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Original Reports

Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants

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Abstract: **Chronic pain is a major health care problem. A better mechanistic understanding and new treatment approaches are urgently needed. In the brain, pain has been associated with neural oscillations at alpha and gamma frequencies, which can be targeted using transcranial alternating current stimulation (tACS). Thus, we investigated the potential of tACS to modulate pain and pain-related autonomic activity in an experimental model of chronic pain in 29 healthy participants. In 6 recording sessions, participants completed a tonic heat pain paradigm and simultaneously received tACS over prefrontal or somatosensory cortices at alpha or gamma frequencies or sham tACS. Concurrently, pain ratings and autonomic responses were collected. Using the present setup, tACS did not modulate pain or autonomic responses. Bayesian statistics confirmed a lack of tACS effects in most conditions. The only exception was alpha tACS over somatosensory cortex where evidence was inconclusive. Taken together, we did not find significant tACS effects on tonic experimental pain in healthy humans. Based on our present and previous findings, further studies might apply refined stimulation protocols targeting somatosensory alpha oscillations.**

Trial registration: ***The study protocol was pre-registered at ClinicalTrials.gov (NCT03805854).***

Perspective: ***Modulating brain oscillations is a promising approach for the treatment of pain. We therefore applied transcranial alternating current stimulation (tACS) to modulate experimental pain in healthy participants. However, tACS did not modulate pain, autonomic responses, or EEG oscillations. These findings help to shape future tACS studies for the treatment of pain.***

Key Words: *Pain, humans, neural oscillations, neuromodulation, tACS.*

Pain is a vital protective phenomenon but can also occur for extended time periods without protecting the body. In such chronic pain conditions, pain represents a highly disabling disorder and is a leading cause of disability worldwide.[20,47] Current treatment approaches are often insufficient and can cause serious side effects as indicated by the current Opioid crisis.[42] Moreover, the development of pain therapeutics is stagnating.[52] Thus, novel approaches for the treatment of chronic pain are urgently needed.[28,45,52]

Recent insights into the brain mechanisms of pain open new perspectives for novel treatments. Accumulating evidence indicates that pain is closely associated

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with neural oscillations.[43] In particular, changes of neural oscillations at alpha (8–13 Hz) and gamma (30–100 Hz) frequencies in somatosensory and prefrontal brain areas have been related to the intensity of longer-lasting experimental pain and chronic pain (e.g.,[15,35,38,41,51,64]). Moreover, animal studies using optogenetics and invasive electrical stimulation have indicated that changes of neural oscillations are causally involved in generating pain.[55,65] Thus, modulating neural oscillations to eventually modulate pain is a promising novel approach for pain treatment.[24]

Transcranial alternating current stimulation (tACS) is an emerging neuromodulation technique which aims at non-invasively modulating neural oscillations in the human brain. During tACS, a weak alternating, sinusoidal current is applied to the scalp with the goal of entraining neural oscillations at the stimulation frequency, thereby increasing their amplitude.[10,44,62] The appeal of tACS is that it is non-invasive, safe, cost-efficient, and potentially mobile which allows for broad clinical applications.[24] Thus, tACS is increasingly explored as a new treatment approach for neuropsychiatric disorders.[28,54,56]

To date, only 2 studies have investigated whether tACS can modulate pain.[1,5] Both studies employed tACS targeting somatosensory alpha oscillations. One study assessed effects on the intensity of clinical pain in patients suffering from chronic low back pain.[1] A first analysis did not show significant effects of tACS on average pain severity or perceived disability. However, subsequent exploratory analyses indicated reduced pain in the tACS compared to the sham condition. In addition, increases in alpha oscillations after tACS were correlated with changes in pain severity. The other study investigated tACS effects on brief experimental pain in healthy participants.[5] Results indicated that tACS can reduce pain intensity, but only when expectations of upcoming pain intensity are uncertain. The 2 studies thus provided preliminary evidence that tACS at alpha frequencies over somatosensory areas can potentially yield analgesic effects. Effects of tACS at other locations or frequencies on pain have not yet been studied.

Gamma oscillations might represent another promising target. Gamma oscillations in prefrontal brain areas encode pain intensity during tonic experimental pain in healthy participants and during chronic pain in patients.[35,38,51] In addition, gamma oscillations reliably track inter- and intraindividual variations of brief experimental pain in humans and rodents.[25] Furthermore, the optogenetic induction of gamma oscillations in the primary somatosensory cortex leads to enhanced pain behavior indicating a causal role for pain.[55] However, although tACS at gamma frequencies is feasible,[3,54] no study has examined its effects on pain so far.

Here, we further explored the potential of tACS to modulate pain. We systematically applied tACS at alpha and gamma frequencies or sham tACS over somatosensory and prefrontal cortices during tonic experimental pain. Thereby, our design extended previous work by targeting a new location and frequency, which have previously been implicated in the processing of pain.[35,38,41,51] We chose a tonic heat pain paradigm that resembles chronic pain conditions more than usual phasic pain stimuli and thus models some aspects of chronic pain.[43]

Methods

Participants

A priori sample size calculations using G*Power[18] determined a sample size of 28 participants for a repeated measures analysis of variance (RM ANOVA) design with 6 conditions (see below), a power of 0.95, an alpha of 0.05, and medium effect sizes of f = 0.25. This corresponds to an η^2 (proportion variance explained) of 0.06.[12] Based on these calculations, the final sample comprised 29 participants (all right-handed, 13 females, age: 25.7 $\pm$ 4.0 years [mean $\pm$ SD]). Overall, 39 healthy human participants were recruited. Ten participants were excluded during the course of experiment due to the absence of pain (n = 3) or intolerable pain (n = 3) during the first session, technical issues (n = 1: thermal stimulation was interrupted due to a broken cable, n = 1: technical defect of recording hardware), or meeting exclusion criteria during 1 of the sessions (n = 2).

Inclusion criteria were age above 18 years and right-handedness. Exclusion criteria were pregnancy, neurological or psychiatric diseases, severe internal diseases including diabetes, skin diseases, current or recurrent pain, regular intake of medication (aside from contraception, thyroidal and, in 1 case, antiallergic medication), previous surgeries at the head or spine, previous syncopes or head traumas resulting in unconsciousness or concussion, metal or electronic implants, and any previous side effects associated with thermal, electrical, or magnetic stimulation. None of the included participants showed signs of clinical anxiety or depression according to the Hospital Anxiety and Depression Scale[66] with a cut-off of 8/21[11] (anxiety: 2.5 $\pm$ 2.0 [mean $\pm$ SD]; depression: 0.7 $\pm$ 0.9 [mean $\pm$ SD]).

Prior to any experimental procedures, all participants gave written informed consent. The study protocol was approved by the local Ethics Committee of the Medical Faculty of the Technical University of Munich and pre-registered at ClinicalTrials.gov (NCT03805854). The study was conducted in accordance with the latest version of the Declaration of Helsinki and recent consensus guidelines for the application of tACS in humans.[3]

Paradigm

In a within-subject design, each participant took part in 6 recording sessions. In line with studies reviewed in recent guidelines for non-invasive brain stimulation,[63] sessions were separated by at least 24 hours to avoid carry-over effects between sessions. Each session comprised a fixed sequence of events (Fig 1A). In each session, tACS was applied over prefrontal cortex (PFC) or somatosensory cortex (S1) (Fig 1B) using alpha frequency (10 Hz) stimulation, gamma frequency (80 Hz) stimulation, or sham stimulation (Fig 1C). Concurrently, a tonic heat pain stimulus of varying intensity was applied to

