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Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation

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ABSTRACT

Transcranial electric stimulation (tES) is a promising tool to non-invasively manipulate neuronal activity in the human brain. Several studies have shown behavioral effects of tES, but stimulation artifacts complicate the simultaneous investigation of neural activity with EEG or MEG. Here, we first show for EEG and MEG, that contrary to previous assumptions, artifacts do not simply reflect stimulation currents, but that heartbeat and respiration non-linearly modulate stimulation artifacts. These modulations occur irrespective of the stimulation frequency, i.e. during both transcranial alternating and direct current stimulations (tACS and tDCS). Second, we show that, although at first sight previously employed artifact rejection methods may seem to remove artifacts, data are still contaminated by non-linear stimulation artifacts. Because of their complex nature and dependence on the subjects' physiological state these artifacts are prone to be mistaken as neural entrainment. In sum, our results uncover non-linear tES artifacts, show that current techniques fail to fully remove them, and pave the way for new artifact rejection methods.

Introduction

Manipulative approaches are much needed in systems neuroscience. Take neuronal oscillations as an example. They are ubiquitous in the brain and have been implicated in various functions (Buzsáki and Draguhn, 2004; Fries, 2005; Jensen and Mazaheri, 2010; Siegel et al., 2012; Singer, 1999; Womelsdorf et al., 2014). However, supporting evidence, especially in humans, remains largely correlative and only few studies have addressed this causally (Helfrich et al., 2014; Marshall et al., 2006; Polanía et al., 2012; Romei et al., 2011, Romei et al., 2010; Voss et al., 2014). One strategy to causally assess potential roles of neural oscillations is to manipulate them and to simultaneously measure the effect on neural activity and behavior. This is technically challenging and well-defined experimental protocols as well as analysis pipelines have not been established yet.

Transcranial electric stimulation (tES) is a non-invasive brain stimulation technique, which provides the possibility to control stimulation strength, frequency and, to some extent, stimulation site (Dmochowski et al., 2011; Kanai et al., 2008; Schutter and Hortensius, 2010; Schwiedrzik, 2009). These features render tES and in particular one of its variants, transcranial alternating current stimulation (tACS), suitable for manipulating specific brain rhythms (Herrmann et al., 2013). During tACS, a sinusoidal electrical current at a specific frequency is applied to the subject through electrodes placed on the scalp. The potential of electrical stimulation to manipulate neuronal oscillations has been shown in animal models (Fröhlich and McCormick, 2010; Ozen et al., 2010). However, in humans, tACS has largely been limited to investigating effects on behavior and on neurophysiological aftereffects (Brittain et al., 2013; Herrmann et al., 2013; Marshall et al., 2011, Marshall et al., 2006; Polanía et al., 2012; Zaehle et al., 2010). A key reason for the limited number of studies directly investigating effects on neural activity during stimulation is the massive electrophysiological artifact induced by the stimulation. These artifacts are particularly problematic when attempting to investigate effects on neuronal activity within the same frequency range as the stimulation frequency (Zaehle et al., 2010).

Recently, different approaches have been proposed to remove tES artifacts from EEG and MEG for studying neuronal activity during stimulation (Helfrich et al., 2014; Neuling et al., 2015; Soekadar et al., 2013; Voss et al., 2014). Based on the assumption of linear stimulation artifacts, these methods follow approaches like template subtraction, component analysis, beamforming or temporal filtering. However, a thorough characterization of stimulation artifacts, which is needed for assessing artifact cleaning procedures, is missing. Here we provide this characterization.

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Materials and methods

Methods outline

We measured EEG and MEG during several different tES conditions. First, we tested if a pure sinusoidal model can explain tES artifacts. Next, we investigated in the time and frequency domain whether heartbeat and respiration modulate tES artifacts. Finally, we used temporal and spectral features of tES artifacts to track them through different stages of available artifact rejection pipelines. The rationale behind each analysis is explained in the Results section.

Participants and experimental protocol

All experiments were conducted in 5 healthy male participants. All subjects gave written informed consent before participating. All experiments were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. The main tACS experiment with small stimulation electrodes was conducted in 4 subjects that each participated in 6 experimental runs. Each run consisted of the following sequential conditions: sham, tACSa, tACSb, sham, tACSb, and tACSa. Each condition lasted 66 s. For each run, 11 Hz tACS and 62 Hz tACS were randomly assigned to tACSa and tACSb conditions to avoid any potential sequence effects. During the first 5 runs, subjects fixated a central fixation spot at the center of a blank monitor (60 Hz refresh rate). In the last run subjects kept their eyes closed. Before start of the experiment, subjects were habituated to transcranial electric stimulation. In one of the four subjects, we performed a control experiment with large rubber electrodes. In this control experiment runs 3 and 6 were measured with eyes closed. We performed two more control experiments on a fifth subject with the same electrode layout as in the main tACS experiment. In both experiments, the subject fixated a central fixation spot. In the first control experiment, we checked for the potential influence of the EEG ground electrode placement on the stimulation artifact during 62 Hz tACS. We recorded 10 min of EEG with ground on the right forearm and 10 min with ground on the forehead (Fpz of 10–10 system). In the second control experiment, we recorded MEG and EEG during cathodal tDCS, anodal tDCS and sham conditions (10 min per condition). Cathodal and anodal are defined based on the polarity of the parietal stimulation electrode.

