



Recovering Brain Dynamics During Concurrent tACS-M/EEG: An Overview of Analysis Approaches and Their Methodological and Interpretational Pitfalls

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Abstract

Transcranial alternating current stimulation (tACS) is increasingly used as a tool to non-invasively modulate brain oscillations in a frequency specific manner. A growing body of neuroscience research utilizes tACS to probe causal relationships between neuronal oscillations and cognitive processes or explore its capability of restoring dysfunctional brain oscillations implicated in various neurological and psychiatric disease. However, the underlying mechanisms of action are yet poorly understood. Due to a massive electromagnetic artifact, overlapping with the frequency of interest, direct insights to effects during stimulation from electrophysiological signals (i.e. EEG/MEG) are methodologically challenging. In the current review, we provide an overview of analysis approaches to recover brain signals in M/EEG during tACS, detailing their underlying concepts as well as limitations and methodological and interpretational pitfalls. While different analysis strategies can achieve strong attenuation of the tACS artifact in M/EEG signals, a complete removal of it is not feasible so far. However, we argue that with a combination of careful experimental designs, robust outcome measures and appropriate control analyses, valid and important insights to online effects of tACS can be revealed, enriching our understanding of its basic underlying mechanisms.

Keywords Transcranial alternating current stimulation (tACS) · Brain oscillations · tACS-M/EEG integration · Online effects · MEG · EEG

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Introduction

The frequency specific modulation of brain oscillations via the transcranial application of alternating currents (a.k.a. transcranial alternating current stimulation; tACS) has emerged as a promising technique for research and clinical applications (Herrmann et al. 2013, 2016). Evidence from in vivo and in vitro electrophysiology in animals and from computational modelling suggests that stimulation with alternating, sinusoidal currents causes temporal alignment of neuronal firing and is thereby able to entrain neural oscillations (Reato et al. 2010; Fröhlich and McCormick 2010; Ozen et al. 2010; Ali et al. 2013; Negahbani et al. 2018). Although a variety of studies was able to demonstrate that tACS causes changes in oscillatory activity measured with M/EEG after stimulation (Zaehle et al. 2010; Neuling et al. 2013; Wach et al. 2013; Vossen et al. 2015; Kasten et al. 2016; Wischnewski et al. 2018), and modulates behavior (Vosskuhl et al. 2015; Lustenberger et al. 2015; Kasten and Herrmann 2017), the precise underlying mechanisms are so far not well understood.

The main reason for this lack of evidence is that recording of electrophysiological signals (i.e. M/EEG), during the application of currents in the range of 1–2 mA to the scalp imposes a major methodological challenge, hindering direct insights to tACS effect on human electrophysiology. The stimulation introduces a massive stimulation artifact, several orders of magnitude larger than the signals of interest (Kasten et al. 2018a). To further complicate the situation, artifact and signal of interest usually overlap in the frequency domain; i.e. the frequency range to be analyzed in M/EEG is usually similar or equal to the tACS frequency. During the past couple of years, different methods have been suggested to recover electrophysiological signals during tACS. The current review aims to provide an overview of different methods to suppress tACS artifacts in EEG and MEG data along with a brief introduction their underlying concepts. Subsequently, methodological and interpretational pitfalls associated with the analysis of tACS online effects in M/EEG will be discussed. It should be emphasized from the beginning that, although a massive reduction of the tACS artifact is feasible with present approaches, currently no method exists that allows complete, reliable removal of the artifact.

Approaches to Recover M/EEG Signals During tACS

To enable analysis methods to tackle the stimulation artifact, some technical requirements on the hardware should be emphasized. EEG and MEG systems are optimized to record small signals originating from the brain. The electromagnetic artifact encountered during tACS, however, is several orders of magnitude larger than these brain signals. It is thus crucial to ensure that the recording hardware offers a sufficient dynamic range to represent the large signals inherent to the tACS artifact and avoids saturation. When recording tACS artifacts using MEG systems, additional care must be taken that stimulation frequency and amplitude are compatible with the systems maximum slew-rate. Stimulation protocols using too high frequencies and high intensities may cause flux trapping and lead to sensor degradation (Soekadar et al. 2013).

