Investigating the Role of Phase-Synchrony During Encoding of Episodic Memories Using Electrical Stimulation

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The multi-sensory nature of episodic memories indicates that communication between a multitude of brain areas is required for their effective creation and recollection. Previous studies have suggested that the effectiveness of memory processes depends on theta synchronization (4Hz) of sensory areas relevant to the memory. This study aimed to manipulate theta synchronization between different sensory areas in order to further test this hypothesis. We intend to entrain visual cortex with 4 Hz alternating current stimulation (tACS), while simultaneously entraining auditory cortex with 4 Hz amplitude-modulated sounds. By entraining these different sensory areas, which pertain to learned audio-visual memory associations, we expect to find that when theta is synchronized across the different sensory areas, the memory performance would be enhanced compared to when theta is not synchronized across the sensory areas. We found no evidence for such an effect in this study. It is unclear whether this is due to an inability of 4Hz tACS to entrain the visual cortex reliably, or whether sensory entrainment is not the underlying mechanism required for episodic memory

tACS,Theta Oscillations,Episodic Memory

1. Introduction

The creation and retrieval of episodic memories depends on communication between a multitude of areas throughout the brain. After information is first received and processed in sensory areas, it is eventually relayed to the hippocampus, a structure that has been implicated in episodic memory processes (Scoville & Milner, 1957). This communication has been suggested to be mediated by oscillatory firing patterns (Parish, Hanslmayr, & Bowman, 2018; Hanslmayr, Staresina, & Bowman, 2016; Hanslmayr, Staudigl, & Fellner, 2012). A prominent frequency range in the hippocampus is the theta rhythm (~4 Hz), which is assumed to be relevant to processes involving episodic memory (Lega, Jacobs, & Kahana, 2012; Jacobs, 2014; Griffiths et al., 2018). Experiments in rodents have shown that certain parts of the theta phase modulate long-term potentiation, which is assumed to be the neural mechanism underlying memory encoding (Hasselmo, 2005).

A recent series of experiments from our lab aimed to show similar effects of theta-phase on memory encoding in humans (Clouter, Shapiro, & Hanslmayr, 2017; Wang et al., 2018). In the first study, video clips accompanied by an arbitrary sound were presented. The participants were instructed to associate the presented videos with the accompanying sound. The videos and sounds were individually theta modulated. For the auditory stimuli, the volume was modulated. For visual stimuli, the luminance was modulated. The visual and auditory information was presented at different phase delays. The video clips could be presented in phase, or out of phase (phase delays of 90°, 180°, and 270°). We found that memory performance was significantly

enhanced for clips where the sounds and videos were in phase compared to the out of phase conditions. In order to control for the possibility that only phase synchrony, independent of theta, drives the memory effect, two non-harmonic frequency bands were introduced as control conditions (1.7 Hz and 10.5 Hz), alongside another stimulation condition with a non-stationary waveform. The memory effect was not observed in any of the control conditions, suggesting that the effect depends on phase synchrony specifically in the theta band. The experiment was replicated in a subsequent study, where we recorded neural activity using electroencephalography during the experiment (EEG) (Wang et al., 2018). In this subsequent study we found that at a single-trial level, strength of entrainment could predict memory performance.

These studies together suggest there is an optimal theta-phase for memory. The underlying assumption is that the flickering stimuli entrain the respective sensory areas within the brain (Rager & Singer, 1998). Based on that assumption we hypothesize that this entrainment ultimately affects how easy the items can be bound and processed in the context of memory. However, given the flickering nature of the stimuli used in these experiments, we cannot exclude the possibility that the observed memory effects are largely driven by inherent properties of our visual system. This mainly relates to findings suggesting that the visual system, but not the auditory system, discretely samples the environment in theta and alpha range frequencies (Landau, & Fries, 2012; VanRullen, Zoefel, & Ilhan, 2014). The implication is that by attempting entrainment with such heavily modulated stimuli, any memory effects might be mediated by purely perceptual effects. If entrainment is indeed the underlying mechanism that produces the observed effects, similar results should be observed when changing the mode of entrainment to a method that modulates the visual system more subtly and is less noticeable to the observer. A method for neural entrainment that has gained prominence in recent years is transcranial alternating current stimulation (tACS). This method is hypothesized to cause neural entrainment by biasing neural populations to fire at certain times, over others, without directly causing any action potentials (Helfrich, et al., 2014a; Antal & Paulus, 2013). This is unlike the flickering stimuli used in the previous studies, which lead to forced overt neural responses in the visual cortex.

