Characterizing low-frequency artifacts during transcranial temporal interference stimulation (tTIS)

von Conta, Jill; Kasten, Florian H.; Ćurčić-Blake, Branislava; Schellhorn, Klaus; Herrmann, Christoph S.

Published in:
Neuroimage: Reports

DOI:
10.1016/j.ynirp.2022.100113

Publication date:
2022

Neuroimage: Reports 2 (2022) 100113

Contents lists available at ScienceDirect

Neuroimage: Reports

journal homepage: www.sciencedirect.com/journal/neuroimage-reports

Characterizing low-frequency artifacts during transcranial temporal interference stimulation (tTIS)

Jill von Conta [a], Florian H. Kasten [a,b], Branislava Ćurčić-Blake [c], Klaus Schellhorn [d], Christoph S. Herrmann [a,b,e,*]

[a] Experimental Psychology Lab, Department of Psychology, European Medical School, Cluster of Excellence "Hearing4All", Carl von Ossietzky University, Oldenburg, Germany
[b] Neuroimaging Unit, European Medical School, Carl von Ossietzky University, Oldenburg, Germany
[c] Department of Biomedical Sciences of Cells and Systems, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
[d] neuroConn GmbH, Ilmenau, Germany
[e] Research Center Neurosensory Science, Carl von Ossietzky University, Oldenburg, Germany

ARTICLE INFO

Keywords:
Non-invasive brain stimulation (NIBS)
Transcranial temporal interference stimulation (tTIS)
Brain oscillations
Artifact

ABSTRACT

Transcranial alternating current stimulation (tACS) is a well-established brain stimulation technique to modulate human brain oscillations. However, due to the strong electro-magnetic artifacts induced by the stimulation current, the simultaneous measurement of tACS effects during neurophysiological recordings in humans is challenging. Recently, transcranial temporal interference stimulation (tTIS) has been introduced to stimulate neurons at depth non-invasively. During tTIS, two high-frequency sine waves are applied, that interfere inside the brain, resulting in amplitude modulated waveforms at the target frequency. Given appropriate hardware, we show that neurophysiological data during tTIS may be acquired without stimulation artifacts at low-frequencies. However, data must be inspected carefully for possible low-frequency artifacts. Our results may help to design experimental setups to record brain activity during tTIS, which may foster our understanding of its underlying mechanisms.

1. Introduction

Transcranial alternating current stimulation (tACS) is a well-established brain stimulation technique to modulate brain oscillations and human behavior (Herrmann et al., 2013; Kanai et al., 2008; Kasten and Herrmann, 2017; Vossen et al., 2015; Zaehle et al., 2010). However, the simultaneous measurement of tACS effects during neurophysiological recordings in human electroencephalography (EEG) and magnetoencephalography (MEG) is challenging due to the strong electro-magnetic artifacts induced by the stimulation current. Those artifacts spectrally overlap with the brain oscillation under investigation and thereby hinder direct insights on the effects of tACS during stimulation (Kasten and Herrmann, 2019). Several analysis approaches, such as template subtraction (Helfrich et al., 2014), or spatial filtering (Kasten et al., 2018a; Neuling et al., 2015) aimed to suppress the stimulation artifact in M/EEG data. The complete restauration of the intrinsic brain activity remains, however, challenging (Noury et al., 2016; Noury and Siegel, 2018).

Recently, amplitude modulated tACS (AM-tACS) has been suggested as an approach to overcome the stimulation artifact at the target frequency (Witkowski et al., 2016). During AM-tACS a high-frequency carrier sine wave is modulated at the target frequency. In theory, AM-tACS exhibits power at the carrier frequency and two side bands (carrier frequency ± modulation frequency), circumventing the artifact at the modulation frequency. Based on simulations, Kasten et al. (2018b) investigated low-frequency artifacts caused by the equipment in electrophysiological measurements during AM-tACS. They showed that non-linear properties of stimulation and recording hardware cause small changes to the AM-waveform which reintroduce low-frequency artifacts at the frequency of the amplitude modulation. If stimulation or recording hardware operate in a non-linear fashion, the output signal of a device is not a linear product of the (e.g. amplified) input signal. Further, the authors showed that low-frequency artifacts were present in phantom- and human-measurements in different experimental setups during AM-tACS. Overall, non-linearities can arise in two main steps, namely in the signal generation step, and in thee signal recording step.