For the recovery of EEG signals during tACS, a template subtraction approach has been suggested (Helfrich et al. 2014; Kohli and Casson 2015). The approach assumes a superposition of a relatively stable tACS artifact with EEG signals originating from the brain, which undergo strong fluctuations over time. By averaging multiple segments of the EEG, time-locked to the same phase of the tACS artifact, a template is created, which captures the shape of the artifact, while the brain signals average out. The subsequent subtraction of the template from the single EEG segments, in turn,

should then remove the artifact while retaining the superimposed brain signals. A general advantage of the technique is that it can be performed on single channels. However, as the approach turned out to leave residual artifacts in the data, some authors performed additional artifact suppression techniques such as PCA (Helfrich et al. 2014), or notch filtering (Voss et al. 2014).

Artifact suppression in MEG recordings is based on the application of spatial filtering (beamforming). Beamformers have been designed to separate signals from different directions. They are widely used in radar and sonar technologies (Van Veen and Buckley 1988) and are commonly used for source localization of M/EEG signals (Van Veen et al. 1997; Gross et al. 2001). For this purpose, spatial filters are designed such that signals originating from a specific location in the brain are passed, while signals from distant locations get attenuated (Van Veen et al. 1997). A spatial map of brain activation is obtained by creating multiple filters with different pass bands for a pre-defined set of possible source locations (Van Veen et al. 1997). TACS artifact suppression in MEG recordings leverages the insensitivity of linearly constrained minimum variance (LCMV) beamforming (Van Veen et al. 1997) to highly correlating sources (Soekadar et al. 2013; Neuling et al. 2015). In LCMV, the spatial filter at each source location is forced to minimize the variance of its output (hence the name). If the signal originates from two or more distinct, highly correlating sources, the covariance of the sources is exploited to minimize the output variance of the filter. Such high correlations of distinct sources are rarely observed in the brain. During tACS, however, the electromagnetic artifact propagates to virtually all MEG sensors with high consistency, allowing the LCMV beamformer to cancel out large proportions of the artifact (Neuling et al. 2015). As a consequence of this mechanism, the artifact suppression performance of the method is naturally limited by the degree to which the recorded signals of the artifact are correlated (or uncorrelated) over sensors (Mäkelä et al. 2017).

While both methods have been demonstrated to cause a strong attenuation of the artifact, it has been shown that they fail to achieve its complete removal (Noury et al. 2016; Noury and Siegel 2017; Kasten et al. 2018a). Specifically, physiological processes such as heart-beat and respiration cause amplitude modulations of the artifact over time, introducing side-bands around the main stimulation artifact that survive the above artifact cleaning approaches (Noury et al. 2016). For EEG recordings, it has been argued that modulations of the artifact arise from changes in body impedance, which can be caused by respiration and heart-beat. For MEG signals, the same physiological processes elicit small head-movements, changing the distance between stimulation electrodes and cables to the sensor array (Noury et al. 2016). Apart from side-bands around the main artifact peak,

the above processes may also explain residual tACS artifacts directly at the stimulation frequency. The template subtraction approach relies on the assumption of a stationary, time-invariant artifact and cannot capture short-term fluctuations. Consequently, any deviation of the artifact in a given, to be cleaned segment from the average/template will remain in the data as a residual artifact. In a similar manner, changing the distance of the tACS electrodes and cables to the MEG sensor array may change the correlation of the artifact over sensors, thereby compromising the artifact suppression capabilities of LCMV beamforming.

Special Waveform Shapes

To improve tACS artifact suppression or avoid spectral overlap between the tACS artifact and the brain oscillation of interest completely, some authors proposed the use of alternative waveforms for stimulation.

The use of tACS with sawtooth-waves has been suggested to improve performance of the EEG template subtraction method (Dowsett and Herrmann 2016). In contrast to sinusoidal waveforms, sawtooth-waves cause strong peaks in the spectrum at harmonic frequencies of the stimulation. Thus, EEG segments contaminated with strong residual artifacts can be identified by large harmonic peaks and rejected from further analysis (Dowsett and Herrmann 2016). This way, sawtooth-waves offer a more objective criterion to evaluate the performance of artifact cleaning. First work on tACS with sawtooth-waves was able to show that this type of stimulation can cause enhancement of endogenous α -power. However, the effect also seemed to depend on the shape of the sawtooth (Dowsett and Herrmann 2016).

