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Effects of transcranial alternating current stimulation in the theta frequency on associative memory consolidation

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Lekensamenvatting

De aanleiding van deze scriptie is de mogelijke toepassing van elektrische wisselstroom stimulatie bij trauma-behandeling gericht op exposure. Bij een exposure-therapiesessie wordt men blootgesteld aan traumatische herinneringen in een veilige omgeving. De mate waarin een traumareactie wordt uitgedoofd hangt af van een geheugenproces in de hersenen. Elektrische wisselstroom stimulatie is een vorm van niet-invasieve hersenstimulatie die op een relatief pijnloze, goedkope, en veilige manier in de kliniek kan worden toegepast om huidige traumabehandelingsmethoden te verbeteren. Bij elektrische wisselstroom stimulatie worden twee elektroden op het hoofd geplaatst waartussen een zwakke wisselstroom gaat lopen. Gebieden in het brein communiceren met elkaar door middel van hersengolven. Het idee achter de werking van elektrische wisselstroom stimulatie is dat hersengolven in het brein zich gaan aanpassen aan het ritme van de toegediende ritmische stroom. Diverse studies hebben aangetoond dat elektrische wisselstroom stimulatie positieve gevolgen kan hebben op het geheugen. Echter, is er nog onvoldoende kennis over de werking op geheugenconsolidatie, het proces waarbij herinneringen worden vastgelegd in het brein. Bij geheugenconsolidatie is met name de communicatie tussen twee hersengebieden van belang: de prefrontale cortex en de hippocampus. Door het ritme van activiteit op elkaar af te stemmen wordt goede communicatie bewerkstelligt tussen deze gebieden wat belangrijk is voor het goed vastleggen van het geheugen. Tijdens de geheugenconsolidatie communiceren de prefrontale cortex en de hippocampus in een specifiek ritme met elkaar: het theta-ritme (4-8 Hz). Met elektrische wisselstroom stimulatie in het theta-ritme zouden we de ritmische activiteit kunnen bevorderen waardoor het geheugen beter opgeslagen kan worden. Het doel van deze studie was om elektrische wisselstroom stimulatie toe te dienen bij gezonde vrijwilligers om meer inzicht te krijgen in de werking van stimulatie op geheugenconsolidatie. De proefpersonen ondergingen een geheugentaak. Eerst kregen ze combinaties van gezichten en omgevingen te zien die ze moesten onthouden. Vervolgens werd de hersenstimulatie toegediend waarbij ook de hersenactiviteit opgenomen werd zodat de activiteit van het theta-ritme in het brein later bekeken kon worden. Na de hersenstimulatie werden verschillende combinaties van de gezichten en omgevingen op het scherm getoond. Proefpersonen moesten aangeven of ze de combinatie al eerder hadden gezien. Onze hypothese was tweedelig: we verwachtten dat elektrische wisselstroom stimulatie het geheugen zou bevorderen en een verhoging van de hersenactiviteit in het theta-ritme teweeg zou brengen. Tegen onze verwachting in bleek elektrische wisselstroom stimulatie in het theta-ritme geen effect te hebben op onze uitkomstmaten. De discrepantie tussen de uitkomsten van de huidige studie in vergelijking met eerdere bevindingen kan onder andere verklaard worden door verschillen in onderzoeksopzet, zoals de locatie van de stimulatie-elektroden. In de toekomst zou bekeken kunnen worden of geïndividualiseerde stimulatie beter werkt. Daarnaast zou de onderzochte populatie, die een goed-functionerend geheugen heeft, als verklaring kunnen dienen voor de afwezigheid van een effect. Wellicht heeft hersenstimulatie meer effect op mensen die een lagere geheugencapaciteit hebben. Voor vervolgonderzoek wordt dan ook geadviseerd om rekening te houden met individuele verschillen op baseline.

Abstract

Neural oscillations in the theta frequency range (4-8 Hz) are thought to underlie effective communication between brain regions subserving associative memory (e.g., prefrontal-hippocampal circuitry). Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation technique that may entrain endogenous theta oscillations. Application of tACS during memory encoding has been shown to modulate associative memory encoding; however, few studies have investigated whether and how associative memory consolidation is affected by tACS at theta frequency (i.e., theta-tACS). Using an interleaved EEG/tACS approach, we have evaluated electrophysiological (i.e., frontal-midline theta power) and behavioral (i.e., memory task performance) effects of frontal theta-tACS, applied during memory consolidation. In a counter-balanced cross-over design, 30 participants (50% F, mean age 24 years) received 20 minutes of either sham tACS or active tACS (5 Hz, 2 mA peak-to-peak) during the consolidation phase of an associative memory task. Results showed no significant difference between stimulation conditions on our outcome measures. Our study provides no evidence of an effect of frontal theta-tACS on associative memory performance or frontal-midline theta power.

Keywords: Transcranial alternating current stimulation; theta-frequency; associative memory, consolidation.

1. Introduction

Standardized treatment (e.g., exposure therapy) is not equally effective among all patients with Post-Traumatic Stress Disorder (PTSD), suggesting the need to explore therapeutic alternatives or adjuvant interventions (McLean et al., 2022). A promising avenue of PTSD clinical research is transcranial electrical stimulation (tES), a form of non-invasive brain stimulation (NBS), given its easy feasibility (e.g., portability, low costs) and safety (Smits et al., 2021). Among tES types, transcranial alternating current stimulation (tACS) seems to be efficacious in targeting endogenous oscillatory brain activity (Tavakoli & Yun, 2017). By applying a weak sinusoidal electrical current between two electrodes attached to the scalp, tACS may induce subthreshold cortical neuron somatic membrane polarization in a frequency-dependent manner (Antal & Herrmann, 2016; Bland & Sale, 2019). Accumulating evidence suggests that tACS-induced synchronization of neural oscillatory networks may give rise to observed modulation of cognitive function (Klink, Paßmann, et al., 2020).

One potential approach for improving therapeutic outcomes may be strengthening of new memory associations, which form the rationale behind exposure therapy: newly formed fear extinction memory traces compete with original trauma-related memories, resulting in fear reduction (Sevenster et al., 2018; Vervliet et al., 2013). Prior studies suggest that tES triggers divergent effects on therapy effectiveness depending on timing of application: targeting the consolidation phase seems to ameliorate fear extinction while stimulation during extinction learning has been associated with deterioration (Abend et al., 2016; van't Wout et al., 2017). Theta (4-8 Hz) oscillatory synchrony within the prefrontal-hippocampal circuit has been shown to underlie associative memory consolidation (Reiner et al., 2014; Roberts et al., 2018; Rozengurt et al., 2017), indicating that tACS-induced entrainment of theta oscillations (theta-tACS) may improve consolidation of newly formed memories.

Although no studies have yet investigated the effect of theta-tACS on memory in PTSD, a recent study has revealed that frontal stimulation during consolidation is able to modulate item-recognition in healthy individuals (Shtoots et al., 2022). Regarding associative memory, effects of theta-tACS in healthy individuals have been mixed, with some studies finding no effect (Ergo et al., 2020) but others finding either an decrease (Lara et al., 2018; Meng et al., 2021) or increase (Alekseichuk et al., 2020; Antonenko et al., 2016; Klink, Peter, et al., 2020; Lang et al., 2019) memory performance. These discrepant results highlight the complexity of research on tACS: no definite conclusion can be drawn as stimulation parameter settings varied in frequency (4-6 Hz), current intensity (1-4 mA) and electrode montages (frontal/temporal/parietal). Apart from heterogeneity in study design, previous studies have all applied stimulation during encoding which further hampers comparability to effects of theta-tACS during consolidation.