The idea for the current study is to attempt entraining the visual system by using tACS over occipital areas while presenting un-modulated videos in unison with theta modulated auditory stimuli. The expectation is that if the previously discussed results are due to entrainment of the sensory modalities, the same effects should be observed, albeit with a smaller effect size since tACS would produce to a more subtle entrainment than a flickering stimulus.

2. Methods

2.1 Participants

In a Bayesian analysis framework it is legitimate to monitor the Bayes factor during data collection, since the Bayes factor is not biased in one direction with increasing sample size (unlike traditional frequentist analysis approaches based on p-values) (Berger & Wolpert, 1988; Rouder et al., 2012; Biel & Friedrich, 2018). Therefore, the number of tested participants was determined by monitoring the Bayes factor of the behavioral memory effect between conditions (with the following maximum and minimum: 30 =< N =< 120). Subjects were tested until the

Bayes factor (BF) either became at least 10 (in favor of the null hypothesis) or < 1/10 (in favor of the alternative hypothesis). A BF > 10 would indicate that the alternative hypothesis is at least 10 times more likely given the null hypothesis, while a BF < 1/10 would indicate the null hypothesis is 10 times more likely than the alternative. These BF values have been chosen based on common practices which are calibrated to produce results at least as stringent as an alpha of 0.02 in traditional frequentist analyses (Jeffreys, 1961).

The tested population was right-handed participants between the ages of 18-35 with normal hearing and normal vision, or corrected-to-normal vision. All participants are screened in accordance to the tACS safety guidelines. They should have no history of any neuropsychiatric disorders or abnormalities, no active implants, and no recent use or previous dependency of any drugs. Participants have been recruited using the University of Birmingham Research Participation scheme at the University of Birmingham as well as flyers and posters distributed on the campus.

All participants were reimbursed for participation. All methods used are approved by the Birmingham University Ethics Committee

2.2 Stimulus material

This study used the same stimulus material as in Clouter and colleagues (Clouter, et al., 2017). The auditory stimulus material is identical to the previously used material, meaning that the volume of the stimuli will be sinusoidally modulated at a 4 Hz frequency. This value was chosen in order to reduce the amount of differences with the studies this experiment tries to replicate. The peaks of the modulated signal oscillated between 0% and 100% of the volume. Moreover, the stimuli always start at 50% volume. The visual stimulus material was left unmodulated. The sounds were randomly paired to a video for each session forming set clips. Every clip (N = 192 clips) is 3 s long.

Stimulus presentation occurred with the Psychophysics Toolbox extension implemented in MATLAB (Brainard & Vision, 1997; Pelli, 1997; Kleiner et al., 2007), on a 19" SynchMaster943B screen with a 75 Hz refresh rate at a 1280 x 1024 resolution connected to a computer equipped with a NVIDIA Quadro K600 636 MB Graphics card. The participants viewed this screen from a distance of approximately 70 cm. Auditory stimuli were presented through a set of ER3C headphones (Etymotic Research) as delivered via a UR22 USB Audiointerface (Steinberg).

Since the tACS stimulation occurs continuously, independent of the modulated auditory stimuli, trials have to be back-sorted (post-hoc) to determine phase alignment. Due to the highly timing dependent nature of the hypothesis, back-sorting was performed using a scalp electrode near the stimulation site to inform on the exact stimulation phase at a given point in time. The trials were binned for conditions where auditory flicker and tACS stimulation was: in-phase, 90° phase-shifted, 180° phase-shifted, or 270° phase-shifted (See Figure 1B). The bins were determined by centering the bin around the mean direction of each bin (0°, 90°, 180°, 270°) and sorted given a bin-size of ±45° around the centre (for trial numbers per condition, see *table 1*). The 0° bin label has been assigned to the bin at the positive peak of stimulation. This categorization is based on the findings in the tDCS literature, showing that anodal (positively charged) stimulation leads to increased excitability, while cathodal (negatively charged) stimulation leads to a decreased

excitability (Nitsche & Paulus, 2000; Nitsche et al., 2008; Brunoni et al., 2012). Therefore, we assume that the excitable 0° phase is comparable to the 0° phase as resulting from exposure to a stimulus. For the auditory stimuli, all bins were calculated with a 10 ms phase-shift in order to account for conduction delays from hair cells to the auditory cortex (Corey & Hudspeth, 1979; King & Palmer, 1985). We also accounted for a 7 ms trigger delay and a 1 ms conduction delay resulting from the soundwaves travelling within the ear-tubes of the earphones.

