UNIVERSITY OF BIRMINGHAM
University of Birmingham Research at Birmingham

On the effectiveness of event-related beta tACS on episodic memory formation and motor cortex excitability

Braun, Verena; Sokoliuk, Rodika; Hanslmayr, Simon

DOI:
10.1016/j.brs.2017.04.129

License:
Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version
Peer reviewed version

Citation for published version (Harvard):
Braun, V, Sokoliuk, R & Hanslmayr, S 2017, 'On the effectiveness of event-related beta tACS on episodic memory formation and motor cortex excitability', *Brain stimulation*, vol. 10, no. 5, pp. 910-918. https://doi.org/10.1016/j.brs.2017.04.129

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.
•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
•Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 07. Nov. 2024

On the Effectiveness of Event-related Beta tACS on Episodic Memory Formation and Motor Cortex Excitability

Verena Braun[a], Rodika Sokoliuk[a], Simon Hanslmayr[a]

[a] School of Psychology, University of Birmingham, Edgbaston, B15 2TT, Birmingham, United Kingdom

Corresponding authors:

Verena Braun

Email: v.braun@pgr.bham.ac.uk, Telephone: 44 (0) 121 414 4901

Simon Hanslmayr

Email: s.hanslmayr@bham.ac.uk, Telephone: 44 (0)121 414 6203

Abstract

Background

Transcranial alternating current stimulation (tACS) is widely used to entrain or modulate brain oscillations in order to investigate causal relationships between oscillations and cognition.

Objective

In a series of experiments we here addressed the question of whether event-related, transient tACS in the beta frequency range can be used to entrain beta oscillations in two different domains: episodic memory formation and motor cortex excitability.

Methods

In experiments 1 and 2, 72 healthy human participants engaged in an incidental encoding task of verbal and non-verbal material while receiving tACS to the left and right inferior frontal gyrus (IFG) at 6.8Hz, 10.7Hz, 18.5Hz, 30Hz, 48Hz and sham stimulation for 2s during stimulus presentation.

In experiment 3, tACS was administered for 10s to M1 at the individual motor beta frequency of eight subjects. We investigated the relationship between the size of TMS induced MEPs and tACS phase.

Results

Beta tACS did not affect memory performance compared to sham stimulation in experiments 1 and 2. Likewise, in experiment 3, MEP size was not modulated by the tACS phase.

Conclusions

Our findings suggest that event-related, transient tACS in the beta frequency range cannot be used to modulate the formation of episodic memories or motor cortex excitability. These null-results question the effectiveness of event-related tACS to entrain beta oscillations and modulate cognition.

Keywords

beta oscillations; episodic memory; motor cortex excitability; transcranial alternating current stimulation (tACS);

Introduction

Brain oscillations represent regular fluctuations in the local field potential and play a crucial role in establishing synchronous firing patterns [1]. Especially oscillations in the beta frequency range (~13-30Hz) have been linked to a variety of cognitive and sensorimotor processes [2–6]. Beta power decreases, for example, have been shown to predict successful memory encoding [7,8]. Such desynchronized activity occurs in highly task relevant regions [9], and is negatively correlated with blood oxygenation level dependent (BOLD) activity [10]. It has further been demonstrated that power and phase of beta oscillations over the motor cortex influences the amplitude of transcranial magnetic stimulation (TMS) evoked potentials (MEPs) [11,12].

Despite the numerous associations between these processes and beta oscillations, the causal relationship between them remains unclear. Transcranial alternating current stimulation (tACS), an increasingly popular non-invasive human brain stimulation technique [13], has been suggested to provide this causal link between brain oscillatory activity and cognitive processes. Recent findings suggest that tACS entrains brain oscillations in a frequency specific way [14,15]. This modulation of underlying oscillatory activity can affect behaviour [16–19], interacts with underlying oscillatory activity [20–24], and elicits frequency specific neuronal spiking [25].

tACS could be a very beneficial and powerful method for cognitive research, if it was also able to modulate brain oscillations in a time-critical way [26,27]. During cognitive tasks brain oscillations show a very dynamic behaviour and are modulated in the range of seconds. However, most studies having demonstrated effects of tACS on behaviour applied tACS throughout cognitive tasks in a sustained way, resulting in stimulation durations of up to

20min [15–18,20,21,28]. This makes it difficult to directly link oscillatory activity associated with specific cognitive dynamics with the results of these tACS studies. In order to demonstrate that tACS is indeed a useful tool for modulating dynamic cognitive processes, tACS should be administered within a short period of time at certain phases of a cognitive task in event-related, randomized designs.

In the present study we sought to investigate the effectiveness of event-related beta tACS. In a series of experiments we explored whether tACS in the beta frequency range is effective in modulating two different processes: the formation of episodic memories and motor cortex excitability.

Beta power decreases and episodic memory formation

Successful memory formation for verbal material has been associated with power decreases in the beta frequency range [8]. This beta desynchronization can be localized to the left inferior frontal gyrus (IFG) [10], a region which has been linked to successful semantic memory encoding in numerous studies [29]. Using rhythmic transcranial magnetic stimulation (rTMS), Hanslmayr et al [30] demonstrated a causal link between beta desynchronization in the left IFG and memory encoding. By artificially synchronizing the left IFG via rTMS in the beta frequency range, memory formation for words was impaired at beta but not at other frequencies. These findings provide a first causal link between beta power decreases and episodic memory. However, due to safety considerations [31] rTMS stimulation could not be applied at higher frequencies, and hence their effects could not be investigated. Therefore, experiments 1 and 2 aimed to replicate and extend these findings and further examine whether tACS may be a useful addition to TMS. In these two experiments, the differential and specific effects of beta tACS to the left IFG on the encoding

of verbal material and the effects of beta tACS to the right IFG on the encoding of non-verbal material were investigated. Several studies report material-specific lateralization during episodic memory encoding with left frontal involvement during the encoding of verbal material and right frontal activation for non-verbal material [29,32,33]. Therefore beta (18.5Hz) tACS should only affect memory performance for words when administered to the left IFG (as has been shown by Hanslmayr et al. [30]) while right IFG stimulation should result in decreased memory performance for non-verbal material only.

Beta phase and motor cortex excitability

Several simultaneous tACS-TMS studies investigated the causal relationship between beta power and corticospinal excitability [23,34], with recent studies investigating whether the phase of 20Hz tACS can modulate MEP amplitude [35–37]. However, in these studies tACS was applied for prolonged periods of time. Raco et al. [35] for instance applied 20Hz tACS for 200s and found a phase-dependent modulation for the last three MEPs only. In experiment 3 we aim to investigate whether 10s of tACS tuned to the individual motor beta frequency can lead to a modulation of the amplitude of TMS evoked MEPs by the phase of the ongoing tACS.

Aims of the studies

Being able to modify beta oscillations within a short period of time in an event-related, randomized manner is crucial for quantifying the effectiveness of beta tACS and ultimately its usefulness for modulating dynamic cognitive processes. Therefore, different stimulation parameters were used to reveal the ideal stimulation set-up for transient tACS. The effects of different electrode sizes on episodic memory formation were investigated in experiments

1 and 2. In order to keep the current density and possible skin sensations comparable, the stimulation intensity was reduced from 1mA (experiment 1) to 0.8mA (experiment 2). Additionally, the specific effects of beta tACS were investigated using four control frequencies as well as sham stimulation. As episodic memory performance is a rather indirect read out for the effectiveness of brain stimulation, we investigated the effect of beta tACS on MEP size in experiment 3. This, more direct way of quantifying the effectiveness of brain stimulation [38–42], allowed us to explore whether traditional montages with one electrode being placed directly at the target area are more effective than montages with both stimulation electrodes surrounding the target area. Although the same electrode size used in experiment 2 was chosen for experiment 3, lower stimulation intensities were used in order to reduce possible side effects, e.g phosphenes that have been reported repeatedly by participants of experiment 2. Finally, the stimulation duration in experiment 3 was increased to 10s compared to experiments 1 and 2, allowing us to investigate whether slightly longer but still relatively short stimulation has effects on motor cortex excitability. Furthermore, in experiment 3, we aimed to optimize tACS parameters by stimulating at the participants' individual beta frequency rather than using a standard frequency (e.g. 20Hz).