The aim of this study was to gain more insight into the effects of theta-tACS during associative memory consolidation in healthy individuals. The current work focuses on electrophysiological (i.e., frontal-midline theta power) and behavioral (i.e., memory sensitivity) effects of frontal theta-tACS using an interleaved EEG/tACS approach. Here, we aim to explore the hypothesis that theta-tACS may upregulate theta power and increase associative memory performance.

2. Material and Methods

2.1 Participants

Participants were recruited on campus (Utrecht University) between June and October 2022. The study was pre-registered on Open Science Framework (see https://doi.org/10.17605/OSF.IO/TYFQ5). The Faculty Ethical Commission Social Sciences of Utrecht University approved the study. Participants were telephonically screened for the following exclusion criteria: contradictions to tACS, including the presence of an implant (e.g., pacemaker or neurostimulator) or metal inside the head, immediate family history of seizures, and pregnancy; severe neurological and psychiatric disorders; psychoactive medication use; alcohol or substance abuse. Participants were instructed to refrain from caffeine intake and use of nicotine substances within three hours prior to the experiment to preclude possible confounding effects.

Based on previous studies (Lang et al., 2019; Marko et al., 2019; Meng et al., 2021) a priori power calculation showed a sample of 36 was required to achieve 80 % power to detect an effects size of Cohen's d= 0.6. Due to time constraints, data from 33 participants was selected for analysis. A post-hoc power calculation yielded a power of 77 %, based on previously reported effect sizes of tACS on associative memory performance (average d=0.6).

2.2 Procedure

2.2.1 Experimental protocol design

Eligible individuals participated in two experimental sessions. A counter-balanced crossover design was used with two conditions: active tACS and sham tACS. The interval between sessions was at least 24 h to minimize carry-over effects (Figure 1A). Both experimental sessions lasted approximately 90 minutes. Participants were reimbursed with study credits or a voucher (30 euros) at the end of the second session.

For each participant, four minutes of resting state (2 minutes eyes-open, 2 minutes eyes-closed) EEG data was recorded prior to the associative memory task and following tACS-EEG paradigm. In order to target associative memory consolidation, participants were randomly assigned to receive sham or active tACS immediately after the encoding phase. Both the investigator and participant were blinded to the stimulation condition due to a five-digit code that was entered in the study mode of the NeuroConn stimulator. The stimulator codes were associated with sham or active stimulation and randomized by an independent person. Concurrent to tACS intervention, a video was shown to reduce individual state-dependent effects and between-subjects' variability in attentional engagement. After thirty minutes of stimulation and four minutes of EEG recording at rest, the retrieval phase of the associative memory task was conducted. Participants needed to complete several questionnaires pertaining to subjective reports of motivation, fatigue, sensations, and blinding. With the aim to increase task motivation, participants were told prior to the memory task that those with the highest score would be granted an extra voucher of 15 euros.

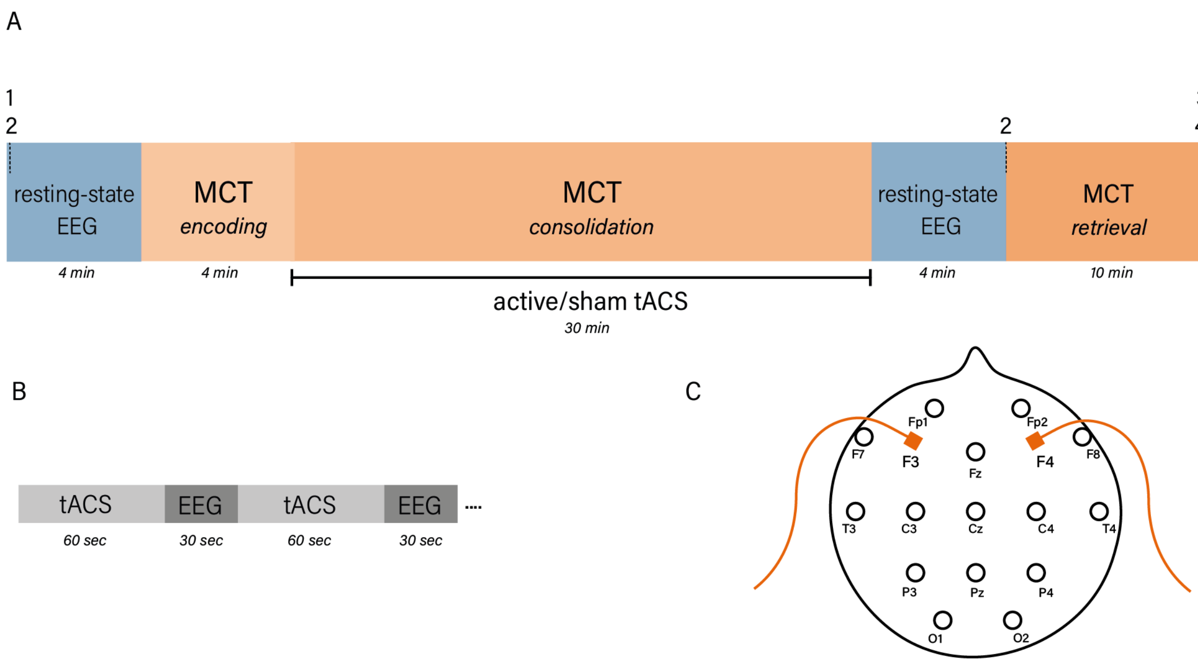

Figure 1. (A) Study Design. Participants underwent a memory contextualization task (MCT), consisting of an encoding, consolidation, and retrieval phase. During the consolidation phase, participants received either active or sham tACS. Multiple questionnaires were conducted throughout the experiment: 1) task motivation; 2) subjective ratings of mental fatigue; 3) tACS sensation; 4) tACS blinding. Asterisk (*) indicates that blinding was assessed only at the end of the second session. (B) Interleaved tACS/EEG protocol. Stimulation blocks (1 minute) were interleaved with no stimulation blocks to record EEG. (C) Schematic overview of tACS montage. Electrodes were positioned over F3 and F4.

EEG = electroencephalography; MCT = memory contextualization task; min = minutes; sec = seconds; tACS = transcranial alternating current stimulation.

2.2.2 Transcranial alternating current stimulation / tACS/EEG Approach

EEG signals were recorded continuously with Biosemi ActiveTwo system (Biosemi B.V., Amsterdam, The Netherlands), using 30[1] scalp electrodes positioned according to the standard 10-20 system locations. Two 3 x 3 cm tACS electrodes in saline-soaked sponges covered by conductive electrode gel were positioned over electrode position F3 and F4 (Figure 1C). The F3/F4 montage was selected to target the frontal cortex, which is implicated in a neural network subserving associative memory processes (Eichenbaum, 2017) and has been used previously in tES-studies that reported cognitive effects (Hsu et al., 2017; Meiron & Lavidor, 2014; Mondino et al., 2020). The peak electric field distribution over the cortical surface was simulated using SimNIBS (Supplementary Figure 1) (Thielscher et al., 2015). The electrode impedance was kept below 10 kOhm. Bifrontal tACS (5 Hz, 2 mA peak-to-peak) was applied using an interleaved tACS/EEG protocol: twenty stimulation blocks (1 minute) were interleaved with no-stimulation blocks (30 seconds) to record electroencephalograms (EEG), adding up to a total stimulation duration of 20 minutes. Each stimulation block consisted of 60 seconds with a ramp-up ramp-down duration of 1 second. For the sham condition, participants received only ramp-up ramp-down to mimic sensations observed with active tACS.