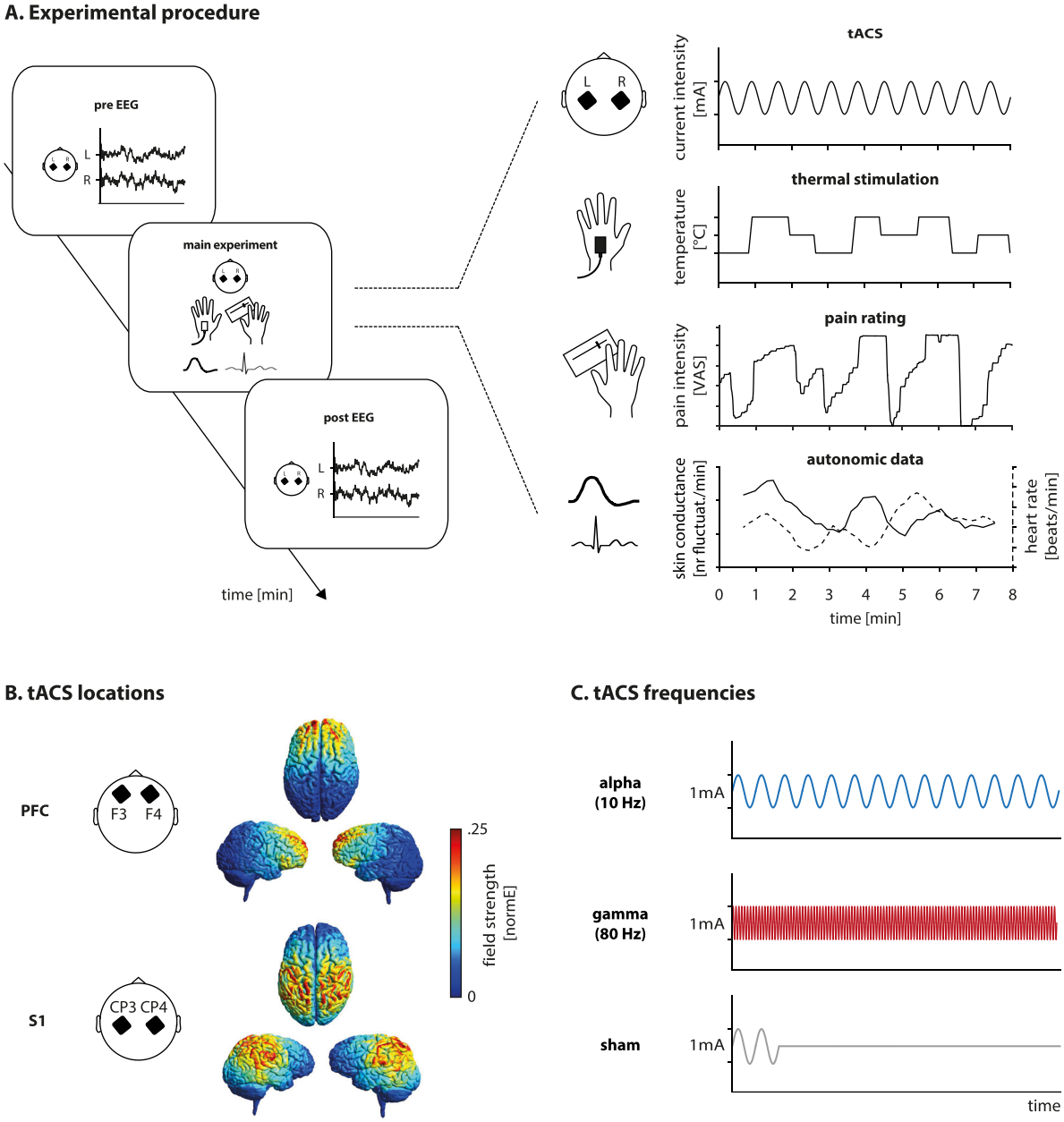

Figure 1. Paradigm. (A) Experimental Procedure. Each participant took part in 6 recording sessions which comprised a fixed sequence of events. During the main experiment, participants received tACS over prefrontal or somatosensory cortices using alpha, gamma, or sham stimulation while a tonic heat pain stimulus of varying intensity was applied to the left hand. Concurrently, participants continuously rated the currently perceived pain intensity and autonomic responses (skin conductance and electrocardiogram) were measured. Before and after the main experiment, 5 minutes of resting state EEG were recorded using the tACS electrodes. (B) tACS locations. Using two 5*5 cm carbonized rubber electrodes placed according to the international 10-20 system, tACS of 1 mA peak-to-peak intensity was applied over PFC (electrode positions F3 and F4) or S1 (electrode positions CP3 and CP4). Electrode placement was validated through simulations performed with SimNIBS 2.1[50] using 1 mA intensity, standard conductivity parameters, and the SimNIBS template head model. Simulations of the induced electrical field strength are shown on the right. Additional views showing coronal, horizontal, and sagittal cross-sections of the head model are displayed in Supplementary Figure S1. (C) tACS frequencies. 1 mA peak-to-peak tACS was applied at alpha or gamma frequencies or using sham stimulation. For alpha frequency stimulation, sinusoidal stimulation with a frequency of 10 Hz was applied. For gamma frequency stimulation, sinusoidal stimulation with 80 Hz frequency was applied. For sham stimulation, 30 s of 10 Hz sinusoidal stimulation were applied at the beginning of thermal stimulation only. All stimulations included 100 cycles fade-in and fade-out. EEG, electroencephalography; L, left; R, right; PFC, prefrontal cortex; S1, primary somatosensory cortex; tACS, transcranial alternating current stimulation; VAS, visual analogue scale.

the left hand. During stimulation, participants continuously rated the currently perceived pain intensity. In addition, autonomic responses (skin conductance and electrocardiogram) were continuously measured. Before and after the stimulation, 5 minutes of resting state EEG were recorded using the tACS electrodes.

Thermal Stimulation

Tonic painful heat stimulation was applied to the participant's left hand for 10 minutes using a thermode (TSA-II, Medoc, Ramat Yishai, Israel). Following an established paradigm,[37-39] a predefined, fixed time

course of stimulation (Fig 1A) consisting of 9 plateaus with 3 temperature levels (low, medium, and high) was applied. Temperature levels were individually adjusted for each participant by adding 0.5, 0.8, or 1.1°C to the individual pain threshold (see below), resulting in 3 intensity levels of thermal stimulation. The stimulation sequence consisted of 3 plateaus of 40, 50, and 60 s duration at each temperature level. The stimulation started from a baseline temperature of 40°C and changed with a rate of 0.1°C/s. All analyses were performed using an 8-minute-time window beginning at the start of the first plateau.

Pain thresholds were determined for the left hand on the first recording day immediately before the pre-stimulation EEG resting state recording. In line with previous studies,[37-39] over the course of 3 minutes, participants continuously adjusted the thermode temperature to their individual pain threshold using 2 buttons of a computer mouse with their right hand. Depending on the side of the button press, the thermode temperature either increased or decreased with a rate of 0.5° C/s. The individual pain threshold was defined as the average stimulus intensity during the last 10 s and was used to determine individual temperature levels for all 6 recording days. Thus, temperature levels were individually adapted but kept constant across all conditions for each single participant. We chose to keep the objective stimulus intensity constant across conditions to rule out different temperature levels as a confounding variable in our analyses. Mean pain threshold temperature was of 44.4 ± 1.7°C [mean ± SD].

Pain Ratings

During the thermal stimulation, participants continuously rated the currently perceived pain intensity on a visual analogue scale (VAS) ranging from 0 ("no pain") to 100 ("worst tolerable pain") using a custom-built finger span device with their right hand. The scale was simultaneously presented on a computer screen by a vertical orange bar, the height of which represented the current pain intensity. Pain ratings were sampled with a frequency of 1000 Hz by a BrainAmp ExG MR amplifier (Brain Products, Munich, Germany).

Autonomic Data

Skin conductance was recorded at the palmar distal phalanges of the left index and middle finger using Ag/AgCl electrodes connected to a GSR-MR module (Brain Products, Munich, Germany) with constant 0.5 V voltage. Participants were instructed not to move the hand during stimulation. Data were recorded in direct current (DC) mode with low-pass filtering at 250 Hz. The *electrocardiogram* (ECG) was measured using a bipolar Ag/AgCl electrode montage with 1 electrode attached below the right clavicle and the other below the sternum. ECG data were band-pass filtered between 0.016 and 250 Hz. Both skin conductance and ECG were sampled at 1000 Hz using the BrainAmp ExG MR amplifier (Brain Products, Munich, Germany).

Transcranial Alternating Current Stimulation (tACS)

Ten minutes of tACS were applied simultaneously to painful heat stimulation. The paradigm, thus, enables the exploration of immediate tACS effects (*online effects*) on pain rather than exclusively relying on after-effects outlasting the stimulation (*offline effects*). tACS intensity was 1 mA peak-to-peak for all participants and conditions. We employed a Neuroconn DC-STIMULATOR MR (Neuroconn, Ilmenau, Germany) and 2 carbonized rubber electrodes with a size of 5×5 cm. To validate electrode placement, electrical fields induced by a 1 mA transcranial current stimulation were simulated beforehand using SimNIBS 2.1[50] with standard conductivity parameters and the SimNIBS template head model (Fig 1B and Supplementary Fig S1). For stimulation of the *PFC*, electrodes were placed at positions F3 and F4 of the international 10-20 system. For stimulation of *S1*, electrodes were attached at positions CP3 and CP4. In line with recent recommendations,[9] electrodes were firmly fixed to the scalp using an even layer of Ten20 conductive paste (D.O. Weaver, Aurora, CO, United States), rendering any additional fixation of electrodes unnecessary. Impedances were kept below 5 kΩ (1.7 ± 0.9 kΩ [mean ± SD across all subjects and conditions]) and were similar for all 3 stimulation conditions of both montages (PFC: $\chi^2(2) = 0.80$, $P = .672$; S1: $\chi^2(2) = 3.07$, $P = .215$; Friedman tests). For *alpha frequency* stimulation, a 10 minute-sinusoidal stimulation with a frequency of 10 Hz was applied. For *gamma frequency* stimulation, a 10 minute-sinusoidal stimulation with 80 Hz frequency was applied. For *sham* stimulation, 30 s of 10 Hz sinusoidal stimulation were applied. All stimulations included 100 cycles fade-in and fade-out. Fade-in always started with the beginning of thermal stimulation. Thus, during the 8 minute-analysis window starting from the first plateau of thermal stimulation, participants received simultaneous, continuous tACS in the alpha and gamma frequency conditions, but no stimulation in the sham condition. For half of the participants, the 3 PFC sessions were performed first, followed by the 3 S1 sessions. For the other half, the order was reversed. Within the 3 sessions of each tACS location, the order of stimulation frequencies (alpha, gamma, sham) was counterbalanced to control for potential sequence effects of stimulation frequency.