Experiments 1 and 2

Material and Methods

Participants

Participants were screened for contraindications against transcranial alternating current stimulation prior to the experiment [43].

36 subjects participated in experiment 1 (24 female; mean age: 20.03 +/- 2.38 years) and 36 in experiment 2 (24 female, mean age: 20.97 +/- 2.22 years).

All participants were right handed, had normal or corrected-to-normal vision and reported no history of neurological disease or brain injury. Informed consent was acquired from each subject prior to the experiment. All were naive to the hypotheses of the study and were fully debriefed at the end of the experiment. The study was approved by the ethics committee of the University of Birmingham.

Stimulus Material

Word stimuli consisted of 270 nouns derived from the MRC Psycholinguistic Database, Version 2.00 [44] and were presented in black. These were divided into 18 lists of 15 words and were matched for word frequency, word length, number of letters, number of syllables, concreteness, and imaginability. Face stimuli consisted of 270 faces drawn from several face databases. The faces were emotionally neutral and were presented in black and white on a black background. These were divided into 18 lists of 15 stimuli and were matched for gender, hair colour, and approximate age. Stimuli were presented in a randomized order, and counterbalanced across subjects. 360 stimuli (180 words, 180 faces) were presented during encoding and retrieval, serving as old items in the retrieval period, 180 stimuli (90 words, 90 faces) were presented during retrieval only, serving as new items (figure 1).

Experimental Setup and Procedure

Participants were seated approximately 80cm from a 19 inch LCD monitor (resolution: 1280 X 1024 pixels, 60Hz frame rate). Stimuli were presented on a grey background on the centre of the screen using the Psychophysics Toolbox extension for Matlab [45].

Before the start of the main experiment participants were familiarized with tACS and desensitized to the stimulation intensity in order to avoid adverse reactions.

Encoding

During encoding, participants had to perform a pleasantness rating of a presented stimulus on a 4-point rating scale (very pleasant – very unpleasant). Answers were given manually by pressing one of four buttons on a computer keyboard using the middle and index finger of both hands; whether the left or right hand corresponded to the *pleasant* or *unpleasant* category was counterbalanced across subjects (figure 1A). During the 2s stimulus presentation, tACS was administered to the left and right IFG at different frequencies. In order to replicate and extend the findings from Hanslmayr et al. [30], tACS was applied at 18.5Hz. Furthermore, the two control frequencies used in Hanslmayr et al. [30] (6.8Hz, 10.7Hz) plus two higher frequencies (30Hz, 48Hz) as well as sham stimulation were chosen as controls, resulting in 15 trials per condition. The sequence of the stimulation conditions was counterbalanced across subjects and pseudo randomized so that the same frequency and the same stimulation site did not occur in more than four consecutive trials.

Retrieval

Following the encoding section, two distractor tasks were used to ensure that the participants did not rehearse the study material. First, the participants were required to count aloud backwards in steps of seven from a 3-digit number for 1min, after which time

they were asked to rate the intensity of the stimulation induced sensations and phosphenes for every stimulation condition separately (see supplementary figure 4). These tasks were followed by the recognition phase. Here, the 360 items presented during encoding, along with 180 new items were presented in a randomized sequence. Subjects were asked to rate how confident they were that an item was old or new on a 4-point rating scale ranging from *very sure old* to *very sure new* (figure 1B). Answers were given manually by pressing one of four buttons on a computer keyboard using the middle and index finger of both hands; whether the left or right hand corresponded to *old* or *new* items was counterbalanced across subjects.

Transcranial Alternating Current Stimulation

In order to investigate the effects of electrode size while keeping the current density underneath the electrodes, and possible skin sensations, comparable, experiments 1 and 2 only differed with respect to electrode size and stimulation intensity. In experiment 1, the stimulation was applied via four donut-shaped rubber electrodes with a diameter of 5cm (14 cm2, NeuroConn, Ilmenau, Germany) at an intensity of 1mA (2mA peak to peak) [46,47]. The unconventional donut shape was chosen to allow measuring the tACS artefact directly from the centre of the stimulation electrodes in future EEG follow-up studies. In experiment 2, the stimulation was applied using round rubber electrodes with a diameter of 3.7cm (10.75 cm2, NeuroConn, Ilmenau, Germany) at an intensity of 0.8mA (1.6mA peak to peak). The resulting estimated current density in the skin underneath the electrodes was in both experiments of approximately 0.07mA/cm2. Transcranial alternating current stimulation was delivered via a 4 channel DC Stimulator MC (NeuroConn, www.neuroconn.de). In both experiments, the stimulation electrodes were placed at EEG electrode positions FP1, C5,

FP2, C6 (figure 2). These positions were selected using a neuro-targeting software (Soterix Medical Inc, New York, USA) which uses a finite-element model of a template adult brain to model the current distribution in the brain. Stimulation sites were chosen to result in the highest target field intensity in the left inferior frontal gyrus (figure 2A). In order to keep the sensations equal between stimulation conditions, the placement for the right IFG stimulation was derived by mirroring the montage for the left IFG stimulation onto the right hemisphere (figure 2B). Impedances were kept below 5kOhm using Ten20 conductive paste (Weaver and Company, Aurora/Colorado). In both experiments, tACS was applied at 6.8Hz, 10.7Hz, 18.5Hz, 30Hz, and 48Hz for 2s at stimulus onset during encoding (see figure1). Additionally, sham stimulation was applied. During sham stimulation, the current was ramped up and down at the beginning and at the end of the 2s stimulation period for around 300ms in all of the five stimulation frequencies.

Data analysis

Correctly identified old stimuli (*hits*) were classified using a receiver operating characteristic (ROC) procedure. In order to control for individual response biases, every subject's neutral response criterion was determined indicating which buttons a participant used for an old response and thus providing a bias free measure of memory strength [8]. This was being accomplished by plotting the diagonal between a Hit rate of 1 and False Alarm rate of 1 in addition to each subject's ROC curve. If the false alarm rate of a given button was lower than the crossing point of the diagonal and the ROC curve, old items associated with this button were classed as hits. Likewise if the false alarm rate of a response was higher than the crossing point, old items associated with this response were classed as misses.

The effects of tACS on memory performance were investigated for verbal and non-verbal material separately using ANOVAs with the within-subjects factors *Stimulation Site, Stimulation Frequency,* and with the between-subjects factor *Experiment.* Additionally, Bayesian analyses were conducted in order to further investigate the amount of evidence for the null and alternative hypotheses [48,49]. The resulting Bayes factors (BF_{01}) indicate how likely it is that the present data can be observed under the null hypothesis (there is no difference between the conditions) as compared to the alternative hypothesis (there is a difference between the conditions) (see supplementary material).

Results

A 3-way ANOVA revealed no interaction between the stimulation frequency, stimulation site, and experiment for verbal material, F(5,350)=0.987, p=0.426. Hence, data of both experiments were merged into one dataset (figure 3A). No interaction between stimulation frequency and stimulation site could be observed, F(5,350) = 1.7, *p*=0.134. We specifically expected a difference between left 18.5Hz stimulation and left sham stimulation [30]. However, the t-test indicated no significant difference between these two conditions; t(71)=0.204, *p*=0.839.