2.2.3 Associative memory task

Associative memory performance was assessed using a task adapted from a memory contextualization task (MCT) (Sep et al., 2019; van Ast et al., 2014), similar to the face-scene task used in previous studies on the effects of theta-tACS on associative memory (Lang et al., 2019; Meng et al., 2021).Participants were shown context stimuli (scenes) with a face stimulus in the foreground, and were instructed to memorize the context-face combinations. Participants underwent 50 encoding trials. On each trial, the context was presented for 1 second, succeeded by combined presentation of facial and context stimuli for 3 seconds. Trials were separated by 0.5 seconds. Total duration of the encoding phase was approximately 4 minutes. Following the interleaved tACS-EEG paradigm, participants completed 150 self-paced retrieval trials, which took on average 10 minutes. The number of trials was based on pilot data in which no ceiling effect was observed, and participants scored above chance-level. A total of 100 different faces depicting a neutral facial expression were divided into two gender-matched subsets: 50 stimuli were identical to encoding (old faces), while the other half was used as lure (new faces) during the retrieval phase. The old faces were presented against either a different encoding context (incongruent trials) or the original encoding context (congruent trials), whereas lure faces were combined with new contexts (lure trials). Congruent trials, incongruent trials, and lure trials were presented in a randomized order. Subjects were instructed to indicate whether the face-context combination was presented during the encoding phase or not (yes/no), and to what extent they were

[1] F3 and F4 were missing due to tACS electrode placement: remaining electrodes: Fp1, Fp2, AF3, AF4, F7, F8, Fz, Cz, FC1, FC2, FC5, FC6, C3, C4, CP1, CP2, CP5, CP6, P7, P8, P3, P4, Pz, PO3, PO4, T3, T4, O1, O2, and Oz.

confident of their choice using a 3-point Likert Scale (1= not confident at all; 2 = somewhat confident; 3 = completely confident).

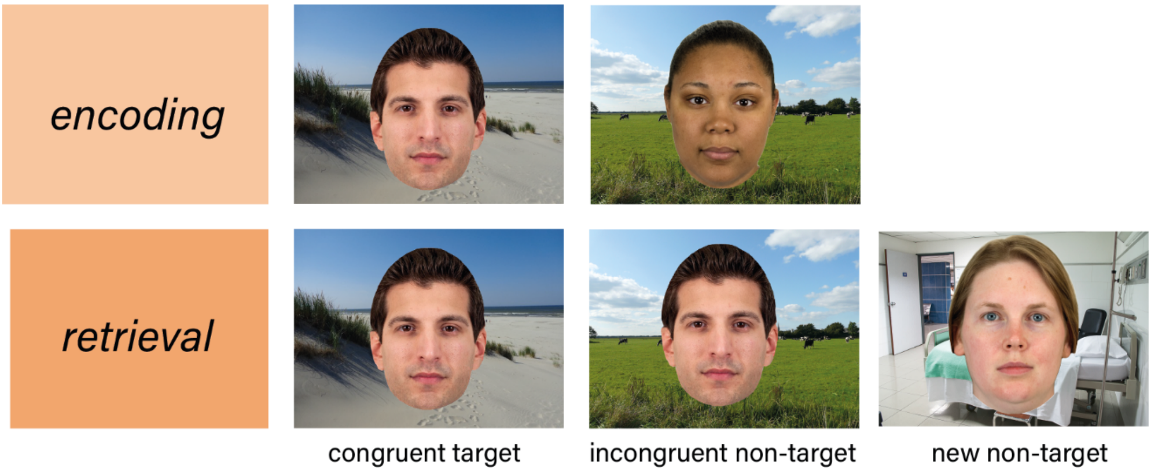

Figure 2. Memory contextualization task.

2.2.4 Subjective measures

To account for individual variation in motivation and mental fatigue, task motivation ("How motivated are you to perform well on the following task?") was rated on a 7-point Likert scale ranging from 1 ("not motivated at all") to 7 ("very motivated") (Hollis et al., 2018; Robinson et al., 2012; Schutter & Wischnewski, 2016) and mental fatigue was rated on the Samn-Perelli fatigue scale (Samn & Perelli, 1982) on a 7-point Likert Scale (1 = fully alert; 7 = completely exhausted, unable to function effectively) at the start of each session. The mental fatigue assessment was repeated at the end of the tACS-EEG paradigm.

At the end of each experimental session, participants completed a set of questions on sensations and adverse effects of tACS (Supplementary Data C). At the end of the final session, subjects were asked to guess in which session they received active and sham stimulation, to assess blinding success.

2.3 Analyses

2.3.1 EEG data analysis

Raw EEG data were processed offline with custom MATLAB scripts using EEGLAB v.2021.1 (Delorme & Makeig, 2004) and ERPLAB v.9.00 (Lopez-Calderon & Luck, 2014). Raw EEG data were down-sampled to 256 Hz, re-referenced to linked mastoids, and high pass (1 Hz) and low pass (35 Hz) filtered by a fourth-order Butterworth filter. Prior to re-referencing, data were manually inspected, and bad channels were interpolated using spherical spline interpolation. A total of 599 one-second epochs were generated and epochs containing major artefacts (e.g., due to movement) were marked and rejected after visual inspection using a semi-automatic method based on the following criteria: max. allowed voltage difference: 150 µV/200 ms; max. allowed absolute amplitude ± 100 µV; max. allowed voltage step: 75 µV/ms (Jung et al., 2016; Rozengurt et al., 2017). Subsequently, independent component analysis (ICA) was applied for eyeblink artifact removal (Delorme & Makeig, 2004). On average, 128/599 trials were excluded.

Power spectral density was computed using a fast Fourier Transform (FFT) approach with Welch's method (Hanning taper, 50 % overlap). The power spectral density ($\mu V^2/Hz$) was averaged over epochs and log-transformed. Average power from the theta band (4-8 Hz) and the stimulation frequency (5 Hz). Statistical analyses were conducted using average log-transformed theta power values (4-8 Hz/ 5H).

2.3.2 Primary outcome measures

Associative memory performance was quantified as memory sensitivity (*d',* d-prime), which takes both false alarms (proportion of misjudging non-target trials as "old") and hits (proportion of correctly recognizing target trials as "old") into account: z-scores of false alarms were subtracted from the z-scores of hits. Frontal-midline theta power was used as electrophysiological outcome. Based on previous research, theta power was averaged over frontal-midline electrodes (Cz and Fz) (Hsieh & Ranganath, 2014; Rozengurt et al., 2017).

2.3.3. Sensations and Blinding

To determine blinding success, participants were asked to guess the order of stimulation (i.e., sequence in which tACS was applied) and rate their confidence at the end of the second session. Moreover, prior expectations and perceived effects of tACS on performance and mental state were assessed (see blinding questionnaire, *Supplementary Data* C). Regarding sensations, participants were asked to indicate the timing and duration of the perceived sensation at the end of each session (see sensation questionnaire, *Supplementary Data* C).