Pre- and Post-Stimulation EEG Recordings

The rationale of tACS is to modulate neural oscillations during tACS. Demonstrating such online effects directly requires the simultaneous measurement of neural oscillations during tACS. However, online EEG measurements are heavily contaminated by tACS artifacts and their significance is therefore uncertain.[62] To nevertheless check for a potential indicator of the neural efficacy of our stimulation, we investigated offline effects of our stimulation. To quantify potential tACS effects on oscillatory brain activity outlasting the stimulation, we recorded 5 minutes of resting state brain activity

immediately before and after tACS (pre- and post-EEG). Participants were asked to stay in a relaxed, wakeful state, without any particular task, keeping their eyes open and their gaze rested on a centrally presented fixation cross. EEG data were recorded using the same 2 electrodes used for tACS, that is, placed at F3 and F4 for PFC sessions and at CP3 and CP4 for S1 sessions. Ag/AgCl electrodes attached to the nose and centrally on the forehead served as reference and ground, respectively. A bipolar Ag/AgCl electrode montage with electrodes below the outer canthus of the right eye and immediately below the hairline at the midline of the forehead was used to record eye movements. EEG data were sampled at 1000 Hz using the BrainAmp ExG MR amplifier (Brain Products, Munich, Germany) and bandpass-filtered between 0.016 and 250 Hz. Impedances were kept below 5 kΩ.

Blinding

Due to the attachment of electrodes, participants and experimenters were not blinded with respect to the location of tACS. However, we aimed at a double-blind design with respect to the tACS frequency (alpha, gamma, sham). To this end, each session was conducted by a main experimenter who was unaware of the stimulation frequency and interacted with the participant and a second experimenter who operated the tACS device. At the end of each session, blinding of the participant was assessed using a short questionnaire consisting of 3 questions: (1) *"Did you have the impression that a continuous brain stimulation was applied today?"*, (2) *"Did you experience sensations at the scalp like tingling, prickling, or pulsing?"*, and (3) *"Did you experience light perceptions (phosphenes) like flickering?"*. Question 1 was answered using a forced-choice format (*yes/no*), whereas questions 2 and 3 were answered using a VAS ranging from 0 ("no") to 10 ("very strongly").

Data Analysis

tACS Effects on Pain

We first assessed whether tACS modulated pain perception (Fig 2). For this purpose, the 8 minute-pain rating and -temperature time courses were smoothed using a sliding-window approach with a window length of 1 s and a step size of 0.1 s. Smoothed pain rating and temperature time courses represented the basis of further analyses. Subsequently, analyses of tACS effects on *pain intensity* were performed. To investigate whether tACS influenced the overall pain level, we computed a summary measure of pain intensity by averaging pain ratings across the 8 minute-interval and compared the resulting averages between conditions. To investigate whether tACS influenced pain intensity at any time during the 8 minutes of thermal stimulation, pain rating

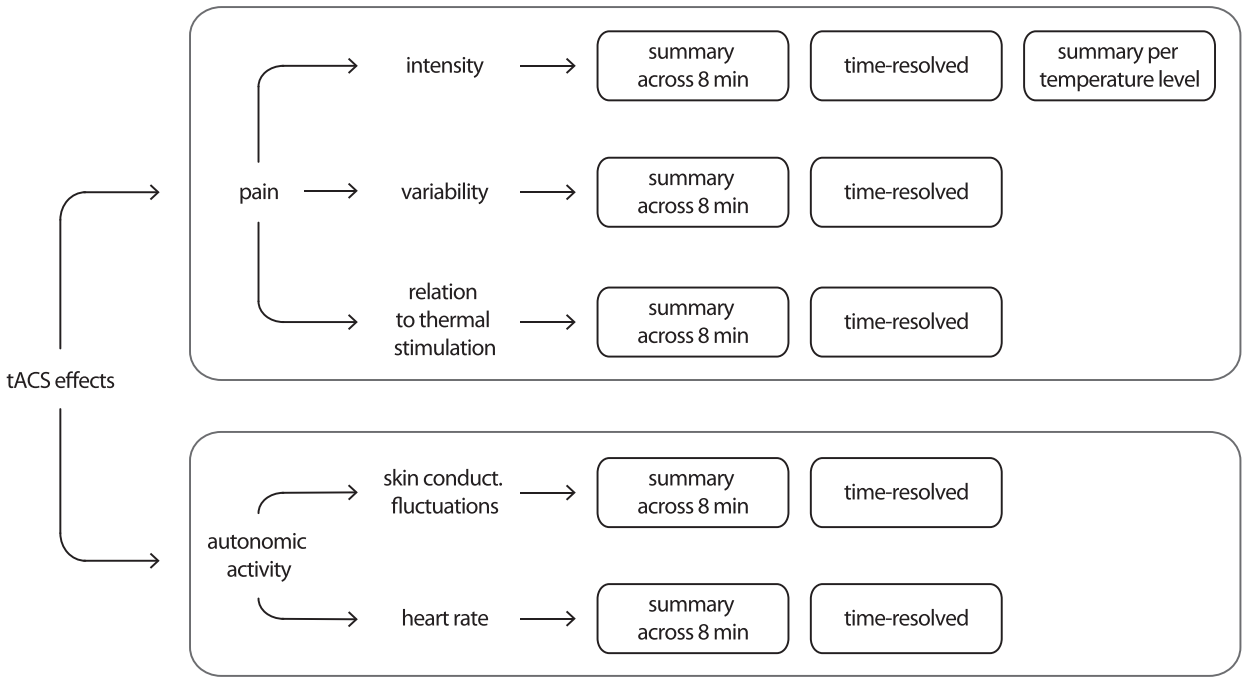

Figure 2. Analysis pipeline. The current study investigated effects of tACS on pain and pain-related autonomic activity. tACS effects on pain were investigated with respect to the intensity of pain, the variability of pain, and the relationship of pain to thermal stimulation. All variables were analyzed across the entire 8-minute analysis interval (summary measures), in a time-resolved fashion, as well as per temperature level in the case of pain intensity. tACS effects on autonomic activity were investigated using the number of skin conductance fluctuations and the heart rate. Again, variables were analyzed across the entire 8-minute analysis window (summary measures) and in a time-resolved fashion. To detect tACS effects, all variables were compared between the 3 tACS conditions (alpha, gamma, sham) separately for both tACS locations (PFC, S1). tACS, transcranial alternating current stimulation.

time courses were compared between alpha, gamma, and sham conditions in a time-resolved fashion. Lastly, we asked whether tACS might selectively alter pain intensity at certain temperature levels and compared the average pain intensity separately for low, medium, and high temperature levels.

Since tACS might also influence the stability of pain ratings or the translation of noxious stimuli into pain rather than pain directly, we next examined tACS effects on pain variability and the relation of pain to thermal stimulation. To this end, we first obtained summary measures across the 8 minute-time course. For *pain variability*, the standard deviation of pain ratings was calculated across the entire time course. For the *relation of pain and thermal stimulation*, Pearson correlations between pain ratings and temperature were calculated. For additional time-resolved analyses, time courses of both measures were calculated by applying a sliding-window approach to the 8 minute-pain rating and -temperature time courses using a window size of 60 s and a step size of 10 s.[37] Subsequently, the standard deviation of pain ratings and Pearson correlations between pain ratings and temperature were calculated for each window.

tACS Effects on Autonomic Activity

Next, we investigated whether tACS modulated pain-related responses of the autonomic nervous system, which are partially independent of pain perception.[37] Based on skin conductance and ECG recordings, the *number of spontaneous skin conductance fluctuations (nSF)* and the *heart rate (HR)* were computed and analyzed.