The conduction of electrical stimulation from the scalp to the visual cortex is assumed to be near instantaneous. This is based on the common assumption that the skin, skull, and brain tissue act as ohmic resistors, an assumption that is supported by intracranial recordings (Logothetis, Kayser, & Oeltermann, 2007; Opitz et al., 2016). It is important to note that this assumption has recently been contested (see for example Gomes, et al. 2016), since there is some minor capacitive (and possibly inductive) effects of neural tissue that are not noticeable in specific circumstances. However, the capacitive effects of neurons do not lead to phase delays exceeding 0.5° for external stimulation at 4Hz (as reported in Opitz et al., 2016). For the purposes of this study, delays of that magnitude are assumed to be negligible, since a 0.5° shift at 4 Hz would correspond to a delay of < 0.5 ms. This is not an uncommon practice, since fundamental neuroscientific models, most notably neuronal cable theories (Rall, 1995), make similar assumptions, and are therefore implicit in more complex analyses such as for example EEG/MEG source modelling methods (Hallez, 2007).

2.3 Stimulation

Stimulation was administered with a NeuroConn DC-STIMULATOR MC through four circular Ag/AgCl electrodes (12 mm diameter). These electrodes were held in place by Soterix HD 1A.2 electrodes filled with Signagel as a conductor. The electrodes were connected to the stimulator using the NeuroConn HDTarget Adaptor Box.

The centrally placed electrode was located at Oz, while the other three electrodes were placed radially at POZ, O9 and O10 (see Figure 2A). This HD-tACS arrangement was chosen due to its high focality compared to more traditional electrode montages (Helfrich, et al. 2014b; Saturnino et al., 2017). The current flow resulting from this montage was simulated using the SimNIBS 2.1.1 software package (Thielscher, Antunes, & Saturnino, 2015). This software allowed us to verify that the resulting current affects the visual cortex while having negligible effects on other areas (Figure 2B). The advantages of such a high focality are two-fold. First of all, the focality ensures that surrounding areas are not affected by stimulation to the same degree as the targeted area. Secondly, the high focality reduces the likelihood that a participant will experience phosphenes, since these are mostly related to currents reaching the retina through the scalp (Schwiedrzik, 2009; Schutter, & Hortensius, 2010). This assertion was confirmed in three pilot sessions in which the subjects were stimulated for 20 minutes with the intended montages (control and experimental montage) with no ongoing task. No phosphenes were reported in any of the pilot sessions, nor during the final experiment, by any of the subjects.

The stimulation intensity was set to 1.5 mA (peak-to-baseline) at the central electrode. Stimulation at this current intensity is high enough to bias cortical firing patterns (Ali, Sellers, and Fröhlich 2013; Huang et al. 2017). This stimulation was ramped up and down for 10 s each

at the beginning and end of each encoding block. Participants were stimulated for a total duration of 288 seconds (approximately 5 min) per block. All impedances were kept under 5 kΩ at all times to reduce any adverse effect stimulation might have on the skin. In order to reduce the skin sensation induced by the stimulation, EMLA cream was applied which has been previously used to reduce electrically induced sensations (McFadden et al., 2011; Khatoun et al., 2018).

Due to the relatively high intensity and low stimulation frequency used, we predicted that this would likely lead to some subjects experiencing somatosensory sensation. This would be problematic since recent research suggests that tACS induced entrainment could result from stimulation of nerves in the skin, rather than stimulation of the underlying cortex (Asamoah, Khatoun, & Mc Laughlin, 2018). In order to control for this, a separate session was performed using a control montage. This montage was mirrored and centered around Cz in order to ensure that the recorded stimulation signal (RSS) resulting from the two montages is comparable. Thus, for this montage the return electrode has been placed at Cz while the surrounding electrodes was placed at CPZ, FC1, and FC2 respectively (see Figure 2A).

2.4 Recorded Stimulation Signal (RSS)

The recorded stimulation signal (RSS) was obtained using a Brainvision professional Brainamp MR plus amplifier at a sampling rate of 1000 Hz and with a scaling of 10 mV. The RSS was only recorded from one Ag/AgCl electrode (as used for standard EEG recordings) placed at location Pz for both montages. The reference was placed at the right mastoid, while the ground was placed at the left mastoid. Offline EEG was preprocessed with the Fieldtrip toolbox for MATLAB (Oostenveld et al., 2011). The data was band-pass filtered at 4 Hz, with a FIR filter with a band-with of 3-5 Hz.We did this, because the signals main purpose is to reliably extract the phase of tACS stimulation at every given trial. We limited ourselves to 4 Hz, because it is the frequency at which both stimuli are modulated. Any other frequencies in the RSS are probably caused by non-linearities. This signal is not relevant for determining the ongoing phase of stimulation. The RSS during the task was epoched to 2 s before and 5 s after onset of the clips. Subsequently, a Hilbert transformation was applied to the data in order to extract the phase angle at every point in time. This was done for both, the RSS signal and a sine-wave fitted to the envelope of the respective trial-specific auditory stimulus. These values were then subtracted from one another to obtain a phase difference value for each time-point. In order to avoid edge effects resulting from the Hilbert transforms, the median of the difference in phase angle was taken for the middle 1 sec period of the 3 second stimulus presentation (time-window of 2-3 s of each trial). The median was taken to counter the slight oscillations resulting from the Hilbert transform, assuming that the phase difference stays constant throughout the time-window, as both stimuli were presented at exactly 4 Hz. This data was then be used to back-sort the trials into the respective conditions as described in section 2.2.

