the reduction of the effect at lower and higher intensities can be explained to the selective targeting of circuits responsible for the early I-waves, or by the disrupting effect of the direct activation of the pyramidal tract.

Alternatively, it could be argued specificity of the 110% condition can be due to the interference of the TMS pulses on the tACS entrainment effects (i.e. TMS induced phase reset), acting at higher TMS intensities, and at the same time, a less reliable estimation of MEP size at intensities below this intensity. Albeit it is hard to distinguish between these two scenarios (I-wave vs interference), we claim that this second hypothesis is less compatible with the finding that TMS effects have been

found to act at very low intensities [34,39]. We conclude that it is unlikely that the stimulus-response curve of the effects we found is the result of the direct interference of TMS on brain oscillations. Based on the literature here reported, there is enough evidence to show that this is due to different TMS intensities targeting specific different neural populations.

The phase of Beta Oscillations encodes corticospinal excitability:

Oscillations in the Beta frequency range (12-25 Hz) have been documented through the different structures of the human motor system [4]. Thus they have been subject of several hypotheses about their role in movement production [2–4,40]. Importantly, during isotonic contractions, EEG signals recorded over motor areas show robust phase-locking to the EMG activity in the beta frequency range [41–43]. Thus indicating that at least part of the motor command reaching the muscles is encoded in the sensorimotor beta oscillations. Specifically, it seems that the phase of beta rhythms recorded over sensorimotor areas [22,23], or as EMG signal [44]**,** predicts the size of the elicited MEPs. Since the phase of beta rhythms is correlated across different areas of the motor system, a simple correlation approach is ineffective to produce evidences supporting a direct causal link between the signal recorded over a certain area and the level of corticospinal excitability. Brain stimulation techniques, on the other hand might target specifically the phase of the sensorimotor rhythms and thus reveal their effects on behavior. By directly entraining the phase of ongoing rhythms by means of external stimulation, we provide a causal link between phase of beta oscillations and the level of corticospinal excitability. It is unlikely that the effects we found might generate somewhere else than the cortical areas targeted by the stimulation. Yet, it is still unclear whether this effect is caused exclusively by local excitability changes at the level of M1, or it is a network modulation that propagates from the motor cortex to the spinal structures.

Future directions and limitations of the current study: As we shown in the previous paragraphs, the entrainment patterns found in our study might be explained by an interference effect, probably due to the use of a fixed stimulation frequency and its interaction with the subjects' individual beta-peaks. Thus, we argue that the implementation of individualized stimulation frequencies in our protocol might have resulted in clearer stimulation effects (e.g. in: Zaehle et al., 2010; Gundlach et al.,

2016; Ruhnau et al., 2016). Nevertheless, the time course of the modulation, seems to indicate that to a certain extent, tACS is able to entrain the frequency of the ongoing rhythms match the stimulation frequency. A similar effect has been documented for the alpha band [13], and it might be responsible for the results found in the studies adopting pre-selected stimulation frequencies (e.g. in: Pogosyan et al., 2009; Kanai et al., 2010; Feurra et al., 2011a, 2011b, 2013; Joundi et al., 2012; Neuling et al., 2012). It could be argued that another limitation of the present study consists in the lack of concurrent EEG recordings capable of detecting spectral changes in response to the tACS stimulation. This is based on the consideration that direct recordings on entrained cortical oscillations are preferable to an indirect measure of cortical excitability as MEPs. Although, we agree on this general statement, there are some considerations to be made:

First, synchronous stimulation and recordings are complicated by methodological problems connected to the rejection of stimulation artifacts [48]. Thus, the assessment of the stimulation effects might be restricted to the post-stimulation period, thereby depending on the robustness of the stimulation after-effects, which might rely on different mechanisms from the online effects [49]. In the context of the motor system, MEP measures offer clear advantages allowing to monitor the stimulation effects online without being affected by the stimulation artifacts.