Transcranial electric stimulation

Stimulation current was applied with an IZ2h stimulator (Tucker Davis Technologies Inc.). Stimulation amplitude was 0.5 mA (i.e., 1 mA peak-to-peak for tACS). Stimulation did not induce flicker percepts. For the main experiment, stimulation was applied through two standard Ag/AgCl EEG electrodes over right occipital and right parietal areas (electrodes O10 and CP4 of the 10–10 electrode system). For the control experiment with large electrodes, 35 cm^2 MR-compatible rubber electrodes (neuroConn GmbH) were placed over occipital and frontal lobes underneath the EEG cap. For all experiments, stimulation electrodes were attached using Ten20 conductive paste (Weaver and Company) and their impedance was kept below 2.5 kΩ. To minimize magnetic artifacts produced by the stimulation current, we carefully twisted all stimulation cables.

Data acquisition and preprocessing

We simultaneously recorded 72-channel EEG (NeurOne system, Mega Electronics Ltd) and 272-channel MEG (Omega 2000, CTF Systems) throughout all experiments at 10,000 Hz and 2343.8 Hz sampling rate, respectively. EEG electrodes were positioned based on the 10–10 electrode system using an EEG cap (EC80, EASYCAP). All signals were in the dynamic range of recording systems and no clipping was observed for either EEG or MEG signals. Due to the interference between stimulation currents and electrical currents of the head-positioning circuits of the MEG system, we could not monitor head movement continuously during the experiment. Instead we measured head positions at the beginning and at the end of each run.

EEG electrodes were attached using Abratyl 2000 conductive gel and impedances were kept below 2.5 kΩ for most electrodes. We referenced EEG electrodes to FCz and, except for one control experiment, positioned a ground electrode on the right forearm. EEG signals were re-referenced to average reference offline. Along with EEG and MEG, we recorded the injected current, the ECG and respiratory movements using bipolar channels of the EEG system. The injected current was indirectly measured by recording the voltage drop across a 200 Ω resistor positioned in series to the head. The ECG was recorded through 2 electrodes placed below the right clavicle and below the left pectoral muscle. Respiration was continuously recorded with a piezo respiratory belt transducer (Vermed-Medizintechnik).

Sinusoidal model subtraction

To remove an optimal sinusoidal model from artifactual signals, we fitted the amplitude, frequency and phase of a sinusoid to the MEG and EEG data and subtracted it from the data. For this, it is important to estimate the stimulation frequency with μHz accuracy. This is because, if the internal clocks of the stimulation and recording system are not synchronized, as in the present case, even small errors of the estimated stimulation frequency lead to strong residual artifacts around the main peak. To this end, we first chose 20 MEG channels with strongest tACS artifacts and split their data into 33 s long segments on which we fitted amplitude, frequency and phase of a sinusoidal model separately for each channel. We estimated the stimulation frequency as the median across all segments and channels (standard deviation of 8.50 and 4.85 μHz, for 11 Hz and 62 Hz tACS, respectively). Next, we defined a new sinusoidal model fixing its frequency at the estimated stimulation frequency. We then separately fitted amplitude and phase of this new model to each segment and channel and removed it from the data.

We followed a similar strategy for EEG. As we also recorded the injected current with the EEG system, this allowed for estimating the stimulation frequency based on the injected current. This is more accurate than estimation based on the EEG signal, because the injected current does not include any brain signals. As for the MEG, we split the injected current into 33 s long segments and estimated the stimulation frequency for each piece. We estimated the stimulation frequency as the median across all segments (standard deviation of 0.10 and 0.66 μHz, for 11 Hz and 62 Hz tACS, respectively).

Spectral analyses

To estimate the power spectral density (PSD) in tACS experiments, we first split the data into either 4 s or 33 s long segments, according to the desired spectral resolution. Then, we applied a Hanning window to each segment and computed its Fourier transform. Finally, we calculated the average power across all segments and scaled the results to PSD (μV^2/Hz and fT^2/Hz for EEG and MEG, respectively). For the case of tDCS and to reveal the spectral structure of artifacts in face of strong low frequency activity of EEG and MEG, we estimated spectra with higher resolution. We split the data into 120 s segments, estimated the Thomson's multitaper PSD of each segment (Slepian tapers with 0.05 Hz bandwidth, NW = 6) and calculated the average power across all segments.

Heartbeat and respiration frequencies

For each subject, heartbeat and respiration rates were defined as the inverse of the median of the temporal intervals between successive ECG R-peaks and respiration ends, respectively.

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