In order to ensure that the signal quality resulting from the RSS electrode would be sufficient for the planned back-sorting procedure, a pilot data-set was collected. For this recording, the subject underwent the described tACS stimulation with both montages (centered at Oz and Cz, respectively) and the described RSS electrode. The data was epoched around regularly administered (yet jittered) triggers. The conclusion from this pilot recording was that the RSS

signal from both montages is suitable to allow for the planned back-sorting procedure (for an example see Figure 3]).

2.5 Procedure

The experiment was administered in two separate sessions which were counterbalanced over the participants. One of the sessions was performed with the electrodes in the experimental montage (centered at Oz). In the control session, the electrodes were placed in the control montage (centered at Cz; see Figures 1A and 2A). In order to reduce any skin sensations that might occur due to tACS stimulation, EMLA cream was applied to the central electrode where the current would be maximal. Following EMLA application, the stimulation electrodes were placed as specified in section 2.4. The participant would then be acquainted with the paradigm. For the encoding trial the instruction stated that the participant should judge how well each sound is suited to the given video in the context of a nature documentary, in order to ensure that the participant was paying attention to the experiment. The response was self-paced. The inter-stimulus interval (ISI) between each trial was able to take on any value between one and three seconds, with the exception of any multiples of 0.25 ± 0.05 s. This ensured that the ISI would never lead to two subsequent trials falling in the same condition.

Each session consisted of 6 blocks. Each block consisted of 16 trials. In each trial the 3 s clips (audio and video) were played and the participants were asked to create an association between the sound and the video. Following the encoding of all 16 trials, a distractor task was performed where the participant was instructed to continuously subtract 3 from a random number as fast as possible for 30 s. Following this, the participants were presented with a sound and 4 images of the clips. The participants then had to choose the correct video clip, i.e. the clip associated with the played sound. The three lures fulfill the following criteria. Firstly, the lure stimuli are stimuli that have been presented in the same stimulus block as the correct video stimulus. Secondly, the lure video stimuli have to have been presented in conjunction with a sound from the same instrumental category. These two measures ensure that the memory that is being tested for is truly episodic and cannot be solved based on familiarity signals. The selection in the retrieval phase was also self-paced. After the recall phase the participant could take a self-paced break. The procedure was then repeated for all blocks.

2.6 Data Analysis

As discussed in section 2.1, subjects recruited to the experiment until the Bayes factor for the Bayesian paired sample t-test between the 0° and the 180° condition for the experimental montage exceeded 10, or fell below 1/10. Initial simulations showed that, assuming the data would show half the effect size of the findings reported in the previous studies, the BF should fulfill the above requirement after about 40 participants. Once data-acquisition is halted, as per the stopping criteria discussed above, further data analysis was performed. Participants with low behavioral performance (< 40 % correct; chance level: 25%) were excluded from further analysis. This relatively high threshold is chosen because it is important that the are participants actively engaged in the task and score high enough in general for any behavioral fluctuations to be visible, especially considering the stimulation method employed in this study. Moreover, any participants reporting phosphenes in the course of the experiment would be excluded from analysis.

To validate that tACS led to phase-dependent memory effects, two Bayesian implementations of the repeated measures ANOVA were applied to the data, one per session/montage. Subsequent Bayesian paired sample t-tests between all conditions would inform which conditions differ significantly from one another regarding their accuracy. These were corrected for multiple testing by fixing the prior probability that the null hypothesis holds across all comparisons to 0.5 (Westfall, Johnson, & Utts, 1997). Moreover, all tests were performed using a uninformative/objective Cauchy distribution as a prior, with r = 0.707 (Jeffreys, 1961; Rouder et al., 2009; Rouder et al., 2012). All Bayesian analyses were performed using the R-based statistical software JASP (JASP Team, 2018; Wagenmakers et al., 2018). We also report the P-values for all the performed tests, as result from their analogous frequentist counterparts.