The second problem concerning the use of direct recordings to assess the tACS effects is the mixing of multiple sources of activity to the same recording electrodes. By effects of volume conduction, a single EEG channel reflects the mixed activity of multiple neural ensemble. Thus the activity of a single source, entrained by the tACS current might be not visible in the EEG signal by effect of a disruptive interference by one or more sources. This is especially a problem if we consider tACS to selectively target a specific subset of neurons (e.g. inhibitory interneurons) within a larger cortical area. Yet, MEPs recorded from a specific muscle might still reflect the activity of a single source entrained by tACS. Thus, MEP analysis might reveal stimulation effects not evident from the analysis of EEG signal.

In our study we found an entrainment of corticospinal excitability which lasts for roughly 30 seconds, before declining. The effect reappears toward the end of the 3 minutes epoch, nevertheless, it is unclear whether this second entrainment period is itself a transitory effect, or it is sustained over time. It is possible that the time course of the modulation reflects the initial adjustment to the stimulation phase and

frequency, and thus the beginning of a long lasting entrainment effect. Nevertheless, based on our protocol we are unable to directly test this hypothesis, future studies might adopt longer stimulation epochs (e.g. > 5 minutes) to clarify this point. Additionally, the effect we found can be characterized further by assessing the excitability level of spinal structures, e.g. by analyzing the effects of the stimulation on the Hoffman reflex. In this way we could assess whether the phase-modulation effects of tACS reflect a local change in M1 excitability, or network-level effects involving spinal structures.

Besides providing evidence in support of the entrainment effects of sensorimotor beta tACS, the present protocol constitutes a solution for achieving phase dependent stimulation of cortical rhythms. This approach can be used to answer several research questions that would otherwise require the use of a dedicated hardware for closed loop recording and stimulation. Specifically, the same protocol could be applied to explore the effects of different stimulation frequencies, or during specific motor tasks which have been shown to modulate corticospinal excitability [50].

REFERENCES

[1] Buzsaki G. Rhythms of the Brain. 2006.

[2] Aumann TD, Prut Y. Do sensorimotor β-oscillations maintain muscle synergy representations in primary motor cortex? Trends Neurosci 2015;38:77–85. doi:10.1016/j.tins.2014.12.002.

[3] Khanna P, Carmena JM. Neural oscillations: beta band activity across motor networks. Curr Opin Neurobiol 2015;32:60–7. doi:10.1016/j.conb.2014.11.010.

[4] Engel AK, Fries P. Beta-band oscillations-signalling the status quo? Curr Opin Neurobiol 2010;20:156–65. doi:10.1016/j.conb.2010.02.015.

[5] Antal A, Paulus W. Transcranial alternating current stimulation (tACS). Front Hum Neurosci 2013;7:317. doi:10.3389/fnhum.2013.00317.

[6] Thut G, Schyns P, Gross J. Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. Front Psychol 2011.

[7] Veniero D, Vossen A, Gross J, Thut G. Lasting EEG/MEG Aftereffects of Rhythmic Transcranial Brain Stimulation: Level of Control Over Oscillatory Network Activity. Front Cell Neurosci 2015;9:477. doi:10.3389/fncel.2015.00477.

[8] Herring JD, Thut G, Jensen O, Bergmann TO. Attention Modulates TMS-Locked Alpha Oscillations in the Visual Cortex. J Neurosci 2015;35:14435–47. doi:10.1523/JNEUROSCI.1833-15.2015.

[9] Fuggetta G, Fiaschi A, Manganotti P. Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG. Neuroimage 2005.

[10] Kanai R, Paulus W, Walsh V. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. Clin Neurophysiol 2010;121:1551–4. doi:10.1016/j.clinph.2010.03.022.

[11] Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. Curr Biol 2008;18:1839–43. doi:10.1016/j.cub.2008.10.027.