2.3.3 Statistical analysis

All statistical analyses were performed in R version 4.1 (R Core Team 2022). To investigate effects of tACS on electrophysiological and behavioral measures, we used a repeated-measures ANOVA with two independent variables: Stimulation (two levels: sham, active) and, to take into account in which sequence stimulation conditions were applied, Order (two levels: sham-tACS, tACS-sham). The dependent variables were frontal-midline theta power and d-prime.

For statistical analysis on interaction effects, participants' results were partitioned into groups based on order of stimulation: tACS-sham or sham-tACS. Pearson's Chi-square tests were conducted to evaluate group differences for gender and blinding.

A MANOVA was conducted to compare sensations felt during active and sham stimulation. A Bonferroni correction was applied to adjust for multiple comparisons.

3. Results

3.1 Demographics

Demographical details are depicted in Table 1. Our results did not show any significant difference in age and sex depending on the stimulation sequence. Subjective ratings of tiredness and motivation were comparable in the sham and active condition (tiredness: $t(62)= -0.4$, $p = 0.7$; motivation: $t(62)=0$, $p = 1$).

Two subjects were excluded from EEG analyses due to presence of salt-bridge artifacts. Outliers (+-2.5 SD from mean) in d-prime (n=2) and theta power (n=1) were removed from further analyses. The final sample for theta power and d-prime analyses included data from 30 and 31 participants, respectively.

Table 1. Participant characteristics.

	N = 33	F/χ^2	p
Demographical details			
Age	24.0 (4.7)	1.46	0.16
Gender (F/M)	17/16	1.54	0.21

Participants' demographical details are comparable between groups based on stimulation sequence (active first or sham first).

3.2 Memory sensitivity

Main effect for order and stimulation x order interaction yielded significant results for memory sensitivity scores (order: $F(1,58) = 17.23$, $p <0.005$, $\eta_p^2 = .23$; stimulation x order: $F(1,58) = 11.06$, $p <0.005$, $\eta_p^2 = .16$), while the main effect of stimulation was not significant ($F(1,58) = 3.58$, $p=0.063$, $\eta_p^2 = .06$) (Figure 1). Follow-up pair-wise comparisons (Bonferroni corrected) revealed that the direction of tACS differed by order (tACS-sham: $t(13) = 2.18$, $p=0.05$; sham-tACS: $t(18) = -3.76$, $p=0.002$), indicating that effects of stimulation on memory sensitivity scores were may be driven by session effects (Supplementary Figure 2). Contrasts showed that impact of session differed depending on session sequence: individuals who received tACS second had higher d-prime scores during the second session ($t(17) = 2.94$, $p=0.00585$) while this pattern was absent in individuals who received tACS first ($t(13) = 1.86$, $p=0.0745$). Thus, current findings demonstrated no impact of theta-tACS on d-prime scores.

3.3 Frontal-midline theta power

The ANOVA on frontal-midline theta power at stimulation frequency showed no significant main effect of stimulation ($F(1,56) = .230$, $p = .633$, $\eta_p^2 = .000$) or order ($F(1,56) = 0.940$, $p =.338$, $\eta_p^2 = .020$), and neither did the interaction between stimulation and order ($F(1,56) = 0.160$, $p =.694$, $\eta_p^2 = .000$) approach the level of significance (Supplementary Figure 3). Main effects of stimulation ($F(1,56) =

0.110, p =.743, η_p^2 = .000), order (F(1,56) = 0.690, p =.409, η_p^2 = .010), and their interaction (F(1,56) = 0.330, p =.570, η_p^2 = .010) yielded no significant results on frontal-midline theta power at theta-band frequencies (Figure 2). Overall, these results show no evidence for an effect of theta-tACS on frontal-midline theta power.

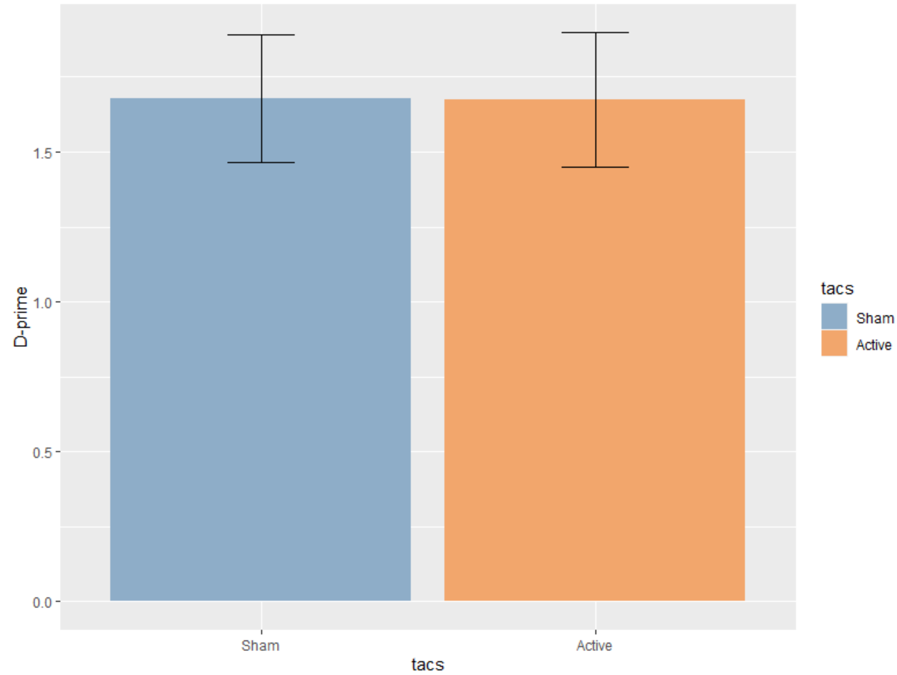

Figure 3 . Associative memory performance. Mean memory sensitivity scores (d-prime) are displayed for both types of tACS stimulation (sham, active). Error bars represent standard errors of the mean.

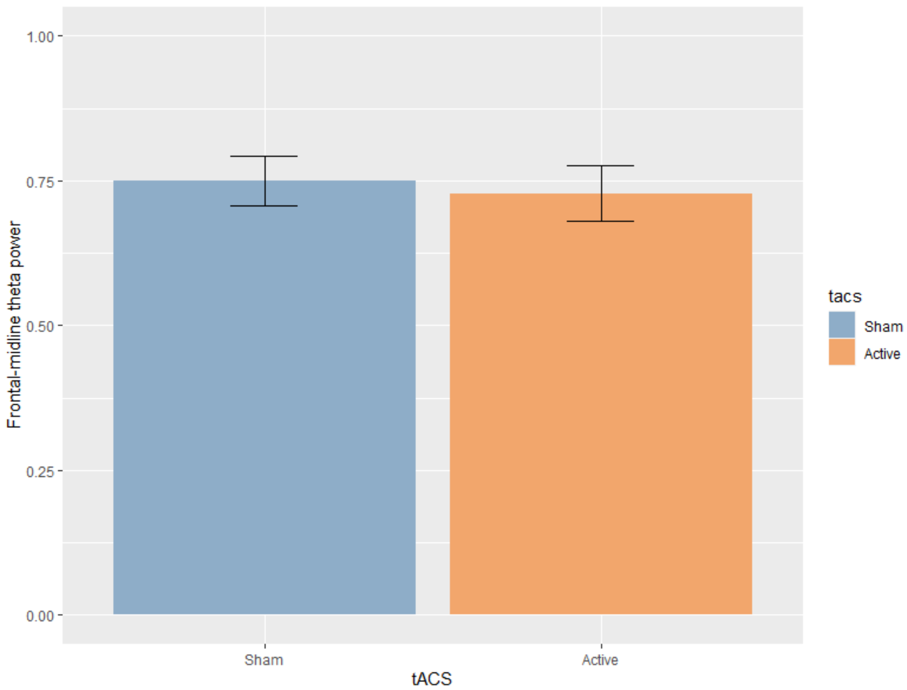

Figure 4. Frontal-midline theta power. Mean log-transformed theta power at theta band frequencies is displayed for both types of tACS stimulation (sham, active). Error bars represent standard errors of the mean.