To obtain the nSF, skin conductance data were visually inspected for movement artifacts, low-pass filtered at 1 Hz using a fourth-order Butterworth filter, and downsampled to 500 Hz. Subsequently, a sliding-window approach with a window length of 60 s and a step size of 10 s was applied to the preprocessed skin conductance time series to obtain nSF time courses.[37] For every window, the nSF was determined by counting spontaneous fluctuations exceeding an amplitude criterion of 0.05 μS with respect to the preceding trough. Windows contaminated with movement artifacts were discarded. To obtain the HR, ECG data were downsampled to 500 Hz and preprocessed using the Matlab toolbox PsPM, version 4.0.2 (bachlab.org/pspm). In PsPM, QRS complexes were detected and a continuous HR time series with a sampling frequency of 500 Hz was created by linearly interpolating the RR interval tachogram. Subsequently, the same sliding window approach used for skin conductance data was applied and the average HR was calculated for every window to obtain HR time courses.[38]

Then, tACS effects on the overall strength of autonomic activity were investigated by comparing summary measures obtained by averaging nSF and HR across the entire 8 minute-time course between tACS conditions. Additionally, a time-resolved analysis was performed by comparing time courses of both measures across conditions.

tACS Effects on Brain Activity

We further investigated whether tACS induced neuronal changes outlasting the stimulation (offline effects). To this end, EEG data obtained before and after tACS were downsampled to 250 Hz. A visual artifact correction was performed, manually rejecting data segments contaminated by muscle activity. All analyses focused on the electrode contralateral to the stimulated hand, that is, F4 for the PFC and CP4 for the S1 electrode montage. In addition, the 60 s data segments closest to tACS were selected, that is, the last minute of the 5 minute pre-stimulation EEG and the first minute of the 5 minutes post-stimulation EEG. Data were cut into 1 s epochs with 50 % overlap and frequency specific power between 1 and 100 Hz was calculated using a Fast Fourier Transformation with a Hanning window resulting in a frequency resolution of 1 Hz. Subsequently, power spectra were averaged across all epochs for pre- and post-stimulation EEGs separately. Pre-stimulation power spectra were then subtracted from post-stimulation power spectra for each of the 6 conditions and each participant individually. During statistical analyses, these difference power spectra were compared between the active tACS conditions (PFC/S1 alpha/gamma stimulation) and the respective sham conditions (see below). In addition, we performed several control analyses. First, we repeated the same analysis calculating difference power spectra based on the complete 5 minutes rather the last and first 1 minute pre- and post-EEG data. A second control analysis used average power spectra across both prefrontal electrodes rather than the contralateral prefrontal electrode since prefrontal activations during pain do not show a clear lateralization.[35,38,51] Third, we log-transformed power spectra before statistical contrasts to account for the non-Gaussian distribution of EEG data. Finally, we also checked for potential tACS effects in the non-targeted frequency band by performing contrasts of gamma power spectra in the alpha tACS conditions and contrasts of alpha power spectra in the gamma tACS conditions. Alpha and gamma oscillations are thought to reflect complementary inhibitory and excitatory feedback processing, respectively.[19] Thus, it is conceivable that tACS targeting one frequency band might alter oscillatory activity in the other, non-targeted frequency band.

Statistical Analyses

Statistical analyses were performed using Matlab (Mathworks, Natick, MA), the Matlab toolbox Fieldtrip,[40] IBM SPSS Statistics for Windows (SPSS), version 26 (IBM Corp., Armonk, NY), and the statistical software package JASP, version 0.11.1 (JASP Team, 2019). Since Shapiro-Wilk tests indicated that some variables were not normally distributed, non-parametric tests were used for statistical analysis. These included Cochran's Q-tests, Friedman tests, and non-parametric cluster-based permutation statistics.[33,34] Post hoc tests with Bonferroni correction were conducted when necessary and included McNemar tests for Cochran's Q-tests and

Wilcoxon matched-pairs signed rank tests for Friedman tests. Cluster-based permutation tests based on F-tests were followed up by pairwise post hoc cluster-based permutation tests based on t-tests. Additionally, Bayesian RM ANOVAs were performed to complement analyses relying on null-hypothesis significance testing.[8] They were followed up by post hoc Bayesian dependent samples t-tests for analyses yielding conclusive evidence for the alternative hypothesis or inconclusive evidence.

Blinding

The blinding of participants was examined using a Cochran's Q-test for question 1, which compared the frequency of yes responses across all 6 experimental conditions. VAS scores from question 2 and 3, which addressed the intensity of skin sensations and phosphenes, respectively, were investigated using Friedman tests. To investigate whether skin sensations and/or phosphenes differed between tACS locations, data from all frequency conditions were aggregated for each location and then compared using a Friedman test with the within-subjects factor location (PFC, S1). To investigate whether skin sensations and/or phosphenes differed between tACS frequencies, data from the 3 frequency conditions (alpha, gamma, sham) were compared using a Friedman test with the within-subjects factor frequency for both locations separately. All subsequent analyses were conducted separately for the PFC and S1 location as blinding questionnaires indicated that participants were successfully blinded for the S1 but not for the PFC location.

tACS Effects on Pain, Autonomic Activity, and Brain Activity

Friedman tests with the factor frequency (alpha, gamma, sham) were used to compare summary measures of pain intensity, pain variability, the relation of pain to thermal stimulation as well as nSF and HR. They were also used to investigate summary measures of pain intensity for each temperature level.

Cluster-based permutation statistics clustering across time were used to investigate tACS effects on time courses of pain intensity, pain variability, and the relation of pain to thermal stimulation, as well as time courses of nSF and HR. Specifically, time courses were compared between alpha, gamma, and sham conditions using cluster-based permutation statistics based on dependent samples F-tests, clustering across time.

Cluster-based permutation statistics clustering across frequencies were used to investigate tACS offline effects on brain activity power spectra. To this end, pre- and post-stimulation difference power spectra from the electrode contralateral to thermal stimulation were compared between the active tACS conditions and the respective sham conditions using non-parametric cluster-based permutation statistics based on dependent samples t-tests, clustering effects across frequencies. Specifically, PFC alpha and gamma conditions were compared to the PFC sham condition and S1 alpha and gamma conditions were compared to the S1 sham condition resulting in 4 pairwise comparisons. When investigating the effect of alpha frequency stimulation, this analysis was applied to a frequency band from 8 to 12 Hz. When investigating gamma frequency stimulation, the frequency band was 70 to 90 Hz.

To control for multiple comparisons, all *P* values were subjected to false discovery rate (FDR) control of Type I error.[7] Corrections were conducted separately for pain, autonomic activity, and brain activity considering the number of all statistical analyses performed for the respective measure (see Fig 2 for an overview for pain and autonomic activity). This resulted in an FDR control for 14 statistical tests (7 tests x 2 tACS locations) for pain ratings, an FDR control for 8 statistical tests (2 tests x 2 tACS locations x 2 autonomic measures) for autonomic activity, and an FDR control for 4 statistical tests (2 tests x 2 tACS locations) for brain activity. Throughout the manuscript, corrected *P* values are reported. Uncorrected *P* values for all analyses are summarized in Supplementary Table S1. If not stated otherwise, statistical tests were performed 2-sided with a significance level (α) of $P < .05$.

Analyses relying on null-hypothesis significance testing were complemented by Bayesian RM ANOVAs. The Bayesian approach to hypothesis testing considers the likelihood of the observed data under the null and the alternative hypothesis. The comparison of the resulting probabilities is reflected by the Bayes Factor (BF_{01} = likelihood of the data given the H0/likelihood of the data given the H1).[8,26] Thus, Bayes factors allow to specifically evaluate evidence in favor of the null hypothesis. Bayesian RM ANOVAs were performed for the pain intensity summary measure as well as the average nSF and HR across 8 minutes. As before, the analyses included the factor frequency (alpha, gamma, sham) and were conducted separately for both tACS locations. For all effects, JASP default prior options were chosen.

Effects as a Function of Responsiveness to tACS

To further investigate potential tACS effects for those participants presumably responding best to the brain stimulation, we first repeated pain and autonomic activity analyses for 2 subgroups.

In a *responder analysis*, analyses were performed for a subgroup of participants showing the strongest evidence for frequency specific tACS offline effects on brain activity quantified by calculating individual alpha and gamma responder ratios. These were based on EEG data from the last minute pre-stimulation and the first minute post-stimulation. To this end, EEG data from the electrode contralateral to stimulation were cut into 1 s-epochs with 50 % overlap, power spectra were calculated using a Fast Fourier Transformation and a Hanning window and averaged across all epochs. To investigate alpha frequency stimulation effects, power values were averaged between 8 and 12 Hz. To investigate gamma frequency effects, power values were averaged between 70 and 90 Hz. In line with previous work,[13] the

ratio between post- and pre-EEG power values was then calculated for the active tACS conditions (alpha, gamma) and normalized by the ratio derived for the respective sham condition:

$$responder\ ratio = \frac{\left(\frac{active_{post}}{active_{pre}}\right)}{\left(\frac{sham_{post}}{sham_{pre}}\right)} * 100$$

These calculations were performed separately for the alpha and gamma conditions and the PFC and S1 electrodes, resulting in 4 responder ratios per participant. Participants with responder ratios above 100 were classified as responders.[13] Overall, 15 of 29 participants were classified as PFC alpha responders, 18 as PFC gamma responders, 18 as S1 alpha responders, and 13 as S1 gamma responders, resulting in 4 condition- and frequency-specific subgroups.