[12] Neuling T, Rach S, Herrmann CS. Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. Front Hum Neurosci 2013;7:161. doi:10.3389/fnhum.2013.00161.

[13] Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Entrainment of Brain Oscillations by Transcranial Alternating Current Stimulation. Curr Biol 2014;24:333–9. doi:10.1016/j.cub.2013.12.041.

[14] Pogosyan A, Gaynor LD, Eusebio A, Brown P. Boosting cortical activity at Beta-band frequencies slows movement in humans. Curr Biol 2009;19:1637–41. doi:10.1016/j.cub.2009.07.074.

[15] Wach C, Krause V, Moliadze V, Paulus W, Schnitzler A, Pollok B. Effects of 10Hz and 20Hz transcranial alternating current stimulation (tACS) on motor functions and motor cortical excitability. Behav Brain Res 2013;241:1–6. doi:10.1016/j.bbr.2012.11.038.

[16] Joundi R a, Jenkinson N, Brittain J-S, Aziz TZ, Brown P. Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol 2012;22:403–7. doi:10.1016/j.cub.2012.01.024.

[17] Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi a., Rossi S. State-Dependent Effects of Transcranial Oscillatory Currents on the Motor System: What You Think Matters. J Neurosci 2013;33:17483–9. doi:10.1523/JNEUROSCI.1414-13.2013.

[18] Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S.

Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J Neurosci 2011;31:12165–70. doi:10.1523/JNEUROSCI.0978-11.2011.

[19] Heise K-F, Kortzorg N, Saturnino GB, Fujiyama H, Cuypers K, Thielscher A, et al. Evaluation of a Modified High-Definition Electrode Montage for Transcranial Alternating Current Stimulation (tACS) of Pre-Central Areas. Brain Stimul 2016.

[20] Henry MJ, Obleser J. Frequency modulation entrains slow neural oscillations and optimizes human listening behavior. Proc Natl Acad Sci U S A 2012;109:2009–100. doi:10.1073/pnas.1213390109.

[21] Ali MM, Sellers KK, Frohlich F. Transcranial Alternating Current Stimulation Modulates Large-Scale Cortical Network Activity by Network Resonance. J Neurosci 2013;33:11262–75. doi:10.1523/JNEUROSCI.5867-12.2013.

[22] Keil J, Timm J, Sanmiguel I, Schulz H, Obleser J, Schönwiesner M. Cortical brain states and corticospinal synchronization influence TMS-evoked motor potentials. J Neurophysiol 2014;111:513–9. doi:10.1152/jn.00387.2013.

[23] Berger B, Minarik T, Liuzzi G, Hummel FC, Sauseng P. EEG oscillatory phase-dependent markers of corticospinal excitability in the resting brain. Biomed Res Int 2014;2014. doi:10.1155/2014/936096.

[24] Raco V, Bauer R, Tharsan S, Gharabaghi A. Combining TMS and tACS for Closed-Loop Phase-Dependent Modulation of Corticospinal Excitability: A Feasibility Study. Front Cell Neurosci 2016;10:1–8. doi:10.3389/fncel.2016.00143.

[25] Siebner HR, Ziemann U, Nitsche MA, Paulus W. Das TMS-Buch: Handbuch der transkraniellen Magnetstimulation. In: Siebner HR, Ziemann U, editors., Berlin, Heidelberg: Springer Berlin Heidelberg; 2007, p. 533–42. doi:10.1007/978-3-540-71905-2_53.

[26] Raco V, Bauer R, Olenik M, Brkic D, Gharabaghi A. Neurosensory effects of transcranial alternating current stimulation. Brain Stimul 2014;7:823–31. doi:10.1016/j.brs.2014.08.005.

[27] Antal A, Herrmann CS. Transcranial Alternating Current and Random Noise Stimulation: Possible Mechanisms. Neural Plast 2016;2016:1–12. doi:10.1155/2016/3616807.