Table 2. Behavioral and electrophysiological outcomes

	Active			Sham		
	M	*SD*	*Range*	*M*	*SD*	*Range*
D-prime	1.674	0.609	0.915-2.93	1.68	0.582	0.507 - 3.05
Theta power (5 Hz)	0.731	0.127	0.440-0.986	0.767	0.125	0.550-1.03
Theta power (4-8 Hz)	0.730	0.136	0.421-0.992	0.770	0.134	0.526-1.03

The overall descriptive statistics of main outcome measures are displayed.
M = mean; SD = standard deviation

3.4 Sensations and blinding

Participants showed above-chance accuracy of stimulation identification (Table 3). Chi-squared analyses showed no differences in participant guesses between session 1 and 2 (χ^2 = 0.1, *p* = 0.70). A total of 4 participants were unable to determine when they had received sham and active tACS (session 1= 3; session 2 = 1). When comparing accuracy of stimulation identification, we found that 81.82 % (27/33) and 73.33 % (24/33) correctly guessed the active and sham stimulation condition, respectively. Sensation data was missing from 1 participant. Active stimulation was not associated with significantly enhanced occurrence of sensations (Table 4). Tiredness (25/32), concentration difficulties (18/32), itchiness (16/32) and burning sensations (12/32) were the most common reported sensations during active stimulation.

Table 3. Summary of results: blinding ratings active vs sham stimulation

	Active	Sham
Correct guess rate	81.82 %	73.33 %
Confidence rate	73.11 %	69.56 %

Adequacy of blinding was assessed by asking participants to guess whether they had received sham or active tACS. Confidence ratings to their judgement (0-100 scale,
0% = not at all; 100 % = absolutely sure) are also reported.

Table 4. Summary of results: statistical results active vs sham stimulation – perceived sensations

	Active	Sham	*F*	df	p	p.adj
Burning sensation	0.375	0.188	-1.38	60.0	0.173	1
Concentration difficulties	0.625	0.781	0.751	54.5	0.456	1
Diziness	0.0312	0.0938	0.825	43.2	0.414	1
Headache	0.344	0.344	0	61.8	1	1
Heat under electrodes	0.344	0.344	-0.689	58.3	0.493	1
Itchiness	0.531	0.219	-1.68	60.0	0.0984	1
Metallic taste	NA	NA	NA	NA	NA	NA
Nausea	0	0.25	1	31.0	0.325	1

Neck pain	0.0312	0.25	2.30	38.4	0.0269	0.296
Pain	0.219	0.0625	-1.35	52.7	0.184	1
Seeing flashes of light	0.0938	0.0938	0	57.8	1	1
Tiredness	1.28	1.41	0.477	61.3	0.635	1

Ratings following active and sham stimulation (5-Likert Scale: 0 = "*none*", 1 = "*slight*", 2 = "*moderate*"; 3 = "*intense*", 4= "*unbearable*"). No participant reported a metallic taste (NA, not applicable). P<0.05 is defined as statistically significant. Bonferroni correction was applied to adjust p-values (P adj) for multiple comparisons. p<0.05, p.adj<0.00417

4. Discussion

Prior studies have highlighted an important role of theta oscillations in associative memory consolidation, yet conclusive evidence on the effectiveness of theta-tACS remains to be elucidated. The aim of the present study was set to investigate the effects of theta-tACS during memory consolidation on associative memory retrieval performance and EEG frontal-midline theta power measures. In contrast to our hypothesis, the results show no significant differences between active and sham tACS on both outcome measures. Collectively, our results provide no definite evidence of an effect of theta-tACS on associative memory performance or theta entrainment.

4.1 Associative memory performance

In an attempt to minimize confounding effects of practice on stimulation effects, we have counterbalanced the order of stimulation across participants. Notwithstanding, we found a sequence-specific session effect: individuals who received active stimulation first showed a session improvement in retrieval performance while such an effect was not visible in individuals who received sham stimulation first. A potential explanation for the observed session-effect may be that participants differed at baseline. Alternatively, it is possible that the effect of tACS on associative memory performance was blunted by a session-effect. To avoid the influence of multiple sessions on intervention, a between-subject design may be more suitable to detect potential stimulation effects.

In contrast to the current findings, several previous studies showed an effect of theta-tACS on associative memory. This difference could be explained by several factors. First, differential susceptibility to the impact of tACS on associative memory may depend on baseline memory performance, similar to findings observed for working memory (Grover et al., 2022; Reinhart & Nguyen, 2019). For instance, Klink and colleagues showed the feasibility of frontal theta-tACS stimulation to improve associative memory performance in older adults, which could be due to the presence of age-related associative memory deficits (Klink et al., 2020). Corroborating this finding, another study stratifying individuals into two age groups (i.e., younger and older adults) found that beneficial effects of tACS emerged solely in older participants (Antonenko et al., 2016). One potential mechanism underlying an age-related decline of associative memory performance in elderly may be the coupling of gamma power along the phase of theta oscillations (i.e., phase-amplitude cross-frequency coupling) (Karlsson et al., 2022). Successful associative memory performance has been associated with gamma power increases close to the peak of the theta oscillation, whereby elderly showed a shift in theta-gamma coupling (Karlsson et al., 2022). Importantly, high performing older adults showed a similar coupling pattern to high performing younger adults, inferring that theta-gamma coupling is dependent on baseline performance. Previous studies have shown that frontal theta-gamma tACS was able to modulate working memory performance to a greater extent than theta tACS (Alekseichuk et al., 2016; Reinhart & Nguyen, 2019); specifically, enhancement was observed when bursts of gamma-oscillations were phase-locked to the peak, not trough, of theta oscillations (Alekseichuk et al., 2016). Somewhat in line, bursts of gamma oscillations nested into the trough of theta were shown to induce deterioration of associative memory in younger adults, albeit no effect was observed in case of phase-locking to the peak (Lara et al., 2018). Thus, stimulation may exert divergent effects on associative memory depending on the degree of theta-gamma coupling: tACS seems beneficial when theta-gamma coupling is compromised (as in low associative memory performers), while optimal theta-gamma coupling (as in high associative memory performers) appears to be considerably less positively malleable to tACS. Future studies should disentangle whether theta-gamma tACS may be more beneficial for enhancement of associative memory, specifically in individuals with low baseline performance.