In a *peak frequency informed analysis*, a selection was made based on the proximity of the stimulation frequency to the frequency of endogenous oscillations in the stimulated brain area, since this proximity can influence the degree of neural entrainment induced by tACS.[62] As power spectra displayed clear peaks of endogenous oscillations at alpha frequencies over S1 exclusively, this analysis was only applied to the S1 alpha condition. Specifically, analyses were performed for a subgroup of participants whose individual alpha peak frequency (IAF) was closest to the 10 Hz-stimulation frequency. To estimate IAFs, the entire 5 minutes, artifact-cleaned pre-stimulation EEG from the S1 alpha condition was cut into 10 s epochs with 50 % overlap, calculating power spectra using Fast Fourier Transformation and Hanning windows. A longer epoch length of 10 s was chosen to increase the frequency resolution to 0.1 Hz. Based on the averaged power spectra, the IAF was defined for each participant as the local power maximum in the frequency range between 8 and 13 Hz. Subsequently, peaks were visually controlled and corrected whenever reasonable. Following this procedure, IAF peaks could be identified for 26 of 29 participants. Next, the 50 % of participants whose IAF was closest to the stimulation frequency of 10 Hz were selected, resulting in a subgroup of 13 participants.

Pain and autonomic activity analyses outlined above were repeated for the 2 subgroups with slight adjustments. First, 3-way comparisons were replaced by 2-way comparisons (e.g., S1 alpha vs. S1 sham instead of S1 alpha vs. S1 gamma vs. S1 sham) because the number of responders differed between stimulation conditions (e.g., $responders_{S1alpha} \neq responders_{S1gamma}$) and IAFs were determined for the S1 alpha condition only. Thus, cluster-based permutation statistics were based on dependent sample t-tests instead of F-tests and the within-subjects factor stimulation was reduced from 3 to 2 levels for all tests. Second, for the peak frequency informed analysis, analyses were repeated for the S1 alpha frequency condition only, contrasting effects in the S1 alpha frequency condition to those obtained in the S1 sham condition. Third, the applied FDR correction was adjusted to the number of statistical tests conducted. For pain ratings, this resulted in an FDR control for 28 tests (14 tests x 2 tACS locations) and for 9 tests (9 tests x 1 tACS locations) in the responder and peak frequency informed analysis, respectively. For autonomic activity, an FDR control for 16 tests (4 tests x 2 tACS locations x 2 autonomic measures) and for 4 tests (2 tests x 1 tACS location x 2 autonomic measures) was applied.

Lastly, we determined the relationship between the responsiveness of the EEG to tACS and main outcome measures across the *entire sample*. To this end, we quantified effects of the active tACS conditions (alpha, gamma) relative to the sham conditions for all pain and autonomic summary measures (Fig 2: pain intensity, variability, relation to thermal stimulation, nSF, and HR) using a modulation index[1]:

$$modulation\ index = \frac{(active - sham)}{(active + sham)}$$

Using Spearman correlations across all subjects, modulation indices were then correlated with responder ratios for each of the 4 active tACS conditions. In addition, modulation indices were related to individual S1 alpha peak frequencies for the S1 alpha condition. FDR correction was applied across all correlations involving pain ratings and autonomic measures, resulting in a correction for 15 and 10 tests, respectively.

Data and Code Availability

All data and code related to this manuscript are available at https://osf.io/pnd6g/.

Results

To investigate whether tACS can modulate pain, participants took part in 6 recording sessions. During each session, 1 mA peak-to-peak tACS was applied at 1 of 2 locations (over PFC or S1) and at 1 of 3 frequencies (alpha [10 Hz], gamma [80 Hz], sham). Concurrently, a tonic heat pain stimulus of varying intensity was applied to the left hand.

Participants were Blinded for tACS Over S1, but not Over PFC

After each session, the blinding of participants was assessed using questionnaires (Supplementary Fig S2). When asked whether a continuous stimulation was applied or not, participants' reports did not differ between tACS frequencies ($\chi^2(5) = 7.46$, $P = .189$). Likewise, skin sensations did neither differ between tACS locations ($\chi^2(1) = .75$, $P = .385$) nor between tACS frequencies for either of the locations (PFC: $\chi^2(2) = 2.11$, $P = .348$; S1: $\chi^2(2) = 0.75$, $P = .688$). However, phosphenes were stronger for tACS over PFC than over S1 ($\chi^2(1) = 7.23$, $P = .007$). In addition, phosphenes differed between frequencies for tACS over PFC, but not over S1 (PFC: $\chi^2(2) = 8.90$, $P = .012$; S1: $\chi^2(2) = 0.19$, $P = .910$). Post hoc tests showed that phosphenes were significantly

stronger in the PFC alpha condition than in the PFC gamma condition ($Z = -3.13$, $P = .006$). Hence, participants were successfully blinded for tACS over S1 but not for tACS over PFC. Thus, all further analyses investigated tACS effects separately for PFC and S1 locations.

tACS did not Modulate Pain

We first investigated whether tACS influenced pain intensity averaged across the entire 8 minutes of thermal stimulation. To this end, we compared average pain intensity during alpha, gamma, and sham tACS for both locations (Fig 3). The results did not show any statistically significant difference, neither during tACS over PFC nor during tACS over S1 ($P > .05$ for all tests; see Supplementary Table S1 for test statistics and uncorrected P values of all pain analyses). We further assessed whether tACS influenced pain intensity at any time during the 8 minutes of thermal stimulation. To this end, we compared pain intensity time courses during alpha, gamma, and sham stimulation for both tACS locations (Fig 3). For both PFC and S1, cluster-based permutation tests did not show significant differences in pain intensity at any time ($P > .05$ for all clusters). We further asked whether tACS might selectively alter pain intensity at certain temperature levels. For instance, tACS might particularly modulate pain at the lowest level at which pain ratings are closest to pain threshold and possibly most uncertain. We therefore compared the average pain intensity separately for low, medium, and high temperature levels (Supplementary Fig S3). However, no significant tACS effects on pain intensity were found for any temperature level ($P > .05$ for all tests). Lastly, we asked whether tACS might influence the stability of pain ratings or the translation of noxious stimuli into pain rather than pain intensity directly. To this end, we investigated whether tACS influenced the variability of pain or the relation of pain to thermal stimulation (Fig 4). Comparisons of summary measures across 8 minutes did not yield significant tACS effects on pain variability or the relationship of pain to thermal stimulation ($P > .05$ for all tests). Likewise, time-resolved analyses of both measures did not show any significant tACS effects at any time ($P > .05$ for all clusters). Taken together, we did not find tACS effects on different measures of tonic pain.

Next, we used Bayesian statistics to evaluate direct evidence for a lack of tACS effects on pain intensity. We specifically performed Bayesian RM ANOVAs with the factor tACS frequency (alpha, gamma, sham) for both PFC and S1 locations. These analyses resulted in a Bayes factor (BF_{01}) which quantifies the relative likelihood of the data given the null hypothesis of no tACS effect over the alternative hypothesis postulating tACS effects. BF_{01} values below 0.33 are commonly classified as evidence for the alternative hypothesis, values from 0.33 to 3 are classified as inconclusive evidence, and values above 3 are classified as evidence for the null hypothesis.[27] The analysis of pain intensity resulted in a BF_{01} of 5.727 for the PFC and a BF_{01} of 2.082 for the S1 location, indicating that evidence for the null hypothesis was

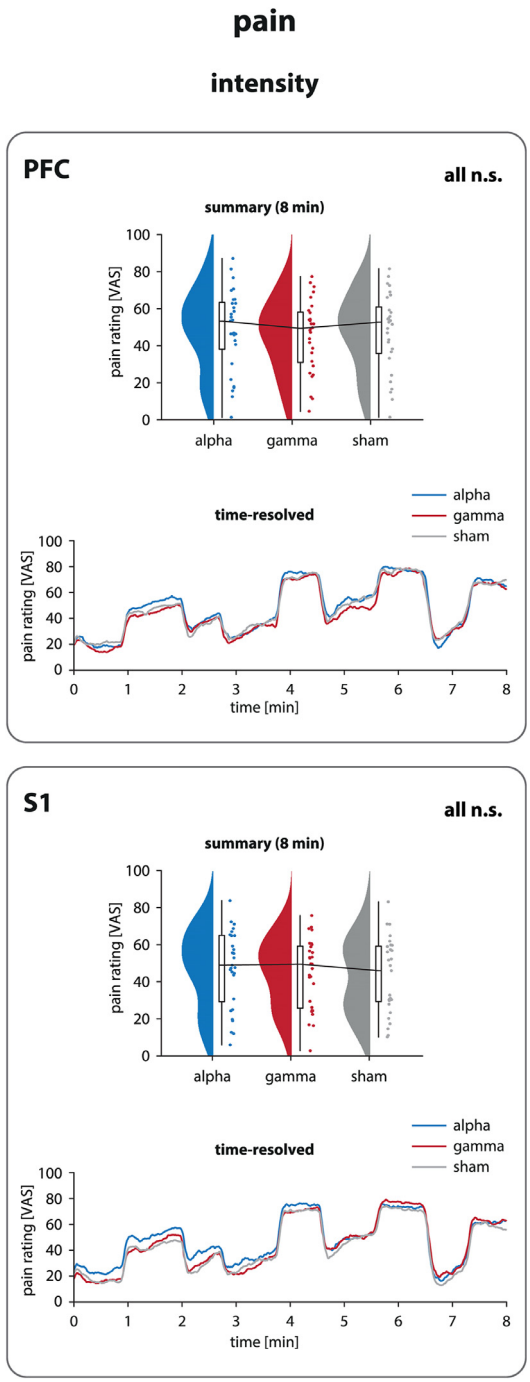

Figure 3. tACS effects on pain intensity. tACS effects on pain intensity are shown separately for PFC (upper panel) and S1 (lower panel) tACS locations. For both locations, upper rows display summary measures obtained by averaging pain ratings (0–100; VAS) across the 8-minute analysis window. Raincloud plots[2] show un-mirrored violin plots displaying the probability density function of the data, boxplots, and individual data points. Boxplots depict the sample median as well as first (Q1) and third quartiles (Q3). Whiskers extend from Q1 to the smallest value within Q1 - 1.5* interquartile range (IQR) and from Q3 to the largest values within Q3 + 1.5* IQR. Lower rows depict time-resolved analyses of pain rating time courses in the alpha, gamma, and sham tACS conditions. None of the analyses revealed significant differences between alpha, gamma, and sham stimulation indicating no tACS effects on the perceived pain intensity ($N = 29$; $PFC_{summary}$: $P = .885$, $PFC_{time\text{-}resolved}$: no cluster found, $S1_{summary}$: $P = .864$, $S1_{time\text{-}resolved}$: $P = .857$; Friedman tests and cluster-based permutation statistics; FDR-corrected P values). n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex; VAS, visual analogue scale.