2.6.1 Exploratory Analysis: Subjective Sensations

While the EMLA cream numbs the skin and reduces the cutaneous sensation resulting from tACS, it does not eliminate this sensation completely in all subjects. For each session, every subject was asked whether they had any cutaneous sensations due to the tACS. This allowed us to partition the data according to cutaneous sensation, allowing us to test for an effect of sensation on behaviour. To this end, we performed an additional ANOVA with an added between-subject factor for 'reported sensation' .

2.6.2 Exploratory Analysis: Individual Sinusoidal Modulation

The above described data analysis assumes that any entrainment resulting from tACS will be consistent across subjects. However, unlike for visual flicker, inter-subject variability could result in different phases of tACS producing the strongest excitatory (or inhibitory) effects. This will depend on many factors that will influence the eventual polarity, such as the folding of the cortex. To further explore this issue in our analysis, we applied the 'MAX-OPP VS MIN-OPP' method, as described in Zoefel et al. (2019). In short, the data is first aligned to a phase-bin with peak performance per subject, whereupon the phase-bin opposite of this peak is subtracted from the mean of the surrounding bins leading to a trough-to-zero-crossing difference value: d1. An equivalent operation is then performed by aligning the data to the bin with weakest trough performance and subtracting the opposite phase bin from the mean of the surrounding phase-bins, leading to a peak-to-zero-crossing value: d2. The two resulting values are subtracted from one another (d1-d2). If there is a sinusoidal modulation present, the distribution for d1 is positive, while d2 is negative, and the resulting difference is positive. Thus, if sinusoidal modulation of memory performance occurs, depending on the phase difference between the auditory stimuli and the tACS, then the resulting d1-d2 distribution should be positive. It is worth noting that the original findings of Clouter et al (2017) did not show a sinusoidal modulation *per se*, but a favored phase bin. As such we sought to explore the possibility that tACS might lead to a sinusoidal modulation with tACS. This analysis was not part of preregistration and is therefore exploratory.

3. Results

3.1 Main Results

As described in Methods, the difference in memory performance between the 0° and 180° phase condition was monitored as the dataset was collected. We found that the Bayes factor never reached a values of at least 10 for our experimental, nor alternative, hypotheses. We therefore collected the maximum preregistered number of subjects for this study (N=120). This Bayes factor difference eventually reached a value of $BF_{01} = 7.43$ in favor of the null hypothesis which is generally interpreted as moderate evidence that no difference exists between the two groups ($p = 0.448$) (see Fig 4). Of these, 38 subjects were excluded from further analysis since their behavioral performance did not meet the required criteria (average performance of > 40%), resulting in an N of 82. Thus, all subsequent analyses were only performed on the 82 subjects who conformed to this requirement. As expected, none of the participants reported any phosphenes.

The Bayesian repeated measure ANOVA testing for phase differences in the experimental condition, suggests that there is strong evidence for the null hypothesis ($BF_{01} = 40.97$; $BF_{10} = 0.024$). This concurs with the frequentist version of the analysis, which has a p-value that would not lead to a rejection of the null-hypothesis with an alpha of 0.05 ($F(3,243) = 0.464$; $p = 0.708$) (see Fig 5 for a plot of the memory performance per phase bin). Similarly, the corresponding Bayesian repeated measure ANOVA for the control condition also favors the null hypothesis ($BF_{01} = 1.49$; $BF_{10} = 0.67$), albeit with weaker evidence (BFs of < 3 are generally considered as anecdotal). This finding stands in contrast with the equivalent frequentist analysis, which would suggest rejecting the null hypothesis at an alpha = 0.05 ($F(3,243) = 2.939$, $P = 0.034$). The holm corrected post-hoc tests suggest a difference between the 0° and 90° condition ($p = 0.033$) and a borderline significant difference between 0° and 270° ($p = 0.058$), but no significant difference between 0° and 180° ($p = 0.8$). Given the Bayesian results and the tendency of p-values to converge towards zero given larger sample sizes, we refrain from further interpreting the results of the frequentist analysis.

3.2 Exploratory Analysis

The inclusion of self-reported cutaneous sensation as a between-subject variable in the model did not provide any evidence for an effect of sensation or an interaction between phase and sensation in either the experimental ($BF_{10}^{phase} = 0.018$, $BF_{10}^{Sensation} = 0.318$, $BF_{10}^{Phase*Sensation} = 0.004$; $p^{phase} = 0.679$, $p^{sensation} = 0.292$, $p^{phase*sensation} = 0.334$) nor the control condition ($BF_{10}^{phase} = 0.474$, $BF_{10}^{Sensation} = 0.138$, $BF_{10}^{Phase*Sensation} = 0.050$; $p^{phase} = 0.053$, $p^{sensation} = 0.987$, $p^{phase*sensation} = 0.278$)).