[28] Reato D, Rahman A, Bikson M, Parra LC. Effects of weak transcranial alternating current stimulation on brain activity-a review of known mechanisms

from animal studies. Front Hum Neurosci 2013;7:687. doi:10.3389/fnhum.2013.00687.

[29] Herrmann CS, Rach S, Neuling T, Strüber D. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front Hum Neurosci 2013;7:279. doi:10.3389/fnhum.2013.00279.

[30] Neuling T, Rach S, Wagner S, Wolters CH, Herrmann CS. Good vibrations: Oscillatory phase shapes perception. Neuroimage 2012;63:771–8. doi:10.1016/j.neuroimage.2012.07.024.

[31] Gundlach C, Müller MM, Nierhaus T, Villringer A, Sehm B. Phasic Modulation of Human Somatosensory Perception by Transcranially Applied Oscillating Currents. Brain Stimul 2016. doi:10.1016/j.brs.2016.04.014.

[32] Pellicciari MC, Miniussi C, Ferrari C, Koch G, Bortoletto M. Ongoing cumulative effects of single TMS pulses on corticospinal excitability: An intra- and inter-block investigation. Clin Neurophysiol 2016;127:621–8. doi:10.1016/j.clinph.2015.03.002.

[33] Paus T, Sipila PK, Strafella AP. Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. J Neurophysiol 2001;86:1983–90.

[34] Van Der Werf YD, Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. Exp Brain Res 2006;175:231–45. doi:10.1007/s00221-006-0551-2.

[35] Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, et al. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 1998;508:625–33.

[36] Ziemann U, Rothwell JC. I-waves in motor cortex. J Clin Neurophysiol 2000;17:397–405. doi:10.1097/00004691-200007000-00005.

[37] McDonnell MN, Orekhov Y, Ziemann U. The role of GABAB receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 2006;173:86–93. doi:10.1007/s00221-006-0365-2.

[38] Garry MI, Thomson RHS. The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. Exp Brain Res 2009;193:267–74. doi:10.1007/s00221-008-1620-5.

[39] Komssi S, Kähkönen S, Ilmoniemi RJ. The effect of stimulus intensity on brain responses evoked by transcranial magnetic stimulation. Hum Brain Mapp 2004;21:154–64. doi:10.1002/hbm.10159.

[40] Mackay WA. Synchronized neuronal oscillations and their role in motor processes. Trends Cogn Sci 1997;1:176–83.

[41] Sanes JN, Donoghue JP. Oscillations in local field potentials of the primate motor cortex during voluntary movement. Proc Natl Acad Sci U S A 1993;90:4470–4. doi:10.1073/pnas.90.10.4470.

[42] Salenius S, Hari R. Synchronous cortical oscillatory activity during motor action. Curr Opin Neurobiol 2003;13:678–84. doi:10.1016/j.conb.2003.10.008.

[43] Kristeva R, Patino L, Omlor W. Beta-range cortical motor spectral power and corticomuscular coherence as a mechanism for effective corticospinal interaction during steady-state motor output. Neuroimage 2007;36:785–92. doi:10.1016/j.neuroimage.2007.03.025.

[44] van Elswijk G, Maij F, Schoffelen J-M, Overeem S, Stegeman DF, Fries P. Corticospinal Beta-Band Synchronization Entails Rhythmic Gain Modulation. J Neurosci 2010;30:4481–8. doi:10.1523/JNEUROSCI.2794-09.2010.

[45] Zaehle T, Rach S, Herrmann CS. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One 2010;5:e13766. doi:10.1371/journal.pone.0013766.

[46] Ruhnau P, Neuling T, Fuscá M, Herrmann CS, Demarchi G, Weisz N. Eyes wide shut: Transcranial alternating current stimulation drives alpha rhythm in a state dependent manner. Sci Rep 2016;6:27138. doi:10.1038/srep27138.