A stimulation waveform suggested to completely overcome the tACS artifact at the frequency of interest is amplitude modulated tACS (AM-tACS; Witkowski et al. 2016). In AM-tACS, a high-frequency carrier waveform is modulated in amplitude by a low frequency modulation signal, tuned to the brain oscillation of interest. In the frequency domain, such a signal contains power at the frequency of the carrier signal and two side-bands at the frequency of the carrier signal \pm the frequency of the modulation signal. At the frequency of the modulating waveform itself, the signal does not exhibit power, thus avoiding the spectral overlap between the targeted brain oscillation and the stimulation artifact (Witkowski et al. 2016). Results from computational modelling suggest that neuronal oscillations entrain to the modulating waveform of AM-tACS similar to conventional sinusoidal stimulation (Negahbani et al. 2018). However, substantially higher stimulation intensities were required for AM-tACS to achieve comparable degrees of synchronization. Recent work demonstrated that, contrary to theoretical considerations, AM-tACS does exhibit an artifact at the

frequency of the modulating waveform and its harmonics in electrophysiological recordings (Minami and Amano 2017; Kasten et al. 2018b). Specifically, non-linear properties of stimulation and recording hardware can reintroduce low-frequency artifacts overlapping with the brain signal of interest, sufficiently strong to be potentially confused with actual effects of the stimulation (Kasten et al. 2018b). As essentially every electronic device exhibits some degree of non-linearity, AM-tACS cannot be assumed to be entirely artifact-free at the modulation frequency. Nevertheless, the observed low-frequency artifacts are several orders of magnitude smaller than the artifact encountered during conventional tACS. It might thus be easier to remove these artifacts from electrophysiological signals using standard techniques such as independent component analysis or spatial filtering (Kasten et al. 2018b). However, research systematically evaluating the performance of such methods in the context of AM-tACS is missing so far.

Methodological and Interpretational Pitfalls

The previous sections provided a general overview of current approaches to tackle the tACS artifact in M/EEG recordings. All presented approaches can achieve a massive reduction of the tACS artifact. However, at the same time none of the methods provides a complete removal of the artifact. Especially AM-tACS exemplifies how a method that in theory seems to provide a perfect solution to the artifact problem can fail in practice due to unexpected mechanisms during the recording process (Kasten et al. 2018b). A more general problem for current and future methods is how to prove that a complete removal of the artifact has been achieved. Residual artifacts in the data cause effects very similar to what would be expected to be observed as an effect of tACS on brain oscillations (increased power at/around the stimulation frequency as well as high phase coherence of electromagnetic activity with the stimulation waveform). Thus, disentangling residual artifacts from effects of stimulation is inherently difficult and would require knowledge of the underlying ground truth to evaluate artifact suppression performance. This, however, cannot be achieved in real-world recordings. Testing methods in recordings from phantom heads would allow a comparison between a ground truth signal and recorded signals after tACS artifact removal. However, the complex physiological processes encountered in humans (Noury et al. 2016; Noury and Siegel 2017) are hard to be simulated in such setups, making predictions about artifact suppression in actual human recordings difficult.

Based on these considerations, it seems reasonable to generally refrain from the assumption that a given approach can achieve complete tACS artifact removal. Rather, experimental procedures should be carried out in a way that the

effects under investigation are robust against the influence of residual artifacts such that valid conclusions can be drawn from the obtained data. For example, there is consensus among different research groups that the achieved artifact reduction might still be sufficient to recover online effects of tACS if different experimental conditions during stimulation are contrasted before comparison with stimulation-free data (Neuling et al. 2017; Noury and Siegel 2018; Kasten et al. 2018a; Herring et al. 2019). It should, however, be emphasized that a robust outcome measure has to be chosen to capture such effects. As laid out in Kasten et al. (2018a), event-related oscillations are often analyzed using relative measures such as event-related (de-)synchronization (ERD/ERS; Pfurtscheller and Lopes Da Silva 1999):

$$ERD/ERS = \frac{R - A}{R} * 100, \quad (1)$$

where R represents the power in a given frequency band during a reference period prior to the onset of a stimulus and A the power in the same frequency band after stimulus onset. In the presence of a residual artifact, R and A represent a mixture of participants' actual brain activity and the residual artifact during the respective time period, which can be expressed the following way:

$$ERD/ERS = \frac{(R_{brain} + R_{artifact}) - (A_{brain} + A_{artifact})}{(R_{brain} + R_{artifact})} * 100. \quad (2)$$