Second, the discrepancy in results could be partly due to electrode montage (Alekseichuk et al., 2020; Lang et al., 2020). Using group-level electrophysiological and functional neuroimaging data to determine stimulation parameters, Alekseichuk and colleagues (2020) found that theta-tACS over the right parietal cortex was associated with augmented associative memory, while this pattern was absent when stimulation was applied over the left parietal cortex. In line with this finding, enhanced associative memory performance was observed following right parietal theta stimulation (Lang et al., 2020). Yet, another study revealed the opposite effect on associative memory when stimulating the left parietal cortex, pointing towards a possible lateralization (Meng et al., 2021). A similar pattern has been observed when stimulating the temporal cortex: right and left hemispheric stimulation have been associated with both enhancement and deterioration of memory performance (De Lara et al., 2018; Antonenko et al., 2016). In contrast to these studies, a bilateral frontal electrode montage was used in this study which may have prevented observation of hemispheric lateralized effects. Apart from lateralization, phase-coupling between electrodes seems to be a necessary factor to take into consideration. Multiple studies suggest that beneficial behavioral effects of bi-electrode tACS are independent of relative phase differences (in-phase or anti-phase) (Hsu et al., 2017; Schutter & Wischnewski, 2016; Wischnewski et al., 2020), whereas others do not (Polanía et al., 2012). Specifically, Polanía and colleagues found that in-phase theta tACS was positively correlated with working memory performance, whereas the opposite effect was observed when anti-phase theta-tACS was applied. Comparing anti-phase and in-phase bi-frontal stimulation (F3/F4), another study reported that behavioral effects were not phase-dependent (Hsu et al., 2019). Definite conclusions with respect to phase-coupling should be drawn with caution: anti-phase and in-phase conditions seem to differ in focality and magnitude of stimulation intensity (Alekseichuk et al., 2019; Saturnino et al., 2017). For instance, anti-phase tACS yielded larger positive behavioral effects compared to in-phase tACS, which may be driven by the observed greater electrical field in frontal-midline areas (Hsu et al., 2019). Future studies should investigate whether effects of our dual-electrode montage (per definition anti-phase) differ from other in-phase montages using an electrode montage which is valid for phase-targeting (e.g., two ring montages) (Saturnino et al., 2017).

4.2 Frontal-midline theta power

In consonance with behavioral results, no effects of theta-tACS on frontal-midline theta power could be found. Specifically, neither EEG power in the theta band nor stimulation frequency were affected by tACS.

Several explanations could be proposed for absence of enhanced theta power, among which stimulation intensity and endogenous oscillation strength seem important factors (Alagapan et al., 2016; Neuling et al., 2013). In general, most studies hinge on the assumption that the working mechanism of tACS relies on the synchronization of endogenous oscillatory activity to the external tACS-induced rhythm (i.e., entrainment). Entrainment may be described by a theoretical concept called the “Arnolds tongue”, referring to a phenomenon that endogenous oscillations are more likely to respond to stimulation frequencies close to the endogenous rhythm, especially when stimulation intensity is low (Antal & Herrmann, 2016). Indeed, some studies unveil evidence of the “Arnolds tongue”. For instance, Alagapan and colleagues (2016) demonstrated tACS-induced entrainment in presence of a low endogenous alpha amplitude (i.e., task state), while an endogenous alpha power enhancement was observed when the endogenous alpha oscillation strength was stronger (i.e., relaxed eyes-open state) (Alagapan et al., 2016). Corroborating findings on state-dependency, other studies found no tACS-induced effect on oscillatory power during states of strong endogenous power, while an effect was observed in a lower power condition (Neuling et al., 2013; Alagapan et al., 2016). Somewhat in line, no bilateral tACS-induced changes in frontal theta band power were observed in high theta power conditions (Hsu et al., 2017). Since memory consolidation is associated with high theta power (Reiner et al., 2014; Rozengurt et al., 2017; Shtoots et al., 2022), ceiling effects may have prevented observing a differential effect of active stimulation on theta power. In agreement with human studies, an optogenetic animal study revealed that application of alternating weak electric fields resulted in enhancement of endogenous power, whereas oscillations at stimulation frequency could only be enhanced by a higher amplitude stimulation or absence of endogenous oscillations (Schmidt et al., 2014). Notably, weak-electric-field-induced effects were solely visible when stimulation frequency matched with endogenous peak frequency; thus, individualized stimulation frequencies seem a prerequisite in human studies limited to low stimulation strength (Neiling et al., 2013; Schmidt et al., 2014). Thus, deviations from individual theta peak frequencies may have reduced the efficacy of tACS-induced entrainment in our study.

Another factor that should be taken into consideration is timing of effect measurement. Conclusive evidence on immediate tACS effects have been impeded by stimulation artifacts (Noury et al., 2016), which limits analysis of EEG data to offline (after stimulation) measurement. Our null results may be due to immediate cessation after stimulation discontinuation. Indeed, a recent study in nonhuman primates suggests that tACS-effects are confined to the stimulation period (Johnson et al., 2020). However, this may not completely explain our null results, considering that other studies have found long-lasting electrophysiological effects: a tACS induced power enhancement remained visible in the timescale of minutes after stimulation was turned off (Kasten et al., 2016; Neuling et al., 2013). A possible explanation for the lack of tACS-induced after-effects in our study may also be the duration of stimulation. Most studies have applied tACS continuously for 10-20 minutes, whereas our interleaved tACS/EEG protocol involved multiple short stimulations of one minute each. Future studies should provide more mechanistic insight into timing and duration of tACS, in light of novel approaches aiming to overcome barriers of concurrent EEG and tACS (Nasr et al., 2022).

4.3 Blinding

Our results point out that blinding was not successful, in line with conclusions from a recent study on blinding success in tACS studies with a ramp-up/ramp-down sham control condition (Sheffield et al., 2022). Literature reports inconsistent findings regarding the effect of correct end-of-study sham guessing on behavioral outcome; therefore, it is difficult to demarcate the precise confounding effect of participant's expectations and beliefs on behavioral outcome (Fassi & Kadosh, 2020; Stanković et al., 2021). Most participants expected either no or a positive effect of active stimulation on memory performance and mental state (Supplementary Data Table 2, 3), which is not entirely reflected in our result. Although blinding did not seem to bias our findings, it seems worthwhile to address blinding issues. Important to note is that our within-subject design allowed participants to compare sensations felt during stimulation, potentially exacerbating difficulties with blinding. Therefore, future studies should attempt to incorporate additional frequencies as an active control, thereby reducing the potential confounding effect of blinding.

4.3 Limitations

Some limitations of the study design need to be acknowledged. Apart from study limitations introduced by session effects and blinding as discussed above, the present study suffered from other limitations. First, although we attempted to consider confounding factors (e.g., motivation), some remaining individual factors (e.g., attention, sleep) may have affected outcome parameters. For instance, individual differences in attentional bias towards specific stimuli could have affected our results. The effect of repetitive transcranial magnetic stimulation (during retrieval) over the dorsolateral prefrontal cortex (our targeted stimulation location) on associative memory was dependent on strategy use (Manenti et al., 2010). Corroborating this finding, evidence of an additive benefit of frontal tES and strategy use has been reported in working memory (Jones et al., 2015). Therefore, future studies should include encoding strategies (Lang et al., 2019).

Second, as aforementioned, brain stimulation seems to have a differential effect on behavioral performance, depending on cognitive ability: higher skill level and lower age were both associated with a deterioration (Furuya et al., 2014; Lara et al., 2018; Meng et al., 2021). Therefore, the incorporation of baseline-performance seems beneficial by reducing the confounding effect of some individual differences. However, in the current study, no baseline could be incorporated in the model: session effects have hampered initial attempts to include behavioral performance during sham as baseline. Future studies could perform a separate baseline session to circumvent session effects.