A 3-way ANOVA for non-verbal material revealed no interaction between stimulation frequency, stimulation site, and experiment F(5,350)=0.992, *p*=0.423. Therefore, data of both experiments were also combined (figure 3B). No interaction between stimulation frequency and stimulation site could be found, F(5,350)= 1.11; *p*=0.355. There was no

difference between right 18.5Hz stimulation and right sham stimulation; t(71)=-1.377, *p*=0.173.

Memory performance for both experiments separately is depicted in supplementary figures 1 and 2. D' values and ROC curves for sham stimulation are shown in supplementary table 1 and supplementary figure 3.

Bayesian statistics

In contrast to Hanslmayr et al. [30], we did not find an effect of left beta stimulation on memory performance for verbal material. Likewise, we did not find effects of right beta tACS on memory performance for non-verbal material. However, traditional null-hypothesis testing cannot provide us with evidence for the absence of an effect. Therefore Bayesian analyses were conducted (JASP Team (2016). JASP (Version 0.8.0.0)[Computer software]) [48].

We specifically expected left 18.5Hz tACS to decrease memory performance for words while right 18.5Hz stimulation should have resulted in decreased memory performance for faces. To quantify evidence for equivalence between conditions, we computed a one-sided JSZ Bayes Factor comparing left 18.5Hz and sham stimulation with default prior scales (r=.707) for verbal material. This comparison revealed substantial evidence for the Null, BF_{01} = 8.982, demonstrating that the data were 8.982 times more likely under the null than under the alternative hypothesis. For non-verbal material, the one-sided JSZ Bayes Factor comparing right 18.5Hz and sham stimulation with default prior scales (r=.707) revealed anecdotal evidence for the Null, BF_{01} = 1.719, demonstrating that the data were 1.719 times more likely under the null than under the alternative hypothesis.

In showing evidence that left beta tACS did not have an effect on memory performance for words, our results conflict with the earlier demonstration by Hanslmayr et al. [30] of a significant difference in memory performance for verbal material between left beta rTMS and sham stimulation. To provide a more direct test as to whether our findings have failed to replicate this study, we also computed a Replication Bayes factor [49] that was calibrated to quantify whether the present results are more congruent with no difference or with a difference comparable to that observed by Hanslmayr et al. [30]. This comparison also revealed strong evidence in favour of the Null, BF01 = 11.89, suggesting that the present experiment has failed to replicate that earlier finding.

Experiment 3

Material and Methods

Participants

Eight participants completed the experiment (all male; mean age: 29.375 +- 4.93 years).

Participants were screened for contraindications against tACS and TMS prior to the experiment [31,43]. All participants were right handed, had normal or corrected to-normal vision and reported no history of neurological disease or brain injury. Informed consent was acquired from each subject prior to the experiment. The study was approved by the ethics committee of the University of Birmingham.

Experimental Setup and Procedure

Due to safety considerations, the experiment was split into two sessions consisting of the same experimental procedure. The break between the sessions was controlled so that both sessions took place at two consecutive days at the same time of the day.

Determination of each participant's motor beta

Before the start of each session, the participants' individual motor beta frequency was determined using a finger tapping task. After a rest period of 2min, participants were asked to tap with the fingers of their right hand prompted by corresponding numbers on the screen for 2min. During this task, EEG was recorded using Ag-AgCl scalp electrodes (NeuroConn, Ilmenau, Germany) at 1000Hz sampling rate over C3, C4, Pz and Cz, referenced to the right mastoid. The EEG recordings were off-line re-referenced against Pz. The data from the tapping and rest condition were subjected to a multitaper frequency transformation using Hanning tapers and then subtracted from each other. As beta power decreases more over contralateral electrodes during the execution of movement as compared to rest [6], the individual motor beta frequency was determined as the frequency in the beta range (13Hz-30Hz) that showed the strongest power decrease in C3 compared to C4.

Transcranial Magnetic Simulation

Transcranial magnetic stimulation (TMS) was delivered with a Magstim Rapid stimulator via a 70mm double coil (magstim; www.magstim.com) to the left motor cortex at 110% motor threshold (identified without active tACS, but over the tACS electrode). The stimulation site (M1) was defined as the position on the scalp that elicited the strongest MEP response: The coil was angled 45° from the midline axis of the participant's head with the handle pointing

backwards. MEPs were recorded from different points at the scalp in order to obtain the position that elicited the strongest response. Motor thresholds were estimated using a modified binary search [50] with amplitude changes of 100 µA peak-to-peak or more being considered an MEP. In every session 420 TMS pulses were delivered randomly every 2.5s – 4.5s (at an average inter stimulus interval of 3.5s) [12] throughout the experiment. The TMS pulses were triggered using an in-house Matlab script triggering the TMS via a USB Data Acquisition Device (Measurement Computing).

Transcranial Alternating Current Stimulation

TACS was delivered via a 4 channel DC Stimulator MC (NeuroConn, www.neuroconn.de). The stimulation was applied using round rubber electrodes with a diameter of 3.7cm (10.75 cm2, NeuroConn, Ilmenau, Germany) at an intensity of 0.7mA (1.4mA peak to peak), resulting in an estimated current density in the skin underneath the electrodes of 0.065 mA/cm2. Two electrode montages were used in order to investigate the efficiency of montages with one electrode directly over the target area [23,34] as compared to montages with the target area in between the stimulation electrodes (as used in the previous tACS memory experiments presented above). Three tACS electrodes were used, which were placed at M1 and EEG electrode positions Pz and Fp1, resulting in two electrode montages. For montage 1 current was being passed between M1 and Pz [23,32], whereas for montage 2, current was being passed between Fp1 and Pz. This setup allowed us to use the same reference electrode, Pz, in both stimulation conditions. In this way a randomized stimulation protocol could be used with only 3 stimulation electrodes. The montage was chosen randomly on a trial-by-trial basis with the restriction of the same montage not occurring in

more than four consecutive trials. Impedances were kept below 5kOhm using Ten20 conductive paste (Weaver and Company, Aurora/Colorado).

tACS-TMS Procedure

During the experiment, participants were seated comfortably in front of a computer screen. No task was involved. Subjects were instructed to keep their hands as relaxed as possible, while looking at a fixation cross in the centre of the screen. Single pulse TMS was delivered throughout the experiment over the tACS electrode placed at M1 (figure 4), while participants received tACS at their individual motor beta frequency. tACS was applied for 10s, followed by a 10s period without stimulation. The electrode montage with which the stimulation was delivered (i.e. FP1-Pz or M1-Pz), was pseudorandomised so that the same montage was never repeated more than twice. Motor evoked potentials were measured from the first dorsal interosseous (FDI) muscle of the right hand using Ag-AgCl EEG electrodes (BrainAmp MR plus, Brainvision). Every 17-18 trials, participants were given a slight break and the TMS coil was cooled down, resulting in four tACS-TMS blocks per session. The tACS artefact was recorded from one Ag-AgCl EEG electrode placed at Cz, referenced to the right mastoid.