The ‘MAX-OPP VS MIN-OPP’ one-sample t-tests provided major evidence for the null-hypothesis, in that the distributions do not differ from 0 in a positive direction (Experimental Condition: $BF_{01} = 7.352$, $BF_{10} = 0.136$, $p = 0.634$; Control Condition: $BF_{01} = 3.602$, $BF_{10} = 0.278$, $p = 0.193$) (see figure 6A). While visually exploring the data, it was noted that the distribution for the experimental condition appeared bi-modal. This could imply that only a subset of subject respond to tACS. Additionally, this would violate the assumption of the performed t-test. This observation was confirmed with an adjusted ‘Hartigan’s dip test’, which is

optimized to detect bi-modal distributions (as described in Kang and Noh (2019)). This test concluded that the distribution of the experimental, but not the control condition was bimodal. The next step was to subtract the unimodal control condition, from the bimodal experimental condition. This was done in order to confirm whether there is a systematic difference between these two conditions, resulting from a subset of participants being successfully entrained by the tACS. If there is a systematic difference between the two conditions, this bimodal shape should be retained. This was not the case, leading to the conclusion that there was no evidence for sinusoidal modulation of memory performance depending on the phase-difference between auditory modulation and ongoing tACS (see figure 6B).

4. Discussion

This study was unable to verify the findings of the previously described visual flicker studies (Clouter, Shapiro, & Hanslmayr, 2017; Wang et al., 2018) using a different method of entrainment of the visual cortex. There could be multiple reasons for this inability to replicate these findings.

Firstly, it is possible that the results of the previous studies on which this study was based, were not inherent to entrainment effects in the brain but rather, due to the intrinsic properties of the presented stimulus material. Essentially this would mean that the original observed effect is not due to memory per se, but rather a perceptual effect. This argument is in line with studies showing direct interactions even between primary sensory cortices (Schroeder & Foxe, 2005; Lakatos et al, 2007; Stein & Stanford, 2008.). It therefore appears possible that the strong rhythmic theta modulation of the stimuli itself impacted somehow on perception, which then had a knock-on effect on memory (i.e. items that are processed less efficiently in the first place, will be more difficult to retrieve later). Albeit it should be noted that in the original studies of Clouter et al. (2017) and Wang et al. (2018) great care was taken to rule out an impact of such perceptual knock-on effects as much as possible.

Another possibility is that tACS does not succeed in entraining the visual cortex as we would have assumed. Much of the 'visual entrainment' tACS literature has focused on modulation of near-threshold level visual performances around the alpha band, since that is the endogenous frequency most dominant in the visual cortex (Kanai et al., 2008; Kanai Paulus, & Walsh 2010; Zaehle, Rach, & Hermann 2010; Helfrich et al., 2014b, Neuling et al., 2015). However, this study performed stimulation alongside natural stimuli (i.e. videos), alongside with stimulation at 4 Hz. Furthermore, we assumed that the tACS entrainment in the visual cortex would propagate downstream to higher level areas (i.e. hippocampus), where its signal is integrated with the auditory signal. It is quite possible that tACS is not able to entrain the brain enough to modulate the visual cortex sufficiently at the desired frequency for this propagation to occur, but rather influences only local activity in a very subtle way through its sub-threshold level modulation.

Furthermore, it is also conceivable that previously reported tACS effects might not result from actual neural entrainment. In order to verify that the underlying mechanisms for tACS effects are due to entrainment, research has been conducted that recorded EEG signal simultaneously with tACS (Helfrich et al., 2014b, Neuling et al., 2015, Soekadar et al., 2013, Voss et al., 2014). However, removing the tACS artefact from the EEG signal is not a trivial task that has arguably

not yet been successfully accomplished (see Noury, Hipp, & Siegel, 2016; Noury, & Siegel, 2017; Neuling, et al. 2017; Noury, & Siegel, 2018 for an in-depth discussion of this issue). One proposed alternative mechanism is the so-called 'rebound' effect, under which tACS induces neural power-changes by compensation effects in individual endogenous frequency bands, following cortical inhibition resulting from the electrical current stimulation (Perkel & Mulloney, 1974; Haberbosch et al. 2019). If tACS does manipulate the brain through such mechanisms other than entrainment, this would explain our inability to find phase-dependent effects in this study

The scepticism about the mechanisms of tACS can even be taken a step further, by questioning the validity of tACS method in general. Recent studies concluded that the electric fields induced by tACS are simply too weak to cause any meaningful modulation of neural activity in general (Vöröslakos et al, 2018; Liu, et al. 2018; but also see Opitz et al, 2016; Opitz et al, 2017; Huang et al. 2016). Under this view, most published tACS effects would be false positives, or result from alternative methods of entrainment such as phosphene and cutaneous sensation (Asamoah, Khatoun, & Mc Laughlin, 2019; Schutter, 2016). The parameters chosen in this analysis would make it unlikely to observe any retinal effects, and cutaneous effects were addressed in our exploratory analysis that included sensory ratings.