* Corresponding author. Experimental Psychology Lab, Carl von Ossietzky University, Ammerländer Heerstr. 114 – 118, 26129, Oldenburg, Germany.
E-mail address: christoph.herrmann@uni-oldenburg.de (C.S. Herrmann).

https://doi.org/10.1016/j.ynirp.2022.100113
Received 7 March 2022; Received in revised form 3 June 2022; Accepted 12 June 2022
Available online 21 June 2022

In the signal generation step, a digital signal has to be converted to an analog signal (digital-analog conversion), and in the signal recording step, an analog signal has to be converted to a digital signal (analog-digital conversion). The properties of the hardware in use limit the linearity of both conversion processes (Vargha et al., 2001). Notably, it is known that all electronic devices exhibit some degree of non-linearity (Maas, 2003). A careful hardware selection by using converters with high digital resolution which operate at high sampling speed helps to overcome those hardware limitations.

Transcranial temporal interference stimulation (tTIS) has recently been proposed as a possible non-invasive technique for selectively stimulating deep brain regions (Grossman et al., 2017; Wessel et al., 2021). During tTIS two high-frequency sinusoidal currents of slightly different frequencies are applied to the brain and interfere in regions of overlap. The resulting sum signal features a signal with a fluctuating envelope depending on the difference of the two high-frequency sinusoids. For AM-tACS, the amplitude modulation is generated by multiplication of the high and low frequency sinusoids before the signal is forwarded to the signal generators (Digital-Analog-Converter (DAC) and the constant current source of the stimulator). For tTIS, the quasi-amplitude modulation (i.e. the expression of the envelope or the beat rhythm at the difference frequency) emerges inside the brain, after the independent generation of two pure high-frequency sine-waves (independently generated).

The aim of the current study is to characterize low-frequency artifacts due to tTIS in neurophysiological recording methods. Due to the generation of the signal (two pure sine-waves are generated independently), tTIS circumvents non-linearities inherent in the signal generation hardware. Therefore, we hypothesized that low-frequency artifacts due to tTIS could be absent or, at least, smaller in size, if we measure with a recording device (such as M/EEG), characterized by high linearity. In order to test this hypothesis, we simulated the resulting signal for tTIS, accounting for non-linearities of the signal generator- and recording-hardware. Moreover, we systematically tested three different recording devices while stimulating a phantom head with tTIS.

2. Methods

2.1. Simulation of non-linear signal generation and acquisition

To assess the role of non-linear hardware components for low-frequency artifacts during tTIS, we adapted the simulation regarding the influence of non-linear input-output function of a system presented in Kasten et al. (2018b). Kasten et al. (2018b) investigated the input-output transfer functions of signal-generator, stimulator output, and EEG. Specifically, the authors fitted a polynomial regression model to the recorded peak amplitudes (V_{out}), depending on the generated peak amplitudes (V_{in}). The results were verified with experimental data. Specifically, we extended the simulation by dissociating the influence of the hardware at the signal generation stage, as well as at the signal recording stage to the stimulation signal. We contrasted the influence of non-linearities (inherent in the signal generation- and recording-hardware) for an amplitude modulated signal (AM-tACS), against the influence of non-linearities for two independently generated sine waves, interfering inside the brain to an amplitude modulated signal (tTIS).

Non-linear input/output properties of the signal generation and recording stages were modelled using 6th degree polynomial functions of the form:

$$f(x) = \beta_6 \cdot x_t^6 + \beta_5 \cdot x_t^5 + \beta_4 \cdot x_t^4 + \beta_3 \cdot x_t^3 + \beta_2 \cdot x_t^2 + \beta_1 \cdot x_t + \beta_0$$

where x is the amplitude of the input signal at time t and β is a weight for the non-linear term. Weights for the function were approximated by using values previously observed for a complete tACS signal generation and EEG acquisition circuit in our previous work (Kasten et al., 2018b).