Assuming that the residual artifact is uncorrelated with the task, it's contribution to both time periods should be approximately similar ($R_{artifact} \approx A_{artifact}$) and thus cancel out in the numerator but remain in the denominator of the equation, introducing a systematic bias to the measure of event-related power change:

$$ERD/ERS = \frac{R_{brain} - A_{brain}}{(R_{brain} + R_{artifact})} * 100. \quad (3)$$

Such systematic bias can lead to erroneous conclusions about the strength or direction of tACS effects on event-related oscillations. To avoid such bias, it has been suggested to use the absolute difference instead (Noury and Siegel 2018; Kasten et al. 2018a). Here, the contribution of the residual tACS artifact cancels out:

$$Power\ Diff. = (R_{brain} + R_{artifact}) - (A_{brain} + A_{artifact}), \quad (4)$$

$$Power\ Diff. = R_{brain} - A_{brain}. \quad (5)$$

The importance of a robust measure to investigate online effects of tACS in the presence of residual artifacts can be illustrated on a set of simulated data (Fig. 1, the corresponding MATLAB code is provided as Supplementary Material). We sampled 100 pairs of random values ranging from 0 to 100 for

R_{brain} and A_{brain} from a uniform distribution with the constraint that $R_{brain} > A_{brain}$ to simulate a systematic power decrease from a reference to a testing period. From these value pairs, ERD and power differences can be computed as in Eqs. 1 and 5, serving as the “ground truth” (blue dots). Subsequently, the contribution of a residual artifact can be simulated by adding an artifact of a certain size to A and R , thus recomputing ERD and power difference according to Eqs. 2 and 4. To capture random fluctuation of the artifact, we allowed a random difference of up to 20% between $A_{artifact}$ and $R_{artifact}$. Figure 1a, b illustrate how ERD and power differences are affected by an artifact ~3 times larger than participants' pre-stimulus α -power (R_{brain}). The residual artifact introduces a systematic bias to the ERD values, resulting in a significant difference between original and artifact contaminated data (Fig. 1a). In contrast, if absolute differences are computed, the presence of the artifact adds noise to the estimated power difference (originating from the random fluctuations of the artifact in R and A), but does not introduce a systematic bias (Fig. 1b).

If the simulation is re-run for different strengths of the residual artifact (expressed as the artifact to brain signal ratios relative to R_{brain}), it becomes clear that already small artifacts introduce systematic differences between true and artifact contaminated ERD values, while absolute differences are relatively robust even for strong residual artifacts (Fig. 1c, d). However, with increasing artifact strength the estimates of the power difference become noisier, which could impair the detection of actual stimulation effects. It should be emphasized that the above simulation has some obvious flaws (R and A values are not created to resemble actual EEG or MEG results and the amount of random fluctuations of the artifact was chosen arbitrarily) and mainly serves to illustrate the impact of residual artifacts on measures of event-related oscillatory dynamics. However, some important lessons can be derived. Relative measures are vulnerable to additional artifact signals and should thus be avoided when analyzing tACS online effects. Absolute difference in contrast, can avoid such systematic bias. However, with increasing artifact strength, more noise is introduced to the estimates. The approach thus has to rely on sufficiently strong artifact suppression.