Even if tACS can modulate brain activity, it is highly likely that the effect sizes for tACS, as with most research, are overestimated by the published literature due to publication bias. This increases the necessity of collecting large sample sizes (Minarik, et al. 2016; Button, et al. 2013; Friston, 2013). However, the trade-off of such increased sample sizes is an increased chance of false positive findings when using traditional frequentist analysis methods. With this fact in mind it is important to encourage the reporting of the Bayes Factor, since Bayesian analyses do not share this property of inflated significance with increased sample sizes (Dienes, 2011; Biel, and Friedrich. 2018). We therefore believe that it is important that null-effects, such as the ones resulting from this study, keep being published. This enables more realistic estimates of the real effect sizes of stimulation methods, allowing for more accurate assessments of their limits. After all, non-invasive brain stimulation methods, such as tACS, have great potential as accessible research tools for the investigation of the underlying neural mechanisms of memory in humans and need to be understood more comprehensively.

Preregistration and data/materials availability

The Stage 1 manuscript was approved and formally registered on April 15, 2019, and may be downloaded from https://osf.io/qha3k, along with the comments raised during the reviewing process. All code that has been used to analyse the data, along with all the behavioral data and all notes made in the lab over the course of the experiment are available at https://osf.io/3ydph/. Legal copyright restrictions prevent public archiving of the movie material used for the experiment. These materials will be shared unconditionally on request to the corresponding author.

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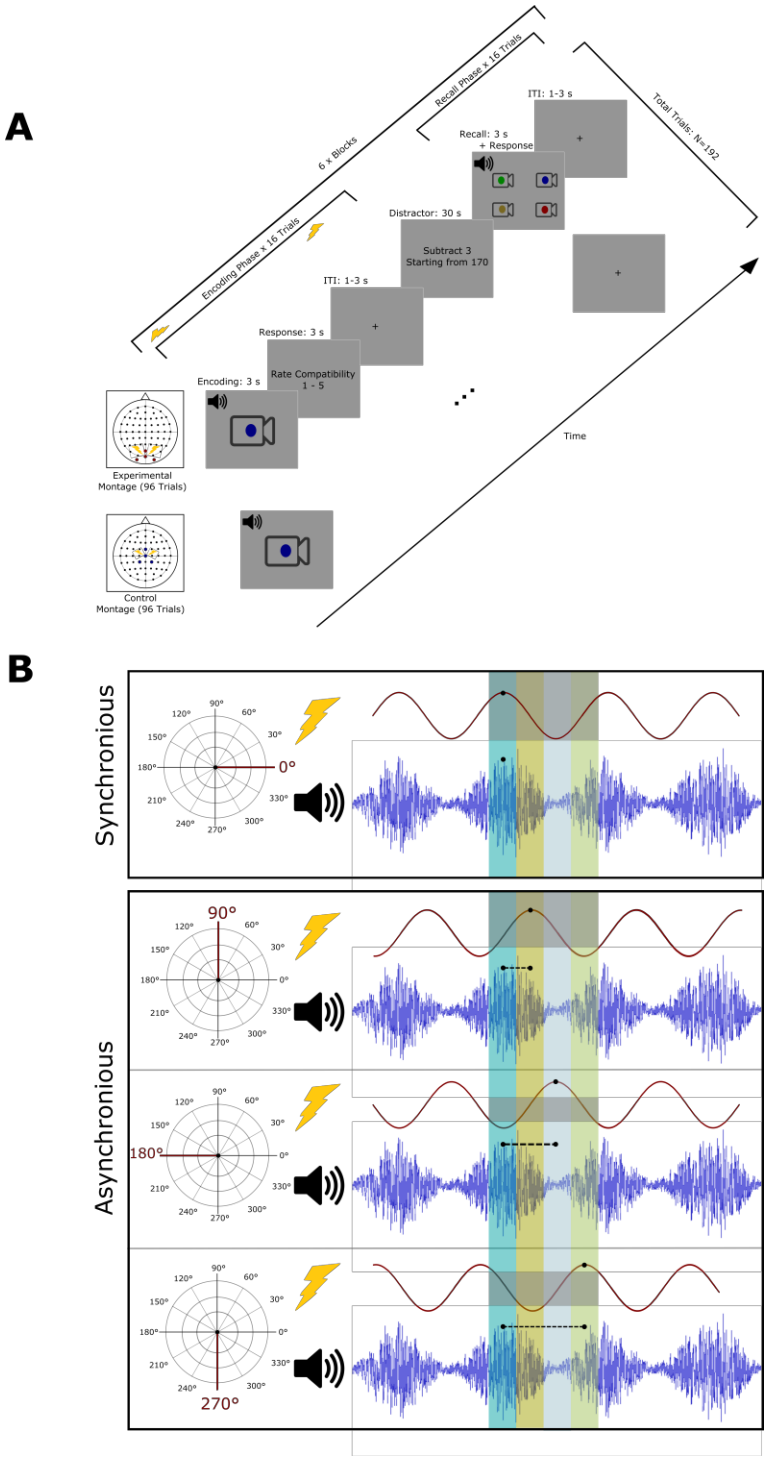