Data Analysis

Data were analysed using FieldTrip [51], the CircStat toolbox [52], and in-house MATLAB scripts. As the tACS stimulator was mains operated while the amplifier used to record the MEPs was battery operated, the different power supplies (different current draws) between these two systems resulted in high levels of noise in the MEP data. Hence, the peak-to-peak amplitudes of the motor evoked potentials were not easily accessible (see supplementary

figure 5). Therefore, MEP data (-0.15s to 0.15s around the TMS pulse) were subjected to a time-frequency composition (20-1000Hz, steps of 5Hz) using Morlet wavelets (width 7) and baseline corrected (baseline window: -0.15s to -0.05s). MEP amplitude was defined as the peak of the mean signal change between 20Hz and 50Hz, 0-50ms following the TMS pulse, which revealed clear MEPs (see supplementary figure 6 and 7). In order to adjust for noise introduced by the breaks and possible changes in position of the TMS coil, MEP amplitudes in every block were z-transformed, ensuring that data from every block were comparable. To extract phase angles of tACS, EEG data recorded from electrode Cz were Hilbert transformed. Due to the TMS artefact distorting the phase estimates (see supplementary figure 9), phase angles were extracted 5ms prior to the TMS pulse. In order to test for phase entrainment effects, MEP amplitudes of every single trial were correlated with the tACS phase 5ms prior to the TMS pulse. These circular to linear correlations between the normalised MEP amplitudes and tACS phase were calculated as implemented in the circular statistics toolbox [52]. Additionally, the data were binned into four different tACS phase bins centred around 0° (*peak)*, 90° (*falling flank*), 180° (*trough*), 270° (*rising flank*) (see supplementary figure 11) [35], and normalised MEP amplitudes at those tACS phase bins were subjected to a repeated measures ANOVA.

Results

Normalised single trial MEP amplitude by tACS phase collapsed across both montages is shown in figure 5B. Circular to linear correlations revealed no correlation between MEP amplitude and tACS phase; overall: $_{cl}$ = 0.0249, *p*=0.4021; Montage 1: $_{cl}$ = 0.0167, *p*=0.8141 (supplementary figure 12A); Montage 2: $_{cl}$ = 0.0442, *p*=0.2394 (supplementary figure 12B).

A 3-way ANOVA with the factors *Session, Montage* and *Phase bin* showed no main effect of the tACS phase (figure 6); F(3,21)=0.223, *p*=0.880. No interaction between the phase bins and tACS montage could be found either, F(3,21)=0.730, *p*=0.546, however, there was a trend towards a main effect for tACS montage with higher MEP amplitudes at M2 (FP1-Pz) than M1 (M1-Pz); F(1,7)=5.338, p=0.054 (see supplementary material). Averaging over the 4 phase bins for montage 1 and 2 separately reveals that overall, there was no significant difference in MEP size between tACS trials in either of the montages and no-tACS trials (figure 5A); Montage 1 (M1-Pz): t(7)=-0.949, *p*=0.374; Montage 2 (FP1-Pz): t(7)=1.121, *p*=0.299 .

Individual motor beta frequencies and resting motor thresholds are depicted in supplementary table 2. The results of the analysis on MEP amplitude prior to z-transformation are shown in the supplementary material.

Bayesian statistics

We expected MEP amplitude to be modulated by the tACS phase. A JZS Bayes factor ANOVA [53] with default prior scales revealed strong evidence for the null compared to the main effects model *Phase bin,* BF_{01} = 20.246.

Discussion

Experiments 1 and 2

In experiments 1 and 2, we aimed to examine whether event-related, randomized tACS can be used to modulate beta oscillations during episodic memory formation. We specifically

expected 18.5Hz tACS to decrease memory performance only for words when administered to the left IFG while right 18.5Hz stimulation should have resulted in decreased memory performance for non-verbal material only. Although similar protocols using TMS were able to show that left beta stimulation impairs encoding of verbal material [30], the present study failed to show such an effect. Beta tACS did not modulate the formation of episodic memories when applied in a temporally sensitive, event-related, randomized manner. This could be partially due to the lower number of trials per condition in these experiments compared to the rTMS study. However, a considerably higher number of participants was tested in order to account for this. Additionally, Bayesian analyses also reveal evidence for the null effect. Therefore, these results indicate that, at this point, tACS in the beta frequency is not a suitable alternative to TMS. As tACS affects neurons in a more subtle fashion than TMS [42], tACS might not be strong enough to interfere with underlying oscillatory activity in such a short period of time [54].

Experiment 3

The aim of experiment 3 was to examine if it is possible to modulate corticospinal excitability via entrainment of beta oscillations in the primary motor cortex using tACS. By applying tACS to M1 at the individual motor beta frequency, we investigated the relationship between TMS induced MEPs and tACS phase. As in the first two experiments and as previous studies indicate [35], we did not find a clear entrainment effect; the MEP size was not modulated by the tACS phase. We believe that this is due to the rather short tACS stimulation period (10s) we used. Studies that reported phase effects of beta tACS on MEP size used longer stimulation periods [35–37], whereas MEP size was not modulated

when applying 30s of slow oscillatory tDCS [55]. Although other studies investigating motor cortex excitability using tDCS had sample sizes similar to this study [55–57], we cannot rule out that a higher number of participants could have been beneficial for finding a phase effect of beta tACS on MEP amplitude. Future studies should therefore consider testing more subjects in order to receive stable results.

Conclusion

During cognitive tasks brain oscillations, particularly those in the beta frequency range (13-30Hz), show a highly dynamic behaviour. For instance beta oscillations decrease in power within a couple of milliseconds during memory processing or movement execution followed by a subsequent increase in amplitude. Such dynamic processes can be studied under constant stimulation (e.g. throughout a cognitive task) in order to reveal state-dependent effects. But to answer causal questions about these specific time sensitive oscillatory processes, our findings would need to show that tACS at beta is capable of modulating oscillatory behaviour in a similar time range. In the series of experiments reported here however, we found that event-related, randomized, transient tACS in the beta frequency range does not modulate the formation of episodic memories or motor cortex excitability.

This failure to find an effect of beta tACS on cognition or cortical excitability—and the finding that such statistically positive effects are unlikely—reveals that the effectiveness of tACS is a complex issue. On the one hand, tACS applied over minutes appears to be effective in modulating behaviour and brain oscillations [15–18,20–22,35]. And indeed, if tACS is used to synchronize and desynchronize distant brain regions, shorter stimulation durations (1s-

1.8s) in other frequencies seem to be successful [26]. On the other hand, the present findings indicate that tACS applied in the range of seconds in order to modulate brain oscillations in one brain area is not effective [54,58]. Before being able to utilize beta tACS to draw conclusions about the causal relationship between oscillatory brain activity and cognitive processes, several issues regarding the use of beta tACS protocols need to be addressed, such as current distribution in the brain, optimal electrode placement, recommended stimulation intensities, recommended stimulation durations etc. Though a growing body of modelling studies addresses these issues [46,59–62], the respective models have to be validated extensively by experimental data, before it will be possible to apply tACS more effectively in cognitive research [63]. Event-related beta tACS could then be a useful and promising method. Yet as long as these problems remain unsolved, tACS may remain ineffective in unravelling the causal relationship between transient beta oscillatory activity and cognitive function.

Acknowledgments

This work was supported by grants awarded to S.H. by Deutsche Forschungsgemeinschaft [Emmy Noether Programme Grant HA 5622/1-1]; and the European Research Council [Consolidator Grant Agreement 647954]. We would like to thank Jonathan Fawcett for his advice on Bayesian analyses.

References

[1] Fries P. A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 2005;9:474–80. doi:10.1016/j.tics.2005.08.011.

[2] Hanslmayr S, Staudigl T, Fellner M-C. Oscillatory power decreases and long-term memory: the information via desynchronization hypothesis. Front Hum Neurosci 2012;6:74. doi:10.3389/fnhum.2012.00074.