Table 1
Recording setups.

System	(1) MEG	(2) Passive EEG	(3) Active EEG
	Elekta Neuromag Triux	brainAmp system, 16-bit amplifier	actiChamp System, 24-bit amplifier
Sampling Rate	5 kHz	5 kHz	10 kHz
Carrier Frequency	500 Hz 1000 Hz	500 Hz 1000 Hz	500 Hz 1000 Hz
Modulation Frequency	10 Hz 11 Hz 23 Hz	10 Hz 11 Hz 23 Hz	10 Hz 11 Hz 23 Hz
Intensity	1 mA	0.1 mA	1 mA

Signals were fed through the function twice to simulate a signal generation and a signal recording stage. For the AM-waveform the signal was created prior to both stages using the following formula:

$$AM_{Signal}\left(t\right) = a_{stim}\left(\left(\frac{\sin(2\pi \cdot f_m \cdot t)}{2} + \frac{1}{2}\right) \cdot \sin\left(2\pi \cdot f_c \cdot t\right)\right)$$

where a_{stim} is the stimulation amplitude, f_m is the modulation frequency and f_c is the carrier frequency. The resulting signal is an AM-waveform with 50% modulation depth. In contrast, for the tTIS signal, two pure sinewaves with frequencies of $f_1 = 195$ Hz and $f_2 = 205$ Hz were created. The two sinewaves were separately fed through the polynomial function simulating the signal generation stage and then linearly combined. Subsequently, the resulting tTIS signal, which now exhibits an amplitude modulation at a frequency equal to the difference of f_1 and f_2, was fed through the polynomial function a second time, to simulate the signal acquisition stage. The carrier frequency of the resulting signal is equivalent to the average of the two sine wave frequencies.

2.2. Phantom M/EEG recordings

In addition to our simulations, we systematically tested three different recording devices while stimulating a phantom head (melon) with tTIS: (1) an MEG, (2) a passive- and (3) an active- EEG system. Specifically, we recorded MEG with a 306-channel whole head MEG system by Elekta Neuromag Triux (Elekta Oy, Helsinki, Finland), sampled at 5 kHz. Moreover, we measured with two different EEG systems: a passive- (16-bit amplifier, BrainAmp system, sampled at 5 kHz) and an active- (24-bit amplifier, actiChamp System, sampled at 10 kHz) EEG system, both manufactured by Brain Products GmbH (Gilching, Germany) with (active) Ag/AgCl electrodes attached to the phantom head at positions roughly corresponding to a nose (reference electrode), forehead (ground electrode) and Pz montage (positions were approximated), while keeping the impedances of the EEG electrodes below 10 kΩ. While measuring each recording device (MEG, passive- and active EEG), we systematically stimulated with two different carrier frequencies (500 Hz and 1000 Hz), as well as with several different modulation frequencies (10 Hz, 11 Hz, and 23 Hz). For an overview across the recording setups see Table 1. The stimulation signal was digitally generated at a rate of 100 kHz, transferred to a digital analog converter (NI-USB 6229 BNC, National Instruments, Austin, TX, USA), which was connected to optically isolated remote inputs of two battery-operated constant current stimulators (Advanced DC STIMULATOR PLUS, neuroConn, Ilmenau, Germany). The impedances of the stimulation electrodes were kept below 10 kΩ. The stimulation intensity was 1 mA for MEG- and active EEG-recordings. Due to limits of the recording hardware (lower amplitude rang for 16-bit digitalization), the stimulation intensity was 0.1 mA for passive EEG recordings.