[47] Feurra M, Paulus W, Walsh V, Kanai R. Frequency Specific Modulation of Human Somatosensory Cortex. Front Psychol 2011;2:1–6. doi:10.3389/fpsyg.2011.00013.

[48] Noury N, Hipp JF, Siegel M. Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. Neuroimage 2016.

[49] Vossen A, Gross J, Thut G. Alpha power increase after transcranial alternating current stimulation at alpha-frequency (α-tACS) reflects plastic changes rather than entrainment. Brain Stimul 2014;8:499–508. doi:10.1016/j.brs.2014.12.004.

[50] Kraus D, Naros G, Bauer R, Khademi F, Leão MT, Ziemann U, et al. Brain State-Dependent Transcranial Magnetic Closed-Loop Stimulation Controlled

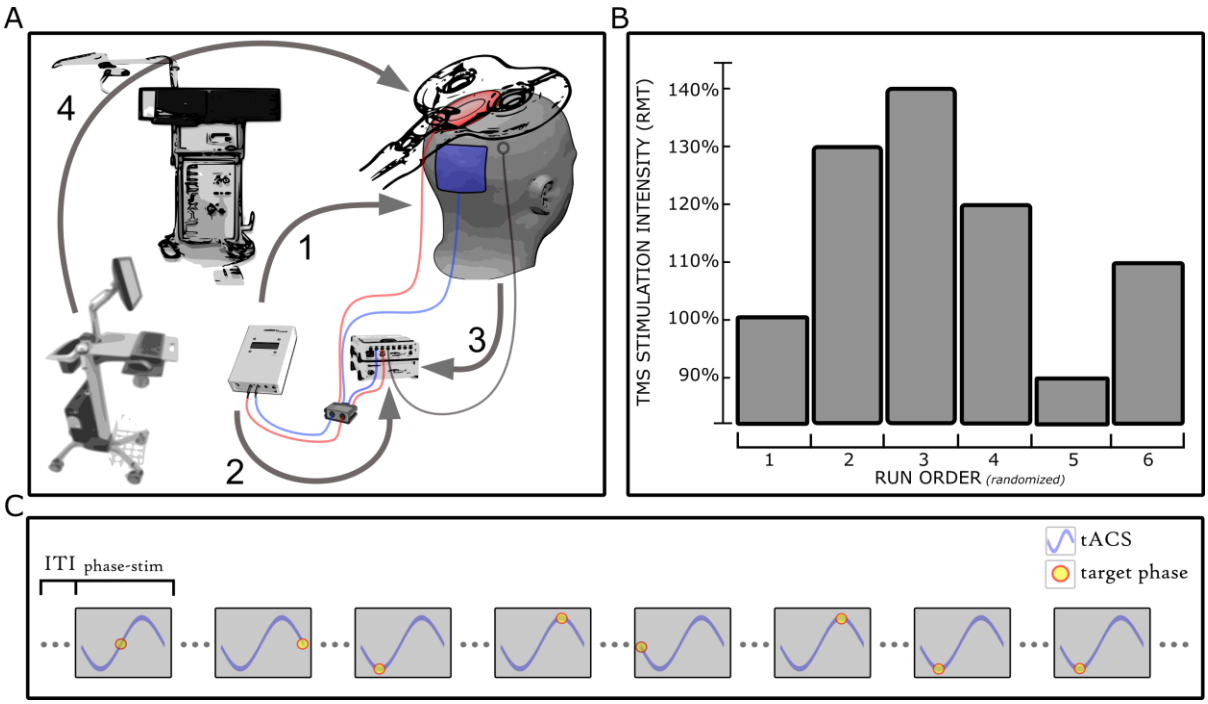
by Sensorimotor Desynchronization Induces Robust Increase of Corticospinal Excitability. Brain Stimul 2015. doi:10.1016/j.brs.2016.02.007.