[3] Brittain J-S, Brown P. Oscillations and the basal ganglia: motor control and beyond. Neuroimage 2014;85 Pt 2:637–47. doi:10.1016/j.neuroimage.2013.05.084.

[4] Kuhlman WN. Functional topography of the human mu rhythm. Electroencephalogr Clin Neurophysiol 1978;44:83–93. doi:10.1016/0013-4694(78)90107-4.

[5] Pfurtscheller G. Event-related synchronization (ERS): an electrophysiological correlate of cortical areas at rest. Electroencephalogr Clin Neurophysiol 1992;83:62–9. doi:10.1016/0013-4694(92)90133-3.

[6] Pfurtscheller G, Neuper C, Andrew C, Edlinger G. Foot and hand area mu rhythms. Int J Psychophysiol 1997;26:121–35. doi:10.1016/S0167-8760(97)00760-5.

[7] Klimesch W. Memory processes, brain oscillations and EEG synchronization. Int J Psychophysiol 1996;24:61–100. doi:10.1016/S0167-8760(96)00057-8.

[8] Hanslmayr S, Spitzer B, Bäuml K-H. Brain oscillations dissociate between semantic and nonsemantic encoding of episodic memories. Cereb Cortex 2009;19:1631–40. doi:10.1093/cercor/bhn197.

[9] Meeuwissen EB, Takashima A, Fernández G, Jensen O. Evidence for Human Fronto-

Central Gamma Activity during Long-Term Memory Encoding of Word Sequences. PLoS One 2011;6:e21356. doi:10.1371/journal.pone.0021356.

[10] Hanslmayr S, Volberg G, Wimber M, Raabe M, Greenlee MW, Bäuml K-HT. The relationship between brain oscillations and BOLD signal during memory formation: a combined EEG-fMRI study. J Neurosci 2011;31:15674–80. doi:10.1523/JNEUROSCI.3140-11.2011.

[11] Schulz H, Ubelacker T, Keil J, Müller N, Weisz N. Now I am ready-now i am not: The influence of pre-TMS oscillations and corticomuscular coherence on motor-evoked potentials. Cereb Cortex 2014;24:1708–19. doi:10.1093/cercor/bht024.

[12] Keil J, Timm J, Sanmiguel I, Schulz H, Obleser J, Schönwiesner M. Cortical brain states and corticospinal synchronization influence TMS-evoked motor potentials. J Neurophysiol 2014;111:513–9. doi:10.1152/jn.00387.2013.

[13] Herrmann CS, Rach S, Neuling T, Strüber D. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front Hum Neurosci 2013;7:279. doi:10.3389/fnhum.2013.00279.

[14] Witkowski M, Garcia-Cossio E, Chander BS, Braun C, Birbaumer N, Robinson SE, et al. Mapping entrained brain oscillations during transcranial alternating current stimulation (tACS). Neuroimage 2015. doi:10.1016/j.neuroimage.2015.10.024.

[15] Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr Biol 2014;24:333–9. doi:10.1016/j.cub.2013.12.041.

[16] Polanía R, Nitsche MA, Korman C, Batsikadze G, Paulus W. The importance of timing in segregated theta phase-coupling for cognitive performance. Curr Biol 2012;22:1314–8. doi:10.1016/j.cub.2012.05.021.

[17] Jaušovec N, Jaušovec K. Increasing working memory capacity with theta transcranial alternating current stimulation (tACS). Biol Psychol 2014;96:42–7. doi:10.1016/j.biopsycho.2013.11.006.

[18] Jaušovec N, Jaušovec K, Pahor A. The influence of theta transcranial alternating current stimulation (tACS) on working memory storage and processing functions. Acta Psychol (Amst) 2014;146:1–6. doi:10.1016/j.actpsy.2013.11.011.

[19] van Driel J, Sligte IG, Linders J, Elport D, Cohen MX. Frequency Band-Specific Electrical Brain Stimulation Modulates Cognitive Control Processes. PLoS One 2015;10:e0138984. doi:10.1371/journal.pone.0138984.

[20] Zaehle T, Rach S, Herrmann CS. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One 2010;5:e13766. doi:10.1371/journal.pone.0013766.

[21] Neuling T, Rach S, Herrmann CS. Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. Front Hum Neurosci 2013;7:161. doi:10.3389/fnhum.2013.00161.

[22] Brittain J-S, Probert-Smith P, Aziz TZ, Brown P. Tremor suppression by rhythmic transcranial current stimulation. Curr Biol 2013;23:436–40. doi:10.1016/j.cub.2013.01.068.

[23] Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi A, Rossi S. State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. J Neurosci 2013;33:17483–9. doi:10.1523/JNEUROSCI.1414-13.2013.

[24] Schmidt SL, Iyengar AK, Foulser a A, Boyle MR, Fröhlich F. Endogenous cortical oscillations constrain neuromodulation by weak electric fields. Brain Stimul 2014;7:878–89. doi:10.1016/j.brs.2014.07.033.

[25] Ali MM, Sellers KK, Fröhlich F. Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. J Neurosci 2013;33:11262–75. doi:10.1523/JNEUROSCI.5867-12.2013.

[26] Stonkus R, Braun V, Kerlin JR, Volberg G, Hanslmayr S. Probing the causal role of prestimulus interregional synchrony for perceptual integration via tACS. Sci Rep 2016;6:32065. doi:10.1038/srep32065.

[27] Ruhnau P, Keitel C, Lithari C, Weisz N, Neuling T. Flicker-Driven Responses in Visual Cortex Change during Matched-Frequency Transcranial Alternating Current Stimulation. Front Hum Neurosci 2016;10:184. doi:10.3389/fnhum.2016.00184.

[28] van Driel J, Sligte IG, Linders J, Elport D, Cohen MX. Frequency Band-Specific Electrical Brain Stimulation Modulates Cognitive Control Processes. PLoS One 2015;10:e0138984. doi:10.1371/journal.pone.0138984.

[29] Kim H. Neural activity that predicts subsequent memory and forgetting: a meta-analysis of 74 fMRI studies. Neuroimage 2011;54:2446–61. doi:10.1016/j.neuroimage.2010.09.045.

[30] Hanslmayr S, Matuschek J, Fellner M-C. Entrainment of prefrontal beta oscillations induces an endogenous echo and impairs memory formation. Curr Biol 2014;24:904–9. doi:10.1016/j.cub.2014.03.007.

[31] Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 1998;108:1–16. doi:10.1016/S0168-5597(97)00096-8.

[32] Floel A. Prefrontal Cortex Asymmetry for Memory Encoding of Words and Abstract Shapes. Cereb Cortex 2004;14:404–9. doi:10.1093/cercor/bhh002.

[33] Kelley WM, Miezin FM, McDermott KB, Buckner RL, Raichle ME, Cohen NJ, et al. Hemispheric Specialization in Human Dorsal Frontal Cortex and Medial Temporal Lobe for Verbal and Nonverbal Memory Encoding. Neuron 1998;20:927–36. doi:10.1016/S0896-6273(00)80474-2.

[34] Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S. Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J Neurosci 2011;31:12165–70. doi:10.1523/JNEUROSCI.0978-11.2011.

[35] Raco V, Bauer R, Tharsan S, Gharabaghi A. Combining TMS and tACS for Closed-Loop Phase-Dependent Modulation of Corticospinal Excitability: A Feasibility Study. Front Cell Neurosci 2016;10:1–8. doi:10.3389/fncel.2016.00143.

[36] Nakazono H, Ogata K, Kuroda T, Tobimatsu S, Buzsáki G, Draguhn A, et al. Phase and Frequency-Dependent Effects of Transcranial Alternating Current Stimulation on Motor Cortical Excitability. PLoS One 2016;11:e0162521.

doi:10.1371/journal.pone.0162521.