Third, as aforementioned, our dual-electrode montage resulted in an anti-phasic (180° phase difference) stimulation. Therefore, one could hypothesize that bilateral induced electrical fields would cancel each-other out along the midline, resulting in a zero current. However, this would not explain previous findings of an effect of bilateral tACS on frontal-midline EEG power (Onoda et al., 2017; Riddle et al., 2022). A potential explanation could be that bilateral tACS is able to exert an indirect effect: the stimulated area (dorsolateral prefrontal cortex) is reciprocally connected with brain areas known to generate frontal-midline theta oscillations (medial prefrontal and anterior cingulate cortex) (Arikuni et al., 1994; Tsuijmoto et al., 2010; Hsieh et al., 2014). Moreover, hemispheric differences in biophysical features (e.g., neuronal orientations, conductivity of tissues) further highlights the complication nature of electric field generation and weakens the credibility of the prior-stated hypothesis of out-cancellation (Gabriel et al., 2009; Opitz et al., 2016).

4.4 Future directions

Following the current thesis, many future research opportunities exist, some of which were highlighted above, while others are discussed now.

The predominant frontal intrinsic oscillatory frequency during resting-state was found to be in the fast beta/gamma band (20-50 Hz), implying that theta may not be an ideal target in our location set-up (Rosanova et al., 2009). Indeed, frontal tACS modulation of gamma, but not theta, oscillations did improve long term memory (Grover et al., 2022). Further substantiating this, frontal gamma-tACS was able to induce cognitive enhancement, whereas no effect was observed using theta-tACS (Santarnecchi et al., 2016). In contrast to findings on gamma, another study found that frontal tES improved memory performance which was accompanied with power changes in frontal-midline beta oscillations (Liang et al., 2021). Future work needs to be carried out to establish whether the fast beta/gamma band may be a more optimal frequency band.

One behavioral study to date has directly measured the effects of frontal (AF4, AF3, FC2, FC1) theta-tACS on memory consolidation (Shtoots et al., 2022). Although they did not look at associative memory specifically, they found that active stimulation improved free recall 2 hours later. It would be interesting to incorporate multiple memory assessments to compare immediate recall of associative memory to delayed recall.

Future work needs to be carried out to establish whether tACS application during consolidation has therapeutic value. Accumulating evidence of aberrant associative memory processing in PTSD, provides a novel opportunity to improve treatment response in PTSD (Lambert & McLaughlin, 2019; Sep et al., 2019). Notably, memory performance may be predictive for therapy response: individuals with poorer baseline memory are less able to benefit from current treatment (Haaland et al., 2016; Nijdam et al., 2015). Therefore, strengthening of new memory associations during exposure therapy seems a promising approach for improving therapeutic outcomes (van‘t Wout et al., 2017). Preliminary evidence suggests that tACS may be more beneficial in individuals who exhibit difficulties with associative memory (Klink, Peter, et al., 2020); thus, future studies should investigate whether tACS is able to improve associative memory in clinical and non-clinical samples.

4.5 Conclusion

To our knowledge, this is the first study reporting both the behavioral and electrophysiological effects of theta-tACS during associative memory consolidation. Our results provide no evidence for effects of bifrontal tACS at 5 Hz on frontal-midline theta power or associative memory performance. Possible explanations for the lack of effects include currently used stimulation parameter settings (e.g., electrode montage, stimulation frequency) and baseline differences. Future studies should consider the potential benefit of individualized stimulation approaches and take individual baseline levels into account. The overall insight from the current thesis may help to optimize the study design of tACS-protocols targetting associative memory consolidation.

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Supplementary data
A Figures

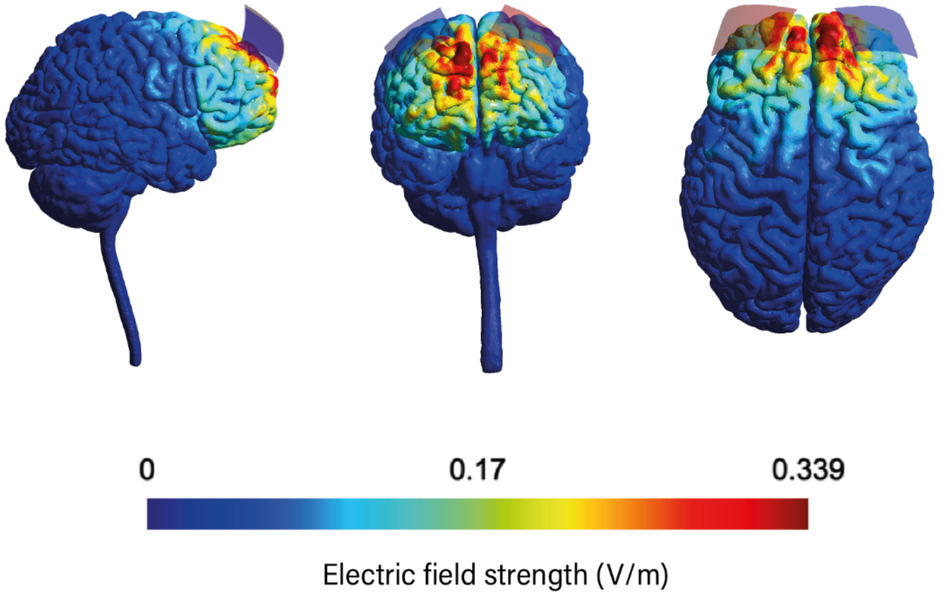

Figure S1. An Electric field simulation. SimNIBS was used to investigate electric field simulation of tACS targeted to F3 and F4.