While initial work on tACS artifact suppression relied on visual inspection, it has recently been suggested to compute an “artifact-to-brain-signal-ratio” (ATBSR) as a more objective measure to characterize the tACS artifact (Kasten et al. 2018a). The rationale behind the measure is to use a sample of participants' brain signal of interest (e.g. power in the α -band) during a stimulation-free recording (B) as a reference and compare the power in a similar interval during stimulation (A):

$$ATBSR = \frac{A}{B}. \quad (6)$$

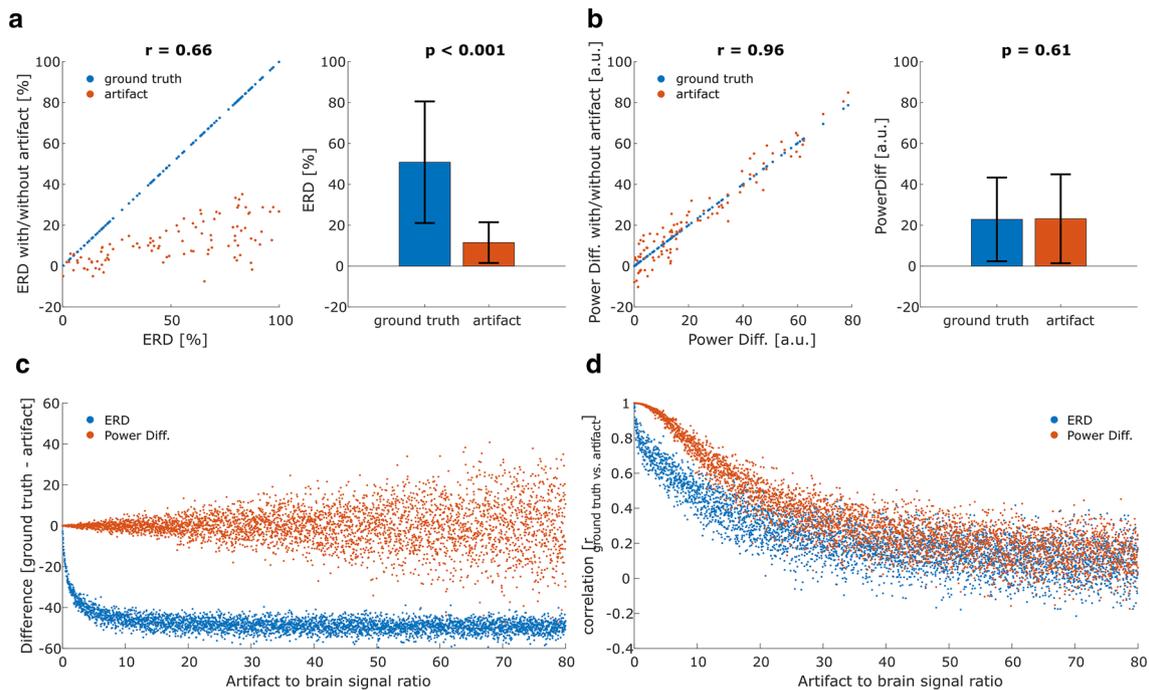


Fig. 1 The influence of residual tACS artifacts on absolute difference and relative change. **a** Effect of a residual artifact on ERD (relative change) values. Compared to the ground truth ERD values (blue), an additional artifact in the data causes systematic underestimation of ERDs, resulting in a significant difference between artifact contaminated data and the ground truth. **b** Effect of a residual artifact on absolute difference. In contrast to ERD, the absolute difference is not systematically biased by a residual artifact. Error bars depict standard deviation. **c** Average difference between original and artifact contami-

nated power difference and relative change for different strengths of the residual artifact relative to R_{brain} (artifact to brain signal ratios). Already for small residual artifacts, ERD values are systematically biased, while power differences become noisier but fluctuate around the ground truth. **d** Correlation between original and artifact contaminated power difference and relative change for increasing strengths of the residual artifact. Power differences show a slower decay in correlation between ground truth and artifact contaminated data as compared to ERD values with increasing residual artifacts

B serves as a reference for the power of participants' natural brain signal, while A contains a mixture of brain signal and artifact:

$$ATBSR = \frac{A_{artifact} + A_{brain}}{B_{brain}}. \quad (7)$$

This way, an upper boundary for the size of the residual artifact can be computed, allowing to judge the achieved artifact suppression and compare it across parameter combinations, artifact suppression methods or measurement modalities. Further, it can be used to inspect the spatial distribution of the artifact.