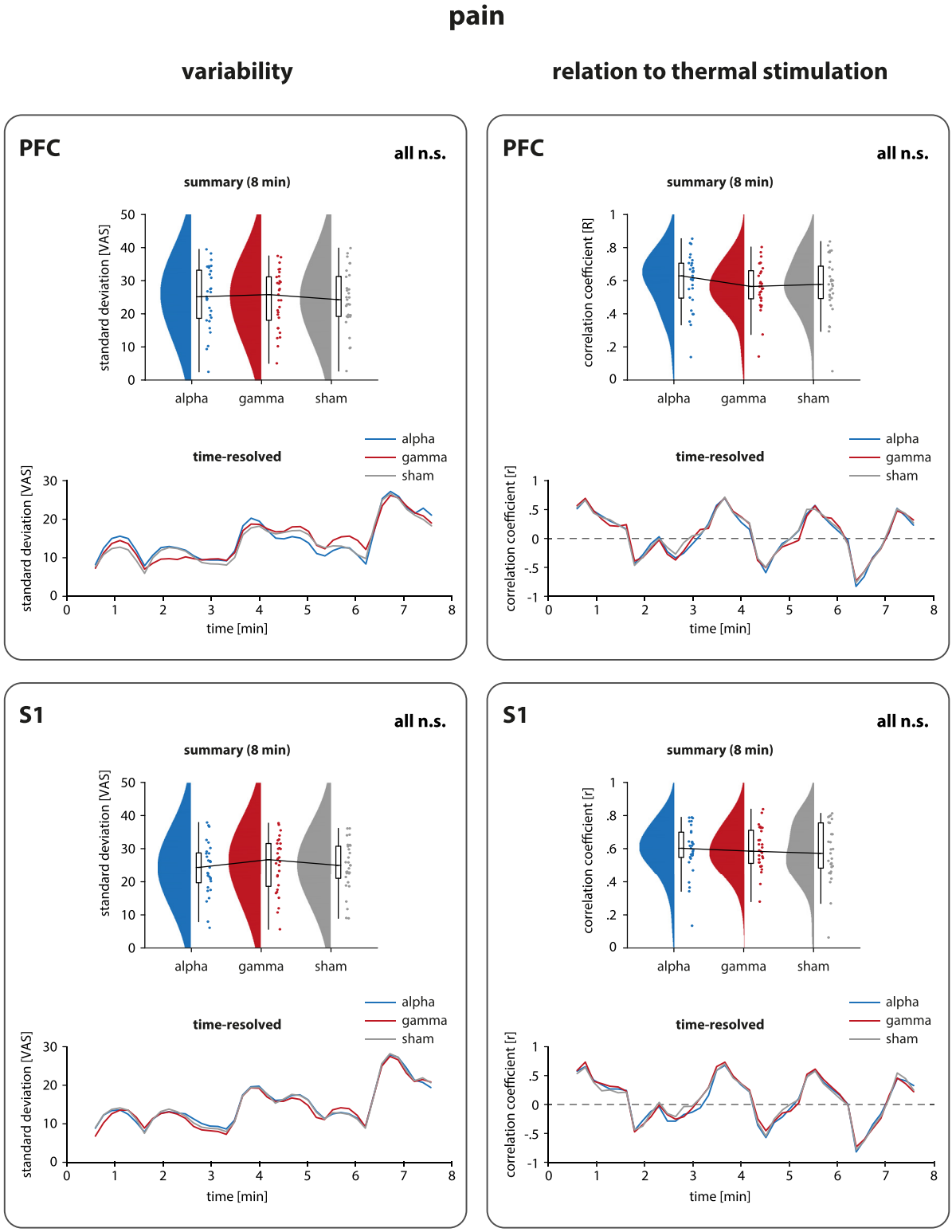

Figure 4. tACS effects on pain variability and the relation of pain to thermal stimulation. In line with Figure 3, tACS effects on pain variability and the relation of pain to thermal stimulation are shown separately for PFC and S1 tACS locations. Results of the analyses based on summary measures obtained across the 8-minute analysis window as well as results of the time-resolved analyses are shown. None of the analyses revealed significant differences between alpha, gamma, and sham stimulation, indicating no tACS effects on the stability of pain ratings or the translation of the noxious stimulus into pain (N = 29; pain variability: $PFC_{summary}$: $P = .864$, $PFC_{time\text{-}resolved}$: $P = .857$, $S1_{summary}$: $P = .857$, $S1_{time\text{-}resolved}$: $P = .864$; relation of pain to thermal stimulation: $PFC_{summary}$: $P = .943$, $PFC_{time\text{-}resolved}$: $P = .857$, $S1_{summary}$: $P = .943$, $S1_{time\text{-}resolved}$: $P = .857$; Friedman tests and cluster-based permutation statistics; FDR-corrected P values). n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex; VAS, visual analogue scale.

moderate for the PFC but inconclusive for the S1 location. To follow up the inconclusive result for tACS over S1, we performed pairwise comparisons between tACS frequencies (alpha, gamma, sham) using Bayesian dependent samples t-tests. These revealed moderate evidence for the null hypothesis when comparing the gamma and sham conditions ($BF_{01\ gamma \neq sham} = 3.893$) but inconclusive evidence for both comparisons entailing the alpha condition ($BF_{01\ alpha \neq sham} = 0.935$; $BF_{01\ alpha \neq gamma} = 2.491$).

Taken together, frequentist statistical analyses did not provide evidence for a modulation of tonic pain by tACS at alpha or gamma frequencies over PFC or *S1*. Bayesian analyses provided moderate evidence for a lack of tACS effects on tonic pain except for alpha tACS over S1 where evidence was inconclusive.

tACS did not Modulate Pain-Related Autonomic Activity

We further examined whether tACS influenced pain-related activity of the autonomic nervous system. Such autonomic responses to noxious stimuli are partially independent of pain perception.[37] Moreover, autonomic responses are mediated by different brain mechanisms than pain perception.[57] To this end, we analyzed tACS effects on nSF and HR (Fig 5). We did not find any tACS effects on these autonomic measures neither when summary measures across 8 minutes, nor when time courses were compared between tACS conditions (Fig 5, $P > .05$ for all analyses; see Supplementary Table S1 for all test statistics and uncorrected *P* values).

In addition, we applied Bayesian RM ANOVAs to evaluate direct evidence for a lack of tACS effects on summary measures of nSF and HR. Analyses of both measures provided moderate evidence for the null hypothesis for both the PFC and the S1 location (nSF: $BF_{01\ PFC} = 3.88$, $BF_{01\ S1} = 4.36$; HR: BF_{01} PFC = 6.59, BF_{01} S1 = 8.95).