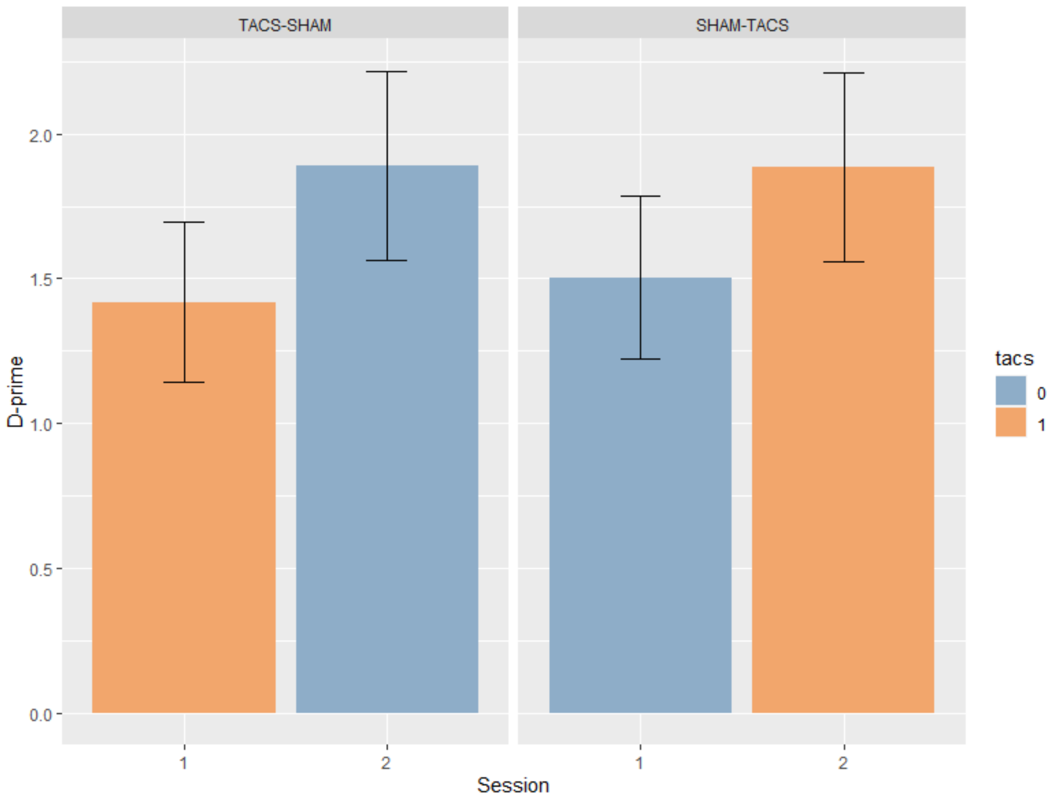

Figure S2 . Associative memory performance. Mean memory sensitivity scores (d-prime) are displayed for both type of stimulation (sham = 0, active= 1) and session . The results are separated into two groups according to order (active first or sham first). Error bars represent standard errors of the mean.

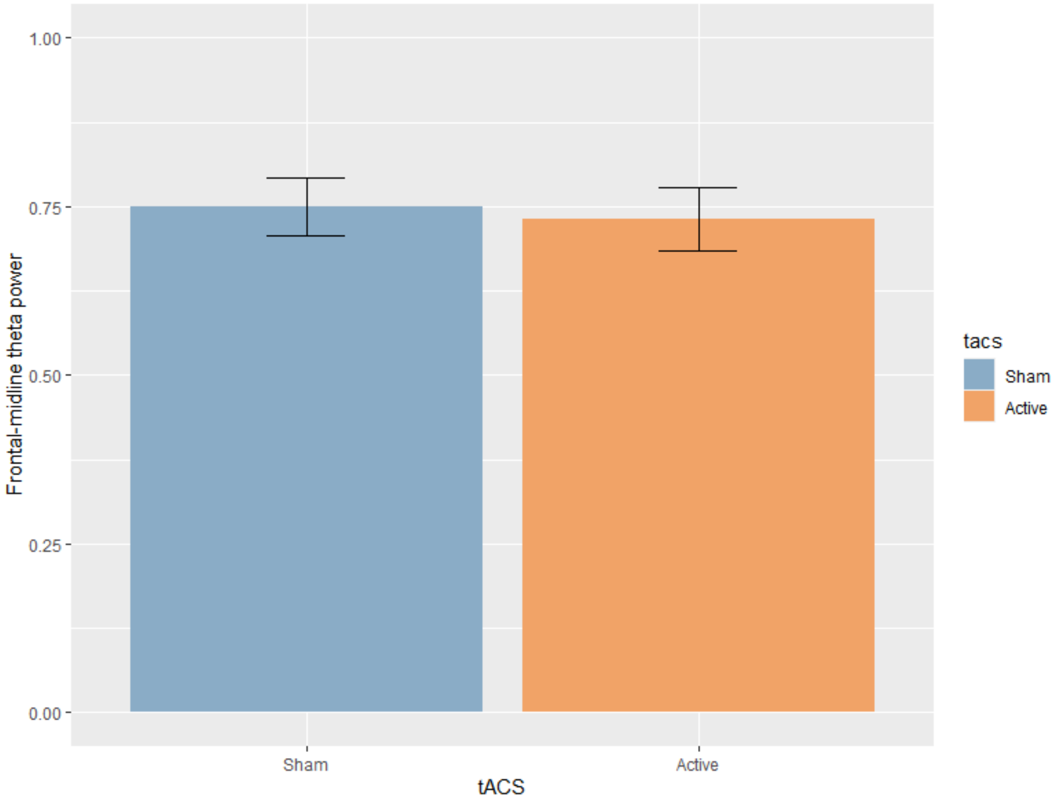

Figure S3. Frontal-midline theta power. Mean log-transformed theta power *at* stimulation frequency is displayed for both types of tACS stimulation (sham, active). Error bars represent standard errors of the mean.

B Tables

Table S1. Blinding

	Active	Sham
Correct guess rate		
Session 1	73.33 %	66.67 %
Session 2	88.89 %	80.00 %
Confidence rate		
Session 1	65.71 %	71.56 %
Session 2	80.50 %	67.57 %

The overall average percentages of correct guessing rates and confidence rates are depicted for active and sham stimulation.

Table S2. Blinding expectations and effect of stimulation

	M	*SD*
Expectations		
Performance	0.433	0.504
Mental state	0.323	0.652
Effect stimulation		
Session 1	0.464 (n=26)	0.508
Session 2	0.483 (n=28)	0.508

Prior expectations of tACS on associative memory performance and mental state are described, whereby mean values vary from 0 (no effect) to 1 (effect). On average, individuals did not expect that stimulation had effect. In addition, the perceived effect of stimulation during both sessions is stated (0= no effect; 1 = effect). No effect of stimulation was found. “I do not know” answers were excluded.

Table S3. Frequency Table of expected directionality of effect

	Frequency
Direction effect	
Performance was worse	2
Performance was better	5
Mental state was worse	2
Mental state was better	2
I do not know	12

C Questionnaires

Blinding

What is your subjectnumber?

____

Do you think you received active or sham (placebo) stimulation?

	Active	Sham	I do not know
during the first session	○	○	○
during the second session	○	○	○

How sure are you?
0% = not at all; 100 % = absolutely sure

	0 10 20 30 40 50 60 70 80 90 100
session 1	
session 2	

Do you think real brain stimulation would affect your performance and/or mental state?

- ○ yes, performance improves
- ○ yes, performance deteriorates
- ○ yes, mental state improves
- ○ yes, mental state deteriorates
- ○ no

Do you think real brain stimulation has affected your performance and/or mental state?

During the first session
- ○ yes, my performance was better
- ○ yes, my performance was worse
- ○ yes, my mental state was better
- ○ yes, my mental state was worse
- ○ yes, but I do not know how
- ○ no
- ○ i do not know

During the second session
- ○ yes, my performance was better
- ○ yes, my performance was worse
- ○ yes, my mental state was better
- ○ yes, my mental state was worse
- ○ yes, but I do not know how
- ○ no
- ○ i do not know

Sensations

What is your subjectnumber?

____

What is the sessionnumber?

○ Session 1

○ Session 2

Did you experience something special during stimulation?
Use the scale to answer the following questions regarding different sensations and the degree to which you felt them:

To what extent did you experience

	none	slight	moderate	intense	unbearable
Itchiness	○	○	○	○	○
Pain	○	○	○	○	○
Burning sensation	○	○	○	○	○
Heat under electrodes	○	○	○	○	○
Metallic taste	○	○	○	○	○
Tiredness	○	○	○	○	○
Headache	○	○	○	○	○
Neck pain	○	○	○	○	○
Seeing flashes of light	○	○	○	○	○
Diziness	○	○	○	○	○
Nausea	○	○	○	○	○
Concentration difficulties	○	○	○	○	○

When did the sensation start?

	At the beginning of stimulation	During the stimulation	At the end of the stimulation
Sensation	○	○	○

How long did the sensation last?

	Only at the beginning of stimulation	During the stimulation	Only at the end of stimulation
Sensation	○	○	○