Besides a sufficiently strong suppression of the tACS artifact in advance, the cancelation of residual artifacts by computing difference measures relies on another important assumption: The strength of the residual artifact must be relatively stable across task conditions and the change of its amplitude must not be correlated with the task (Kasten et al. 2018a). As outlined by Noury et al. (2016), physiological processes such as heart-beat and respiration modulate the strength of the tACS artifact and impair artifact

suppression. The mechanism behind this modulation of the tACS artifact has been proposed to originate from changes in skin conductance (for EEG) and small head movements (in MEG). Based on these mechanisms, a variety of processes may in fact be capable of modulating the tACS artifact, some of which could be affected by different task conditions. Especially paradigms involving strong motor responses or emotional/fearful stimuli may be capable of modulating the tACS artifact in a task dependent manner by changing heart-rates, respiration frequencies, skin conductance or by triggering subtle head or body movements (Palomba et al. 1997; Pollatos et al. 2007). It is thus crucial to rule out that such systematic modulations of the tACS artifact introduce systematic bias to difference measures which might be confused with an online effect of tACS. For this purpose, it has been suggested to compute the event-related change of the artifact envelope before artifact suppression, which allows to test for systematic modulations of the artifact strength by the task (Kasten et al. 2018a). In a next step, the event-related change of the artifact strength can be correlated with the strength of the observed online effect. If a high correlation between

the modulation of the artifact with an online effect is observed, this may indicate that an online effect might likely be driven by a systematic modulation of the artifact by the task. For simple visual experiments, there has so far not been evidence for systematic modulations of the tACS artifact by task conditions (Kasten et al. 2018a; Herring et al. 2019). Yet, further research is needed to explore the possibility of such modulations during physiologically more demanding (e.g. emotional or motor) tasks.

The cancellation of artifacts by computing contrasts between experimental conditions is obviously not innovative. Nevertheless, it may provide a powerful tool to compensate for the imperfections of current tACS artifact suppression approaches. Importantly, the fact that standard analysis approaches such as contrasting of conditions or source localization tools (LCMV beamforming) have artifact suppression/cancellation features is especially important to acknowledge when new methods are presented. The application of such methods after applying a tACS artifact suppression method adds a second, implicit layer of artifact suppression. This can mask residual artifacts present in the data after the application of the to be evaluated method and may lead to exaggerated expectations about the performance of the primary method. While it is valid to combine different analysis methods in order to optimize tACS artifact suppression performance, especially when new methods are presented, it is crucial that the artifact suppression capabilities of each single processing step are properly acknowledged to allow judgement of the range of possible applications and limitations of the method.

Conclusions and Recommendations for Data Reporting

Based on the previous considerations we would like to propose some general recommendations on experimental designs, data analysis and reporting of concurrent tACS-M/EEG studies:

- (1) Very generally, it should not be assumed that complete artifact removal can be achieved. Already when conceptualizing a study, the presence of residual tACS-artifacts has to be expected and accounted for, e.g. by implementing appropriate contrast and control conditions.
- (2) For all outcome measures that are computed, it should be carefully evaluated and discussed whether the presence of a residual tACS-artifact may lead to systematic bias and thus may artificially drive (or mask) effects.
- (3) In order to allow judgement of the achieved artifact suppression, the size of the tACS-artifact before and after artifact cleaning should be transparently reported

and compared. It should be discussed whether the size of the residual artifact may hinder subsequent analysis (e.g. the computation of contrasts may not work with excessively strong residual artifacts).

- (4) Online effects of tACS should be accompanied by appropriate control analyses, aiming to rule out that residual tACS-artifacts could have driven the effect.
- (5) Especially when new approaches to concurrently record tACS and M/EEG are presented, the artifact suppression capabilities of each different analysis step should be disclosed (e.g. if contrasts are computed after the primary method) to avoid false or exaggerated expectations about a methods performance and the range of its application.

Recent years have seen the development of different approaches to recover oscillatory brain activity during tACS. Although these methods are able to achieve substantial reduction of the tACS-induced electromagnetic artifact, its complete removal has turned out to be hard, if not impossible to achieve (and to prove). Carefully designed experiments and analysis pipelines in combination with rigorous control analyses can, however, still provide important insights to online effects of tACS necessary to sharpen our understanding of its basic underlying mechanisms.

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Compliance with Ethical Standards

Conflict of interest CSH holds a Patent on brain stimulation and received Honoraria as Editor from Elsevier Publishers, Amsterdam. FHK declares no competing interests.

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