Taken together, we did not find significant tACS effects on autonomic activity during tonic pain. Bayesian analyses provided moderate evidence for a lack of tACS effects on pain-related activity of the autonomic nervous system.

tACS did not Yield Outlasting Effects on Brain Activity

We next investigated whether tACS induced neuronal changes outlasting the stimulation (offline effects) as a potential indicator of the neural efficacy of our tACS protocol. To this end, we calculated power spectra of EEG activity during the last minute before and the first minute after stimulation (Fig 6). We further calculated post − pre difference power spectra of the electrode contralateral to the thermal stimulation and compared them between the active tACS conditions (PFC/S1 alpha/gamma stimulation) and the respective sham conditions. Cluster-based permutation statistics did not show any significant clusters for tACS over PFC or S1 in the targeted frequency bands ($P > .05$ for all clusters, 1-sided). Control analyses including the entire 5 minutes pre- and post-EEG data for power spectra calculation, using both prefrontal electrodes, and log-transforming power spectra before contrasts confirmed this finding ($P > .05$ for all clusters, 1-sided). Likewise, no tACS effects in the non-targeted frequency bands were observed ($P > .05$ for all clusters, 1-sided). Hence, tACS did not evoke effects on brain activity outlasting the stimulation.

tACS did not Influence Pain and Autonomic Activity When Taking the Responsiveness to tACS and Individual Peak Alpha Frequencies into Account

To enhance our sensitivity, we investigated whether tACS influenced pain or autonomic activity in selected participants who might have responded particularly strongly to tACS. We therefore performed two subgroup analyses. First, although the group-level analysis did not show tACS effects on brain activity, we tested for tACS effects in those participants who showed the highest frequency-specific post/pre ratio of brain activity (*responders*). Repeating the whole-group analyses for these subgroups did not reveal any tACS effects on pain or autonomic activity ($P > .05$ for all analyses). Second, we investigated tACS effects in those 50 % of participants whose individual alpha peak frequency was closest to the 10 Hz tACS alpha stimulation. As the individual alpha peak frequency can be reliably identified over S1 only, this analysis was only performed for the S1 alpha and S1 sham condition. Repeating the whole group analyses for this subgroup did not reveal any tACS effects on tonic pain or autonomic activity ($P > .05$ for all analyses). Lastly, we examined the relationship between the responsiveness of the EEG to tACS on the one hand and pain and autonomic data on the other hand. We did not observe significant relationships between responder ratios or S1 alpha peak frequencies and modulation indices quantifying the degree of tACS effects on summary measures of pain and autonomic data ($P > .05$ for all correlations; data not shown).

Discussion

The current study systematically explored whether tACS can modulate pain and pain-related autonomic activity in healthy human participants using a tonic heat pain paradigm. In 6 recording sessions, participants received tACS over PFC or S1 using alpha, gamma, or sham stimulation while pain ratings and autonomic responses were collected. Analyses showed that, using the current setup, tACS did not modulate the perceived pain intensity, the stability of pain ratings or the translation of the noxious stimulus into pain. Likewise, tACS did not influence autonomic responses. Bayesian statistics further supported a lack of tACS effects in most conditions including prefrontal and gamma tACS. The only

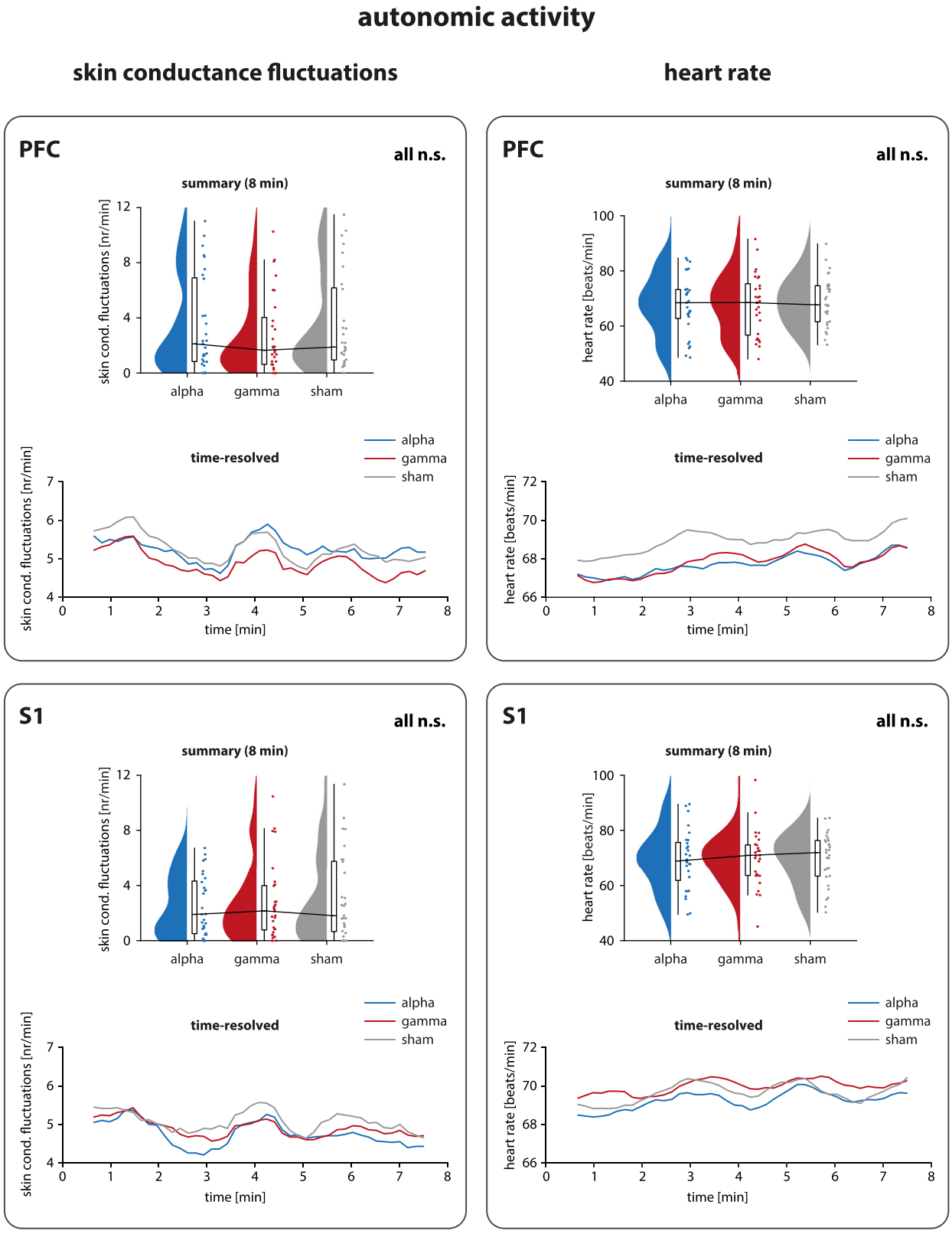

Figure 5. tACS effects on autonomic activity. In line with Fig 3, tACS effects on the number of skin conductance fluctuations and the heart rate are shown separately for PFC and S1 locations. Results of the analyses based on summary measures obtained across the 8-minute analysis window as well as results of the time-resolved analyses are shown. Due to the chosen windowing approach with a window length of 1 minute and a step size of 10 s, time-courses of autonomic data span 7 minutes only and are depicted from 0.5 to 7.5 minutes. None of the analyses revealed significant differences between alpha, gamma, and sham stimulation, indicating no tACS effects on pain-related autonomic activity (N = 29; number of skin conductance fluctuations: $PFC_{summary}$: $P = 1.0$, $PFC_{time\text{-}resolved}$: no cluster found, $S1_{summary}$: $P = 1.0$, $S1_{time\text{-}resolved}$: no cluster found; heart rate: $PFC_{summary}$: $P = 1.0$, $PFC_{time\text{-}resolved}$: no cluster found, $S1_{summary}$: $P = 1.0$, $S1_{time\text{-}resolved}$: no cluster found; Friedman tests and cluster-based permutation statistics; FDR-corrected P values). n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex.