Table 1

	INTERCEPT			SLOPE		
	Phase-shift	**Amplitude**	**P-value**	**Phase-shift**	**Amplitude**	**P-value**
90% RMT	-25.6566°	0.1085	0.7742	173.3703°	0.1409	0.6392
100% RMT	-146.8694°	0.2756	0.1760	2.3300°	0.3513	0.0626
110% RMT	65.5464°	0.4760	0.0062*	-125.1993°	0.5099	0.0012*
120% RMT	-3.4175°	0.1408	0.6490	175.4104°	0.2694	0.2040
130% RMT	4.1976°	0.0940	0.8276	15.0468°	0.1173	0.7418
140% RMT	-103.2444°	0.2449	0.2540	107.5136°	0.1324	0.6770

Table 1 shows the results of the sinusoidal fitting for all intensities. Amplitude, phase-shift to the tACS (in degrees), and p-values of the permutation test are shown for slope and intercept. Significant tests are highlighted by asterisks (Bonferroni corrected).

Figure 1



The figure shows the setup and study design of the experiment. Figure 1a shows the hardware used to achieve phase-dependent stimulation. tACS is delivered to the subject (1), and part of the current is rerouted from the stimulator to the recording amplifier (2) via a custom made splitter box. An electrode is positioned on the subject´s head to record the exact moment of the TMS stimulation artifact (3). The artifact of the two stimulators are synchronized by the computer and the exact timing is used to trigger the TMS system to specific tACS phases (4). The experiment is divided in 6 runs applied in a randomized order (figure 1b). Each run is characterized by a different TMS intensity and divided in 40 trials. During each trial a TMS pulse targets one of the four different tACS-phases. Each condition is repeated ten times and the order is randomized across each run. The inter trial interval (ITI) between two stimulation pulses is set to 5 seconds with a ± 500ms jitter (figure 1c).

Figure 2

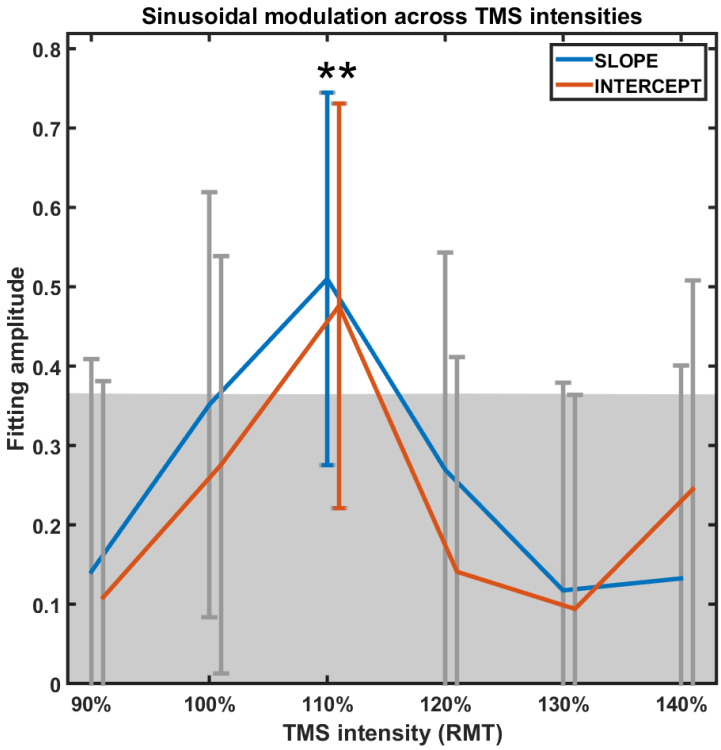


Figure 2 shows the intensity dependent effect of the stimulation for both slope and intercept. The amplitude of the fitted sinus (Y axis) provides a measure of the phase consistency of the regression parameters' modulation across the subjects. The gray patch represents the 95th percentile of the distribution of amplitudes resulting from the permutation test (i.e. the alpha-level: p= 0.008). Values exceeding the alpha-level are highlighted by a colored error-bar and asterisks.