2.3. Data analyses

To assess the frequency content of the simulated stimulation

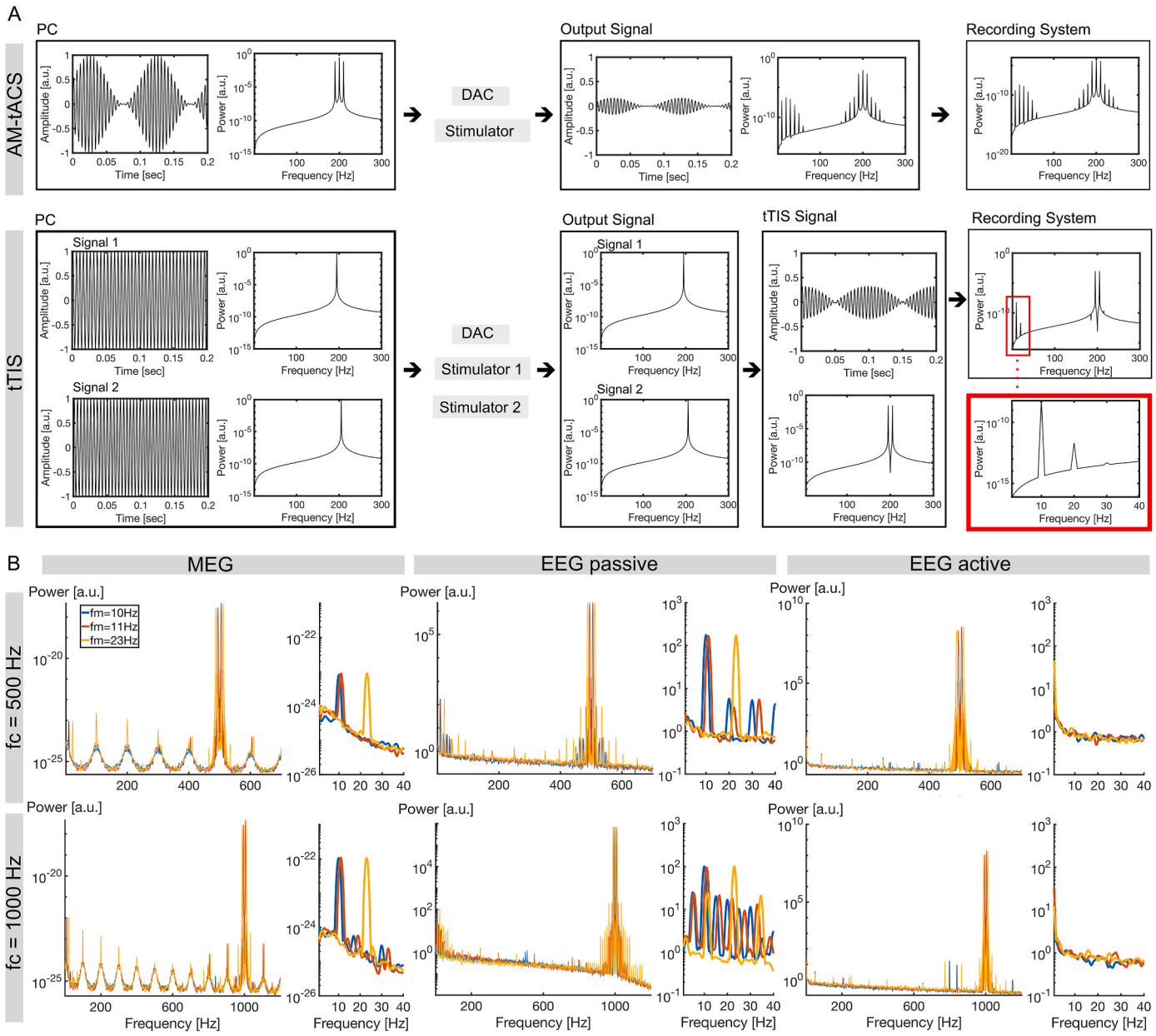

Fig. 1. Results. A. Simulation results. For AM-tACS, the amplitude modulated signal is generated (carrier frequency of 200 Hz, modulation frequency of 10 Hz) within the computer, characterized by power at the carrier frequency and two side bands (carrier frequency ± modulation frequency). The signal is forwarded to the simulation of the signal generators (DAC and stimulator). The output signal shows that the power spectra of the amplitude modulated signal exhibits low frequency artifacts at the modulation frequency, and harmonics. After simulating a non-linear measurement device, we can clearly see the low frequency artifacts in the spectra (top row). For tTIS, two sinusoidal signals (Signal 1 oscillates at 195 Hz, Signal 2 oscillates at 205 Hz) are generated and forwarded to the simulation of the signal generators (DAC and stimulators). The output signals of both single sinusoids show one peak at the stimulation frequency (195 Hz and 205 Hz). Adding the sinusoids to the amplitude modulated signal, we can see no low-frequency artifact in the signal. We solely can see the peaks at the initial frequencies of both sinusoids (195 Hz and 205 Hz). If, however, we simulate a non-linear measurement device, we again can see low-frequency artifacts in the data (at the modulation frequency and its harmonics as indicated in the red boxes). B. Recording results for MEG, passive EEG, and active EEG during tTIS. The low-frequency artifact is clearly visible at different modulation frequencies (f_m) for MEG (visualized for one gradiometer channel), and passive EEG (left and middle). In contrast, no low-frequency artifact is visible in active EEG recordings (right). Note that all frequency spectra are displayed in log scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

waveforms at different stages of the signal generation-recording circuit, power spectra were computed using Fast Fourier Transforms. Simulations were computed for 1 s segments of data, resulting in a frequency resolution of 1 Hz. Spectra were assessed for the original waveforms (before being fed through any non-linear function), after the signal generation stage, and after the signal acquisition stage.

M/EEG data were analyzed using MATLAB (2018) (The Mathworks Inc., Natick, MA, USA) and the Fieldtrip toolbox. Since amplifier saturation can result in low frequency artifacts, we checked the raw signals for clipped channels prior to data analysis (for details see Supplementary Materials A). Specifically, M/EEG data were high pass filtered at 1 Hz, and the signals were cut into 1-sec non-overlapping epochs. This results in a frequency resolution of 1 Hz for the spectra computed by an FFT. On each of these 60 segments, FFTs (4-sec zero-padding, Hanning window) were computed and averaged. This results in five data points per Hz spectral resolution. For the MEG data,