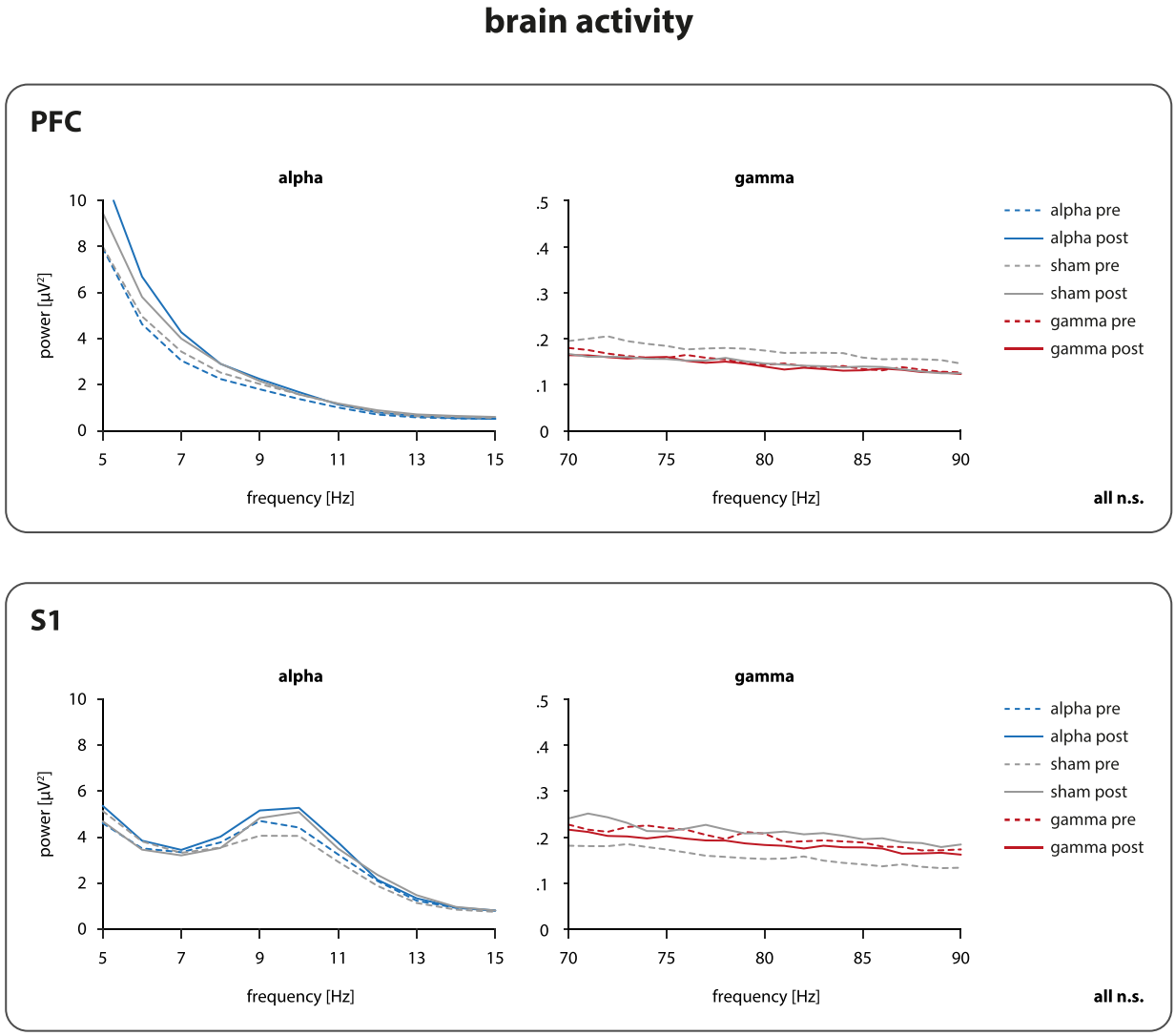

Figure 6. tACS effects on brain activity. Power spectra of pre- (dashed lined; based on last minute of the recording) and post-stimulation EEGs (solid line; based on first minute of the recording) are shown separately for PFC (upper panel) and S1 (lower panel) locations. Left and right plots display power spectra for alpha and gamma frequency bands, respectively. For statistical analysis, pre-stimulation power spectra were subtracted from post-stimulation power spectra for each of the 6 conditions and each participant individually (not shown here). Subsequently, the resulting difference power spectra were compared between the active tACS conditions and the respective sham conditions (PFC_{alpha} vs PFC_{sham}, PFC_{gamma} vs PFC_{sham}, $S1_{alpha}$ vs $S1_{sham}$, $S1_{gamma}$ vs $S1_{sham}$). Analyses did not reveal significant power increases in the targeted frequency bands indicating that tACS did not evoke effects on brain activity outlasting the stimulation (N = 29; PFC_{alpha}: no cluster found, PFC_{gamma}: no cluster found, $S1_{alpha}$: $P = .240$, $S1_{gamma}$: no cluster found; Cluster-based permutation statistics; 1-sided FDR-corrected P values). n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex.

exception was alpha tACS over S1 where evidence for tACS effects on tonic pain intensity was inconclusive.

The present study complements two previous tACS studies in the context of pain.[1,5] Both studies applied 10 Hz alpha stimulation targeting somatosensory areas, which has also been done in the current study. One study indicated an analgesic effect of tACS on the perceived pain intensity induced by brief experimental stimuli but only when pain intensity was uncertain.[5] The other study applied tACS to chronic back pain patients and primarily showed that tACS-induced changes of alpha activity correlated with pain intensity.[1] In addition, exploratory analyses of autonomic activity recorded in this study indicated that HR variability was increased.[46] Thus, these studies revealed tentative evidence for an effect of somatosensory alpha tACS on pain intensity. Our study extends these findings in two important aspects. First, we not only targeted somatosensory alpha oscillations but systematically assessed tACS effects at alpha and gamma frequencies over somatosensory and prefrontal brain areas. Thereby, our design for the first time tested potential analgesic effects of tACS at a new location and frequency. Second, we employed a tonic heat pain paradigm, which models at least some aspects of chronic pain. This allows to investigate new stimulation parameters and to identify the most promising ones in a healthy sample before moving on to a clinical cohort. In addition, our study followed a careful design with strong methodological rigor. Sample size was based on a priori sample size calculation. Targets and frequencies of tACS were clearly motivated by previous studies on the role of neural

oscillations in the processing of pain.[35,38,41,51] Sham conditions controlled for unspecific tACS effects. After each session, blinding was assessed using post-stimulation questionnaires. Extensive analyses of pain and autonomic activity were performed and adequately corrected for multiple comparisons. Finally, analyses were complemented by Bayesian statistics to strengthen the interpretability of negative findings.[8] However, using our stimulation protocol, we did not find significant tACS effects on tonic experimental pain in healthy human participants. Although some methodological differences like the tACS montage, duration, or intensity might have played a role, our findings in general complement the rather weak tACS effects on pain reported so far.[1,5,46]

The lack of tACS effects on pain might be due to different factors. First, the tACS parameters used in the present study might not have been optimal. To ensure that a tACS paradigm is able to modulate neural oscillations, simultaneous EEG recordings are desirable. However, such simultaneous EEG recordings are heavily contaminated by tACS-induced artifacts.[62] In the present study, we therefore performed EEG recordings immediately after tACS. The results did not show significant changes of EEG activity after tACS. However, as EEG after-effects are not consistently observed,[36,61] their lack does not preclude tACS effects during stimulation. It is nevertheless possible that the tACS parameters of the present study were not optimal for modulating pain. For instance, our fixed 2-electrode-setup (without a third return electrode) might have put the left and right hemisphere into anti-phase synchrony, which might not be ideal. Related, our electrode montages potentially caused stimulation of brain areas beyond the targeted areas (see Supplementary Fig S1 for simulations of field strength throughout the brain), which might confound results. Moreover, a stimulation intensity of 1 m A peak-to-peak might not have been sufficient. However, a range of previous studies did find effects using this intensity[3,5,6,21,22] and choosing a stimulation intensity always represents a trade-of between potential effect size and successful blinding of participants.

Second, our tACS setup might in principle be able to modulate neural oscillations and pain but with a different pain paradigm than the one chosen here. The tonic pain stimulus we applied evokes strong decreases in alpha oscillations in early somatosensory areas,[38,51] which, together with previous tACS studies, led us to target S1 alpha oscillations using tACS. However, in addition to the frequency of the targeted oscillations, neuronal entrainment by tACS also depends on their amplitude.[48,53] Consequently, tasks which strongly suppress oscillations might be less susceptible for modulations using tACS. Future studies might therefore use other pain paradigms which yield weaker and/or shorter suppressions of oscillations.

Third, while our tonic heat pain paradigm models some aspects of a sustained pain experience,[31] tonic pain is not identical with chronic pain. It is conceivable that tACS may not modulate pain in healthy participants but only in patients when altered neural circuits can be restored, for example by reversing a slowing of the dominant frequency.[49]

Fourth, we cannot rule out carry-over effects of stimulation from one session to the next. After-effects of tACS have been shown up to 70 minutes after stimulation.[29] Longer-lasting effects have not been shown so far.[23,29,59] However, tACS effects might occur even later than 24 hours after stimulation and might have influenced the present findings.

Lastly, it is still debated by some researchers whether currents applied in human low-intensity tACS studies are sufficiently strong to pass through the skull and modulate brain activity.[32,60] However, behavioral and neural evidence for the effectiveness of tACS is continuously growing.[9,62]

In conclusion, our findings do not provide evidence that tACS can modulate tonic experimental pain in healthy human participants using the current stimulation protocol. Thus, future studies investigating analgesic effects of tACS should aim to optimize the experimental setup. For instance, we chose standardized tACS locations and stimulation intensities with the goal of a broad clinical usability in mind. However, individualized tACS parameters might be more effective. Future studies might for example consider individual anatomical scans for current simulations as well as the individual peak frequency of the targeted neural oscillations to optimize electrode placement and stimulation frequency for every individual.[16,30] In addition, presumably more focal, high-definition electrode montages might enhance effects.[9] Moreover, increasing tACS intensity, duration, and/or performing repeated stimulation sessions might increase its neural efficacy. With respect to gamma oscillations, transcranial random noise stimulation[4] rather than sinusoidal stimulation at a specific frequency might be more effective due to the broad-band, burst-like nature of neuronal gamma activity.[58] Beyond, individual characteristics like placebo/nocebo expectations or a person's suggestibility[14,17] might modulate stimulation effects and could be incorporated in further studies. Considering the urgent need for novel pain treatments, the conceptual plausibility, and potentially broad clinical applicability of tACS to modulate pain, and the relative lack of alternatives, we feel that such follow-up studies are warranted. However, just like any single chronic pain treatment approach, tACS will likely not represent a standalone treatment but rather a valuable part of a combined bio-psycho-social treatment approach. Based on the present and previous studies,[1,5,46] alpha oscillations in somatosensory areas remain the most promising target for future tACS studies.

Supplementary data

Supplementary data related to this article can be found at 10.1016/j.jpain.2021.03.150.

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