[37] Guerra A, Pogosyan A, Nowak M, Tan H, Ferreri F, Di Lazzaro V, et al. Phase Dependency of the Human Primary Motor Cortex and Cholinergic Inhibition Cancelation During Beta tACS. Cereb Cortex 2016;26:3977–90. doi:10.1093/cercor/bhw245.

[38] Barker AT, Jalinous R, Freeston IL. NON-INVASIVE MAGNETIC STIMULATION OF HUMAN MOTOR CORTEX. Lancet 1985;325:1106–7. doi:10.1016/S0140-6736(85)92413-4.

[39] Pascual-Leone A, Tormos JM, Keenan J, Tarazona F, Cañete C, Catalá MD. Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol 1998;15:333–43.

[40] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527:633–9. doi:10.1111/j.1469-7793.2000.t01-1-00633.x.

[41] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57:1899–901. doi:10.1212/WNL.57.10.1899.

[42] Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: State of the art 2008. Brain Stimul 2008;1:206–23. doi:10.1016/j.brs.2008.06.004.

[43] Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current

stimulation concerning healthy subjects and patients. Brain Res Bull 2007;72:208–14. doi:10.1016/j.brainresbull.2007.01.004.

[44] Coltheart M. The MRC psycholinguistic database. Q J Exp Psychol A Hum Exp Psychol 1981;33A:497–505. doi:10.1080/14640748108400805.

[45] Brainard DH. The Psychophysics Toolbox. Spat Vis 1997;10:433–6. doi:10.1163/156856897X00357.

[46] Mehta AR, Pogosyan A, Brown P, Brittain J-S. Montage matters: the influence of transcranial alternating current stimulation on human physiological tremor. Brain Stimul 2015;8:260–8. doi:10.1016/j.brs.2014.11.003.

[47] Brittain J-S, Probert-Smith P, Aziz TZ, Brown P. Tremor suppression by rhythmic transcranial current stimulation. Curr Biol 2013;23:436–40. doi:10.1016/j.cub.2013.01.068.

[48] Rouder JN, Speckman PL, Sun D, Morey RD, Iverson G. Bayesian t tests for accepting and rejecting the null hypothesis. Psychon Bull Rev 2009;16:225–37. doi:10.3758/PBR.16.2.225.

[49] Verhagen J, Wagenmakers E-J. Bayesian tests to quantify the result of a replication attempt. J Exp Psychol Gen 2014;143:1457–75. doi:10.1037/a0036731.

[50] Tyrrell RA, Owens DA. A rapid technique to assess the resting states of the eyes and other threshold phenomena: The Modified Binary Search (MOBS). Behav Res Methods, Instruments, Comput 1988;20:137–41. doi:10.3758/BF03203817.

[51] Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for

advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011;2011. doi:10.1155/2011/156869.

[52] Berens P. CircStat : A MATLAB Toolbox for Circular Statistics. J Stat Softw 2009;31:1–21. doi:10.18637/jss.v031.i10.

[53] Rouder JN, Morey RD, Speckman PL, Province JM. Default Bayes factors for ANOVA designs. J Math Psychol 2012;56:356–74. doi:10.1016/j.jmp.2012.08.001.

[54] Strüber D, Rach S, Neuling T, Herrmann CS. On the possible role of stimulation duration for after-effects of transcranial alternating current stimulation. Front Cell Neurosci 2015;9:311. doi:10.3389/fncel.2015.00311.

[55] Bergmann TO, Groppa S, Seeger M, Mölle M, Marshall L, Siebner HR. Acute Changes in Motor Cortical Excitability During Slow Oscillatory and Constant Anodal Transcranial Direct Current Stimulation. J Neurophysiol 2009;102:2303–11. doi:10.1152/jn.00437.2009.

[56] Jeffery DT, Norton JA, Roy FD, Gorassini MA. Effects of transcranial direct current stimulation on the excitability of the leg motor cortex. Exp Brain Res 2007;182:281–7. doi:10.1007/s00221-007-1093-y.

[57] Furubayashi T, Terao Y, Arai N, Okabe S, Mochizuki H, Hanajima R, et al. Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. Exp Brain Res 2008;185:279–86. doi:10.1007/s00221-007-1149-z.

[58] Vossen A, Gross J, Thut G. Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (α-tACS) Reflects Plastic Changes Rather Than

Entrainment. Brain Stimul 2015;8:499–508. doi:10.1016/j.brs.2014.12.004.

[59] Dmochowski JP, Datta A, Bikson M, Su Y, Parra LC. Optimized multi-electrode stimulation increases focality and intensity at target. J Neural Eng 2011;8:46011. doi:10.1088/1741-2560/8/4/046011.

[60] Miranda PC, Lomarev M, Hallett M. Modeling the current distribution during transcranial direct current stimulation. Clin Neurophysiol 2006;117:1623–9. doi:10.1016/j.clinph.2006.04.009.

[61] Saturnino GB, Antunes A, Thielscher A. On the importance of electrode parameters for shaping electric field patterns generated by tDCS. Neuroimage 2015;120:25–35. doi:10.1016/j.neuroimage.2015.06.067.

[62] Opitz A, Paulus W, Will S, Antunes A, Thielscher A. Determinants of the electric field during transcranial direct current stimulation. Neuroimage 2015;109:140–50. doi:10.1016/j.neuroimage.2015.01.033.

[63] Datta A, Truong D, Minhas P, Parra LC, Bikson M. Inter-Individual Variation during Transcranial Direct Current Stimulation and Normalization of Dose Using MRI-Derived Computational Models. Front Psychiatry 2012;3:91. doi:10.3389/fpsyt.2012.00091.

Figure Legends

Figure 1. The experimental design for experiments 1 and 2 is shown. 360 stimuli (180 words, 180 faces) were presented during the encoding block (A). Participants had to rate the pleasantness of a stimulus on a 4-point rating scale *(very pleasant – very unpleasant).* During the 2s stimulus presentation, tACS was administered to the left and right IFG at 6.8Hz, 10.7Hz, 18.5Hz, 30Hz, 48Hz, as well as sham stimulation. The material was counterbalanced across subjects so that every stimulus was paired with every stimulation condition equally often throughout the experiment. During the retrieval block (B) the 360 stimuli presented during encoding as well as 180 new items (90 words, 90 faces) were shown. Subjects were asked to rate their confidence of an item being old or new on a 4-point rating scale (*very sure old - very sure new)*.

Figure 2. Stimulation electrode configurations for the left inferior frontal gyrus (A) and right inferior frontal gyrus (B). Optimal electrode placement for the left IFG (BA 9) was mirrored onto the right hemisphere. Current field intensity is shown using a finite-element model, provided by Soterix Medical Inc. The field intensities are shown for a stimulation of 2mA, whereas in the present experiments stimulation intensities of 1mA and 0.8mA were used.

Figure 3. Memory performance for words (A) and faces (B) split by stimulation condition and stimulation site (data of experiments 1 and 2 combined). Bayesian t-tests indicate no difference in memory performance between left beta stimulation and left sham stimulation for words (A) and between right beta stimulation and right sham stimulation for faces (B). Error bars show standard errors of the mean.

Figure 4. tACS-TMS procedure for experiment 3 is shown. Two different tACS montages were used: M1-Pz and FP1-Pz (A). TMS pulses (depicted in green) were delivered throughout the trial every 2.5s-4.5s to the left motor cortex over the tACS electrode placed at M1. tACS was applied at the individual motor beta frequency (depicted in orange) for 10s followed by a 10s period without tACS (B). MEPs were measured from the first dorsal interosseous (FDI) muscle of the right hand (C). Each session consisted of 70 trials.