results are shown for an exemplary magnetometer channel (*MEG0732*).

3. Results

The simulation suggests, that if the AM-waveform is created directly in the stimulator (as for AM-tACS), we clearly see low-frequency artifacts at the modulation frequency after the signal generation and the signal recording stage (see Fig. 1A, *top row*). If, however, we send multiple pure sine waves through the system (as for tTIS), no low-frequency artifact can be found in the spectrum after the signal generation stage (see Fig. 1A, *bottom row*). This shows a clear advantage of tTIS over traditional AM-tACS. Nonetheless, if the resulting AM-wave is then sampled by another non-linear recording device (e.g. M/EEG recording device), the low-frequency artifact is reintroduced to the recorded signal (see Fig. 1A, *bottom row*).

For MEG and the passive EEG system, we can clearly see the low-frequency artifact in the data at the different modulation frequencies (10 Hz, 11 Hz, and 23 Hz) for both carrier frequencies (500 Hz and 1000 Hz) (see Fig. 1B left and middle, zoomed spectra 1–40 Hz). In contrast, when recording with the active EEG system, the low-frequency artifacts at the different modulation frequencies are not visible in the data for neither one of the carrier frequencies (see Fig. 1B right). In line, low-frequency artifacts at the modulation frequency are not visible for higher carrier frequencies (for details see supplementary material B).

4. Discussion

Our simulation confirms that during tTIS we avoid artifacts caused by the non-linearity of the stimulators (due to the nature of its signal generation), but the non-linearity of the recording system is crucial for the occurrence of low-frequency distortions during tTIS. This indicates that low-frequency artifacts in the recorded signal is mainly caused by non-linearities inherent in the recording hardware (not in the signal generation hardware). Indeed, experimental data show that the artifact arises in the recorded signal due to non-linearities inherent in the recording hardware for MEG, and the passive EEG system. In contrast, data recorded with the active EEG system show no low-frequency artifact. Note that the active EEG system has a larger dynamic range and, therefore, has a bigger range where the device can sample linearly.

Two main reasons exist for non-linearities in transfer functions, i) amplitude distortions in the time domain, e.g. when approaching the limits of the specified AD range, and ii) amplitude distortions in the frequency domain e.g. when residual artifacts are not suppressed by the characteristic of the anti-aliasing filter or sampled well enough with a higher frequency sampling rate.