Figure 3

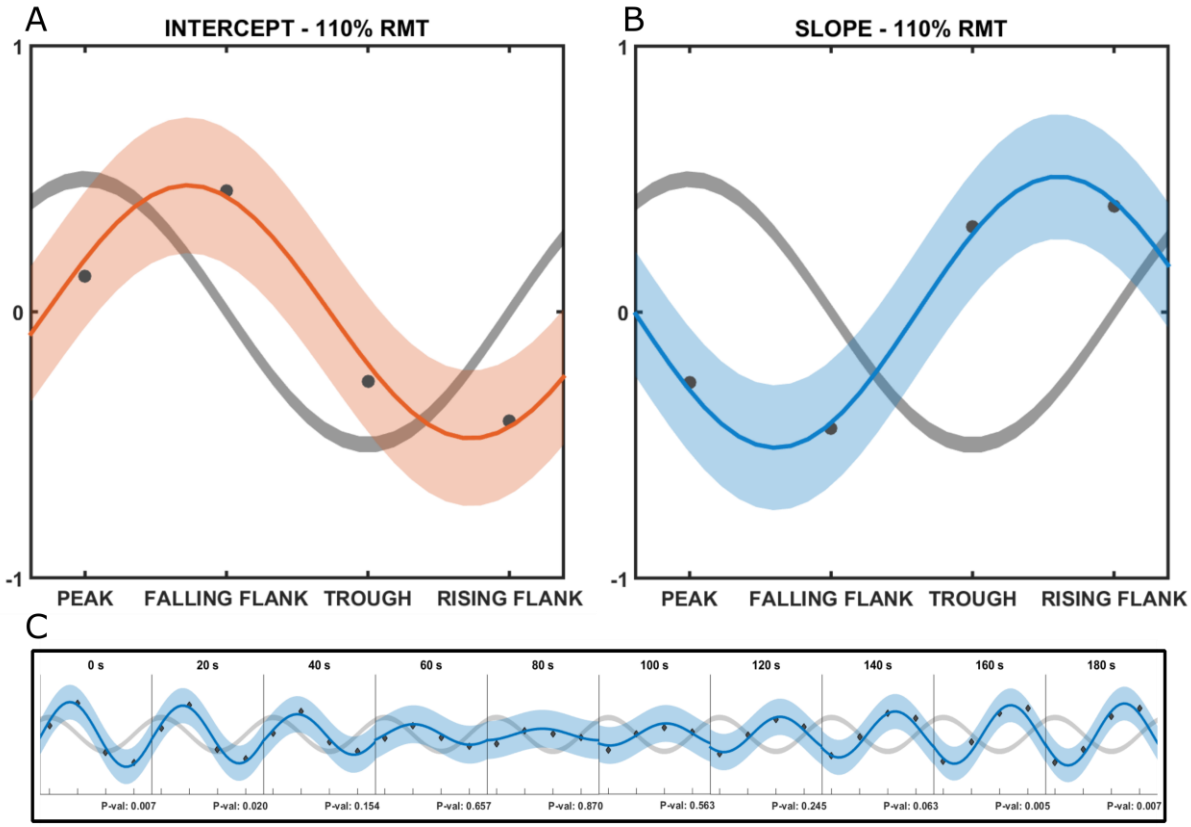


Figure 3a visualizes the results of the sinusoidal fitting for the 110% RMT intensity condition, for both intercept (a) and slope (b). The thick colored lines show the sinusoidal fitted on the subjects' regression parameters, and the patches indicate the confidence intervals returned by the fitting. Black dots represent the normalized values of slope and intercept averaged across the subjects. The gray sinus is the reconstructed tACS waveform. Figure 3c shows the time-course of the modulation. The regression line is reconstructed from the regression parameters for specific time bins while repeating the fitting procedure for each bin. The results of the fitting are shown in the figure and the relative p-values are reported below.