Figure 5. (A) Normalised mean MEP amplitude split by tACS condition. Error bars show standard errors of the mean. (B) Single trial MEP size by tACS phase.

Figure 6. Mean normalised MEP amplitude split by 4 different tACS phase bins: 180° (trough), 270° (rising flank), 0° (peak), 90° (falling flank). (A) tACS Montage 1: M1-Pz. (B) tACS Montage 2: FP1-Pz. Error bars show standard errors of the mean.

Figures

Figure 1

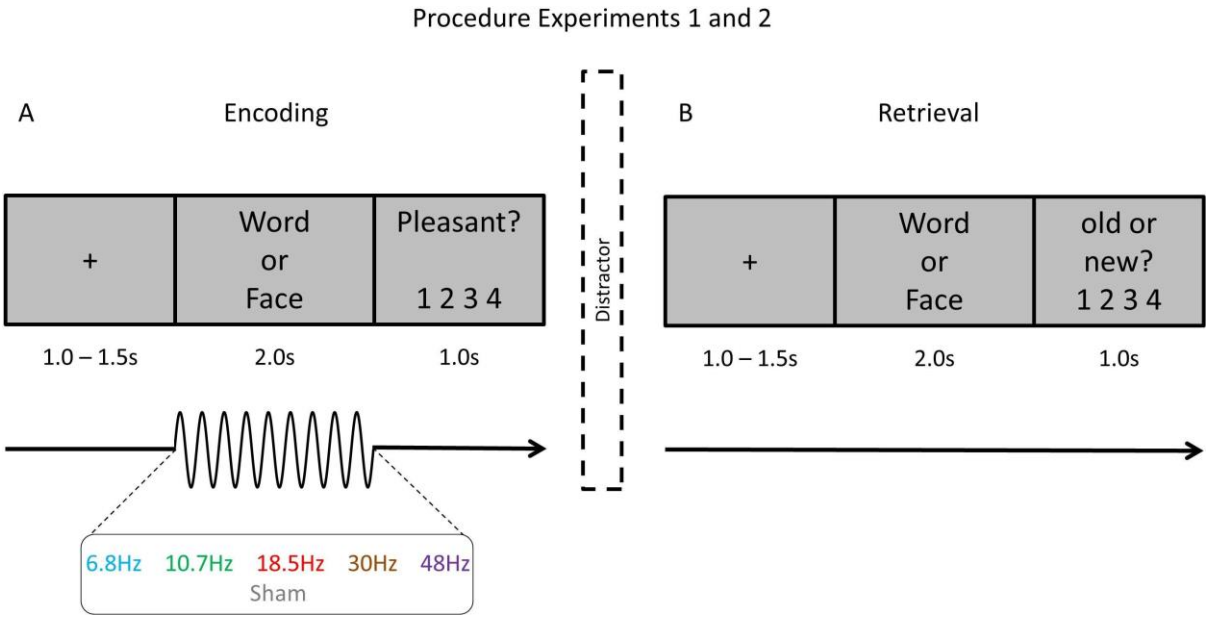

Figure 2

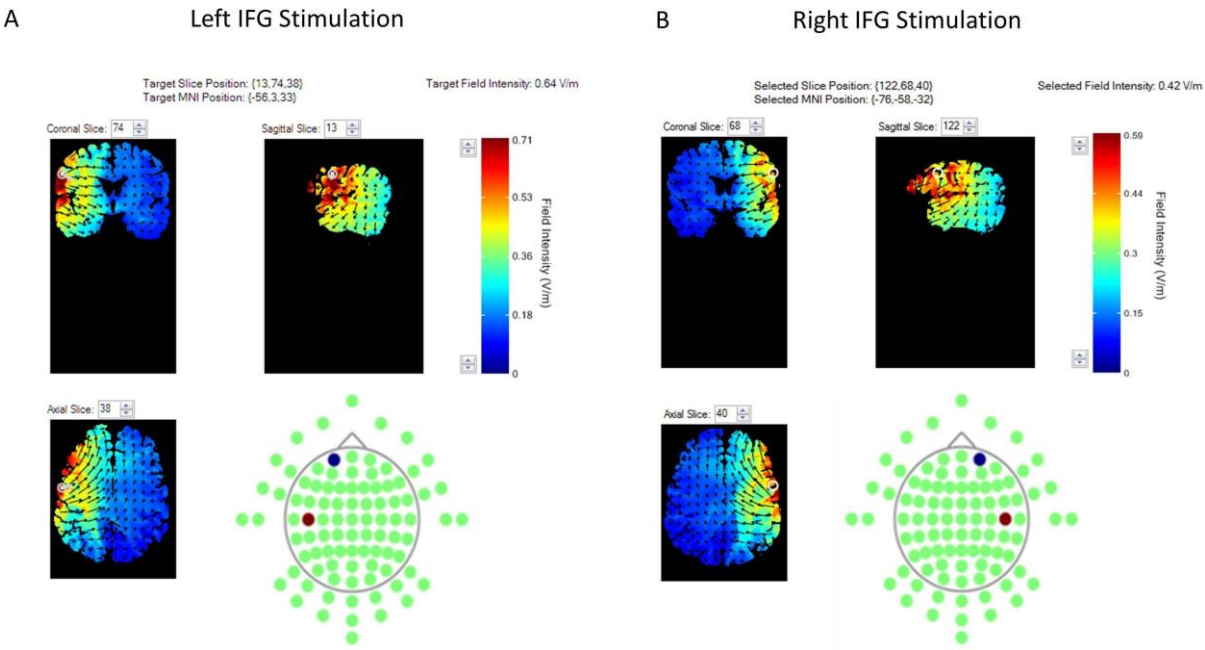

Figure 3

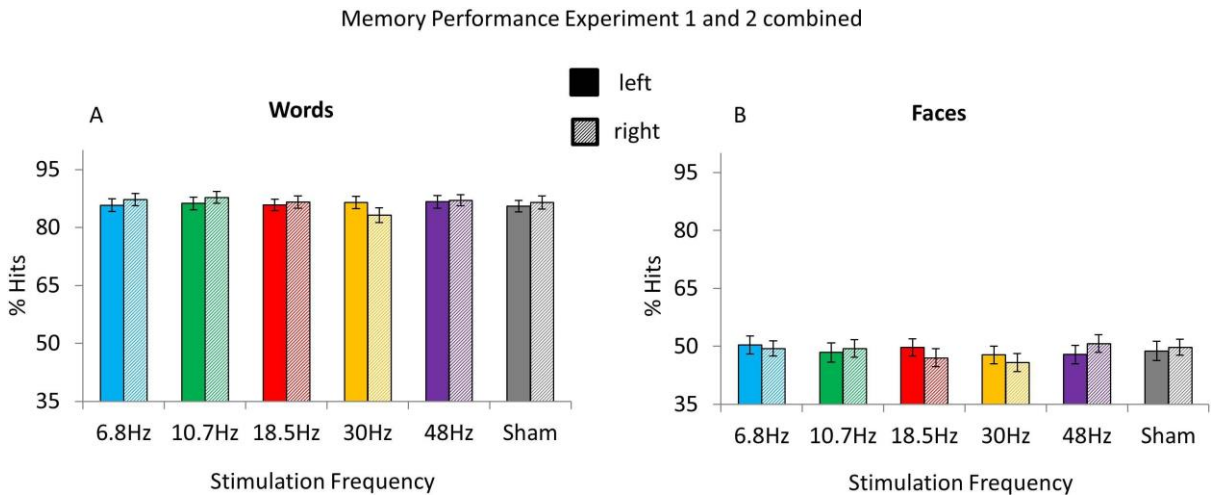

Figure 4

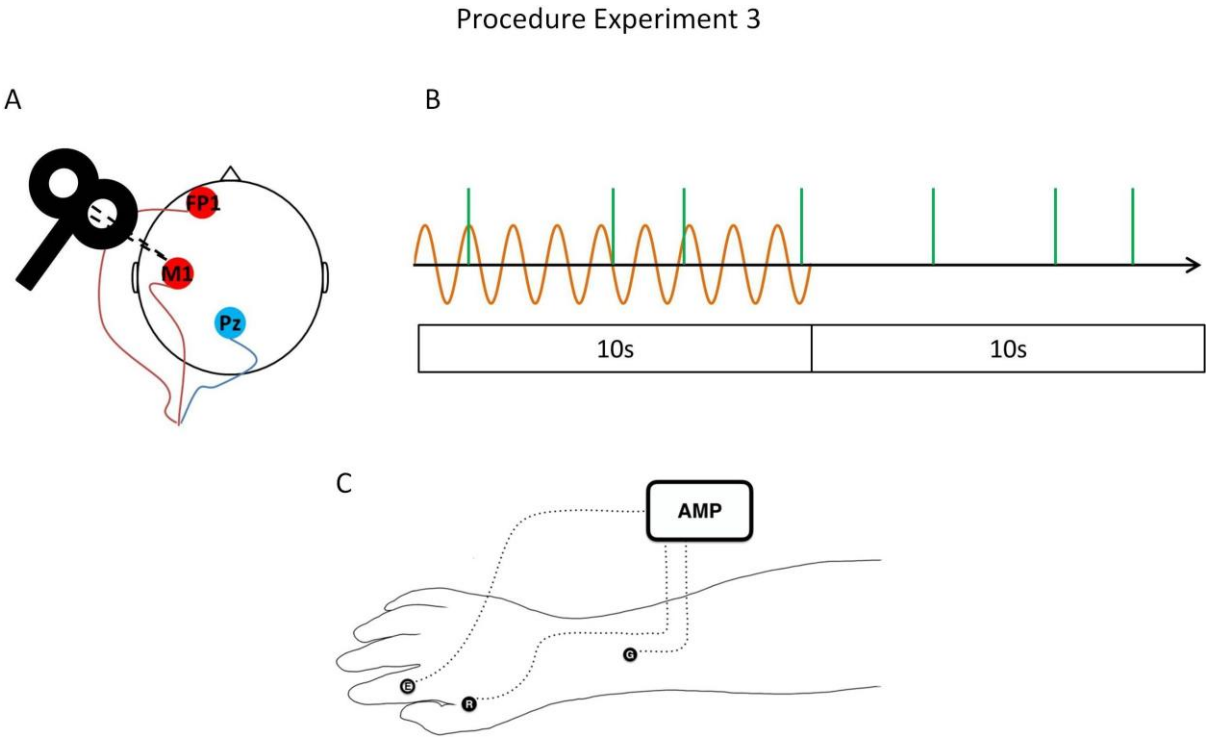

Figure 5

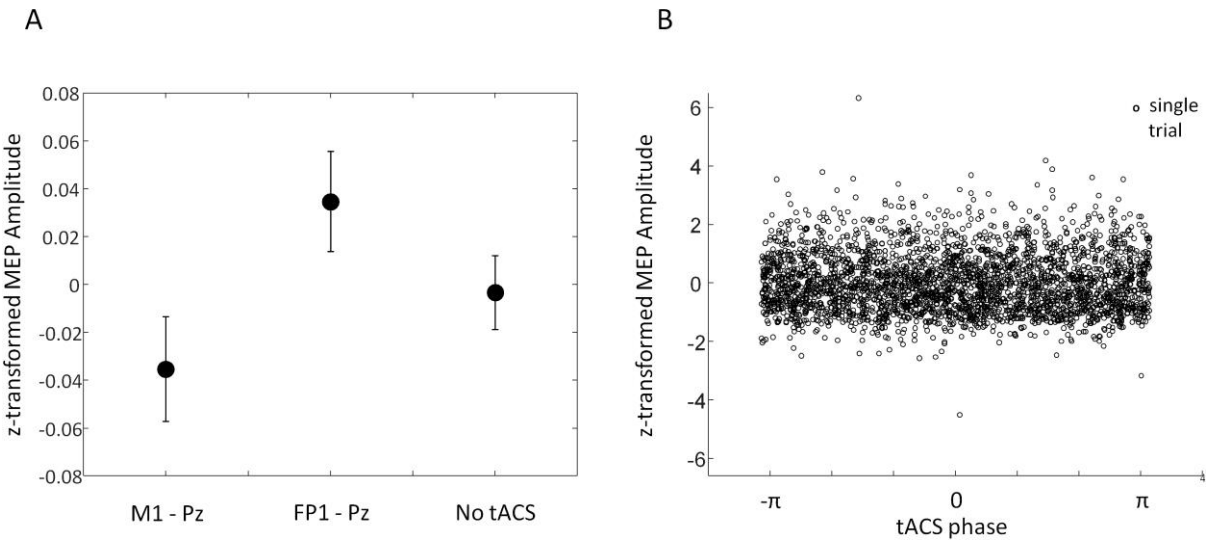
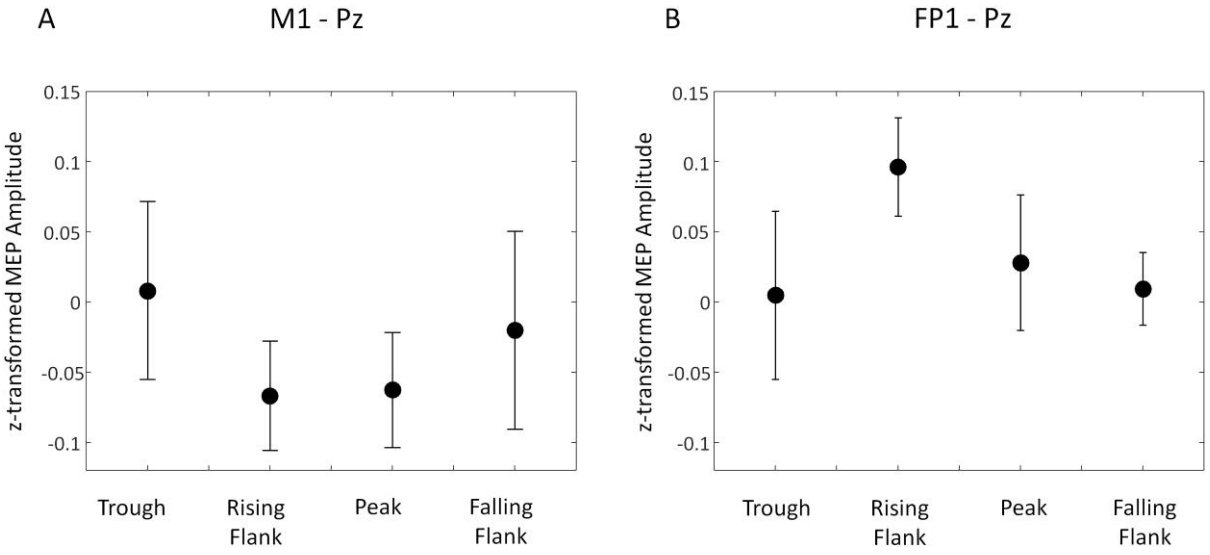
Figure 6