I) The analog input of an analog-to-digital converter has to be protected against overvoltage. This is typically achieved by Schottky- or Zener-diodes or other clamping circuits. Unfortunately, as we have observed, theses clamping circuits already start to clamp the input signal slightly before it exceeds the specified AD range. In that case, the analog signal is not clipped which would be clearly visible. Instead, it is only slightly distorted. Technically, this represents a non-linearity in the transfer function and is the topic of the current manuscript. Importantly, this type of non-linearity occurs independent of the phenomena in the frequency domain.

II) As the Nyquist criterion states, an analog signal has to be sampled with a sampling frequency more than two times the highest frequency contained in the signal. While this is sufficient to capture the frequency of the fastest signal, it is by no means sufficient to capture amplitude variations. Therefore, analog signals are typically over-sampled. The higher the sampling frequency, the lower the sampling error. In addition, every device has a frequency response and higher frequencies tend to be suppressed in amplitude. Technically, both phenomena result in distorted amplitudes. These phenomena in the frequency domain, however, are not the scope of our manuscript.

The results of the current investigation suggest that it is possible to record human brain activity during tTIS without a low-frequency artifact present in the data masking the brain activity at frequencies that are in the range of regular neural frequencies. However, the quality of the neurophysiological data during tTIS highly depends on the recording hardware that is used. As reported for the MEG- and the passive EEG-system, the artifact at the modulation frequency can be easily introduced to the data on the recording level. However, if the measurement is done using an almost linear recording device (such as the active EEG system in the current investigation), the artifact at the modulation frequency can be minimized making the signal useful for investigation of brain functions. The noise floor of at the modulation frequency can be compared, e.g. with the well-researched alpha peak reported in previous studies. Specifically, Stecher et al. (2017) reported alpha power peak amplitudes of at least 2.5 μV^2/Hz with the same recording system. The noise floor of the active EEG at the different modulation frequencies is always <0.1 μV^2/Hz for all carrier frequencies.

Note, the current investigation is limited to phantom data with specific stimulation parameter (stimulation intensity, frequencies). Therefore, future neurophysiological studies with simultaneous tTIS must be inspected carefully in regard to possible low-frequency artifacts. Overall, it has to be noted that the non-linearities of the used recording devices are extremely small and negligible in regular recordings of human brain activity. However, they do become relevant when additional signals at high intensities such as during transcranial stimulation are introduced to the recordings, which is arguable not the main purpose these devices were designed for.

The results of the current investigation suggest that it is feasible to simultaneously stimulate using tTIS and record good quality data without a low-frequency artifact, given a linear recording device. While tTIS is a novel method and little is known about its efficacy in humans, our work may help to design recording setups that allow recording of brain activity during stimulation and foster our understanding of its underlying mechanisms.

Author contributions

JC, FHK, BCB, KS and CSH conceived the study. JC analyzed the data. JC, FHK, BCB, KS and CSH wrote the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CSH holds a patent on brain stimulation. KS is the manufacturer of the advanced DC stimulator plus (Neuroconn, Ilmenau, Germany). JC, FHK, BCB, declare no competing interests.

Acknowledgements

This research was supported by the Neuroimaging Unit of the Carl von Ossietzky University Oldenburg funded by grants of from the German Research Foundation (3 T MRI INST 184/152-1 FUGG and MEG INST 184/148-1 FUGG). This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2177/1 – Project ID 390895286.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynirp.2022.100113.

References

Grossman, N., Bono, D., Dedic, N., Kodandaramaiah, S.B., Rudenko, A., Suk, H., et al., 2017. Noninvasive deep brain stimulation via temporally interfering electric fields. Cell 169 (6), 1029–1041. https://doi.org/10.1016/j.cell.2017.05.024 e16.

Helfrich, R.F., Schneider, T.R., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K., Herrmann, C.S., 2014. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr. Biol. 24 (3), 333–339. https://doi.org/10.1016/j.cub.2013.12.041.

Herrmann, C.S., Rach, S., Neuling, T., Strüber, D., 2013. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front. Hum. Neurosci. 7 (MAY), 1–13. https://doi.org/10.3389/fnhum.2013.00279.

Kanai, R., Chaieb, L., Antal, A., Walsh, V., Paulus, W., 2008. Frequency-dependent electrical stimulation of the visual cortex. Curr. Biol. 18 (23), 1839–1843. https://doi.org/10.1016/j.cub.2008.10.027.

Kasten, F.H., Herrmann, C.S., 2017. Transcranial alternating current stimulation (tACS) enhances mental rotation performance during and after stimulation. Front. Hum. Neurosci. 11 (January), 1–16. https://doi.org/10.3389/fnhum.2017.00002.

Kasten, F.H., Herrmann, C.S., 2019. Recovering brain dynamics during concurrent tACS-M/EEG: an overview of analysis approaches and their methodological and interpretational pitfalls. Brain Topogr. 32 (6), 1013–1019. https://doi.org/10.1007/s10548-019-00727-7.

Kasten, F.H., Maess, B., Herrmann, C.S., 2018a. Facilitated event-related power modulations during transcranial alternating current stimulation (tACS) revealed by concurrent tACS-MEG. Eneuro 5 (3), 18. https://doi.org/10.1523/ENEURO.0069-18.2018, 2018, ENEURO.0069.

Kasten, F.H., Negahbani, E., Fröhlich, F., Herrmann, C.S., 2018b. Non-linear transfer characteristics of stimulation and recording hardware account for spurious low-frequency artifacts during amplitude modulated transcranial alternating current stimulation (AM-tACS). Neuroimage 179 (May), 134–143. https://doi.org/10.1016/j.neuroimage.2018.05.068.

Maas, S.A., 2003. Nonlinear Microwave and RF Circuits. Artech house, Boston.

MATLAB, 2018. Version R2018b. The MathWorks Inc., Natick, Massachusetts.

Neuling, T., Ruhnau, P., Fuscà, M., Demarchi, G., Herrmann, C.S., Weisz, N., 2015. Friends, not foes: magnetoencephalography as a tool to uncover brain dynamics during transcranial alternating current stimulation. Neuroimage 118, 406–413. https://doi.org/10.1016/j.neuroimage.2015.06.026.

Noury, N., Hipp, J.F., Siegel, M., 2016. Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. Neuroimage 140, 99–109. https://doi.org/10.1016/j.neuroimage.2016.03.065.

Noury, N., Siegel, M., 2018. Analyzing EEG and MEG signals recorded during tES, a reply. August 2017 Neuroimage 167, 53–61. https://doi.org/10.1016/j.neuroimage.2017.11.023.

Stecher, H.I., Pollok, T.M., Strüber, D., Sobotka, F., Herrmann, C.S., 2017. Ten minutes of α-tACS and ambient illumination independently modulate EEG α-power. Front. Hum. Neurosci. 11, 257.

Vargha, B., Schoukens, J., Rolain, Y., 2001. Static nonlinearity testing of digital-to-analog converters. IEEE Trans. Instrum. Meas. 50 (5), 1283–1288.

Vossen, A., Gross, J., Thut, G., 2015. Alpha power increase after transcranial alternating current stimulation at alpha frequency (α-tACS) reflects plastic changes rather than entrainment. Brain Stimul. 8 (3), 499–508. https://doi.org/10.1016/j.brs.2014.12.004.

Wessel, M.J., Beanato, E., Popa, T., Windel, F., Menoud, P., Beliaeva, V., et al., 2021. Evidence for temporal interference (TI) stimulation effects on motor striatum. Brain Stimul. 14 (6), 1684. https://doi.org/10.1016/j.brs.2021.10.307.

Witkowski, M., Garcia-Cossio, E., Chander, B.S., Braun, C., Birbaumer, N., Robinson, S.E., Soekadar, S.R., 2016. Mapping entrained brain oscillations during transcranial alternating current stimulation (tACS). Neuroimage 140, 89–98. https://doi.org/10.1016/j.neuroimage.2015.10.024.

Zaehle, T., Rach, S., Herrmann, C.S., 2010. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One 5 (11), 1–7. https://doi.org/10.1371/journal.pone.0013766.