Figure 1:**A.** Visual representation of the experimental procedure. The Experiment will be performed in two sessions, each with a different electrode montage. There will be a total of 192 trials spread over 12 blocks (6blocks per sessions) each containing 16 encoding and retrieval trials which are separated by a distractor task during which they have to countdown in steps of three from a random number. During the encoding phase of a block the participant will be instructed to judge how well a given auditory stimulus and visual stimulus fit together. For the retrieval phase participants will be instructed to match a cued sound to the correct video in a choice of four different videos. **B.** Illustration of the different conditions. Each condition depends on the phase relationship between the current (red) and the amplitude of the sound (blue). The conditions are assigned through the relative phase distance between the auditory and the electrical stimulation (illustrated by the dotted line). Colored sections represent the width of the bins determining what condition a given trial is assigned to.

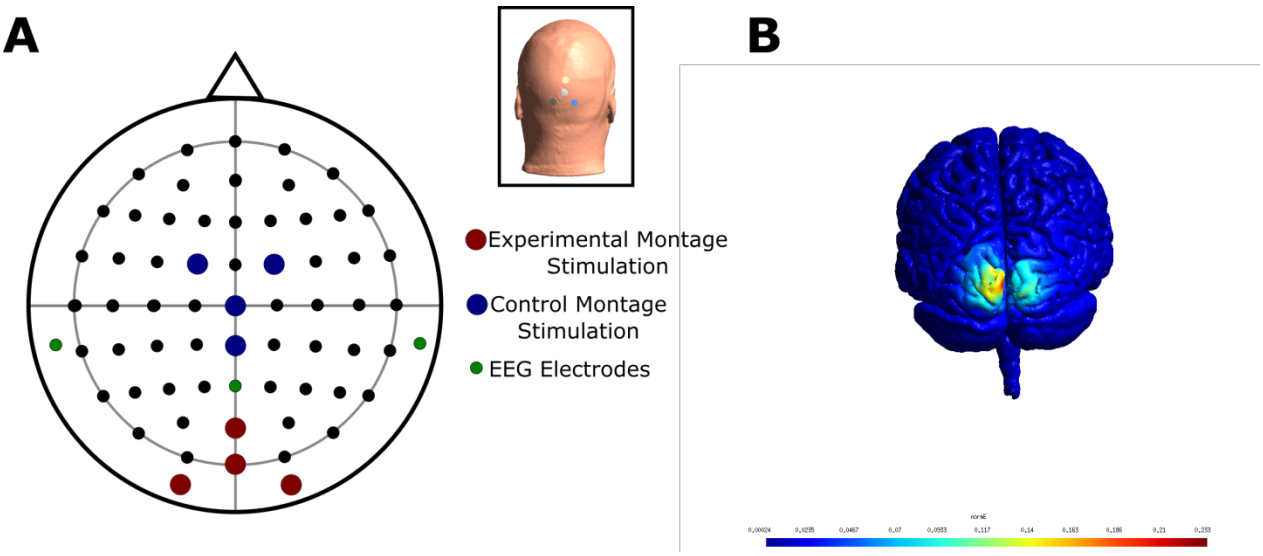

Figure 2: **A.** Figure demonstrating the electrode montage. The coloured circular patches illustrate the location of the electrodes. The ground and reference of the RSS electrodes are each placed on the left and right mastoid respectively. **B.** Simulation of the normalized Electric field resulting from the montage in A. Note that the montage leads to quite a high focality.

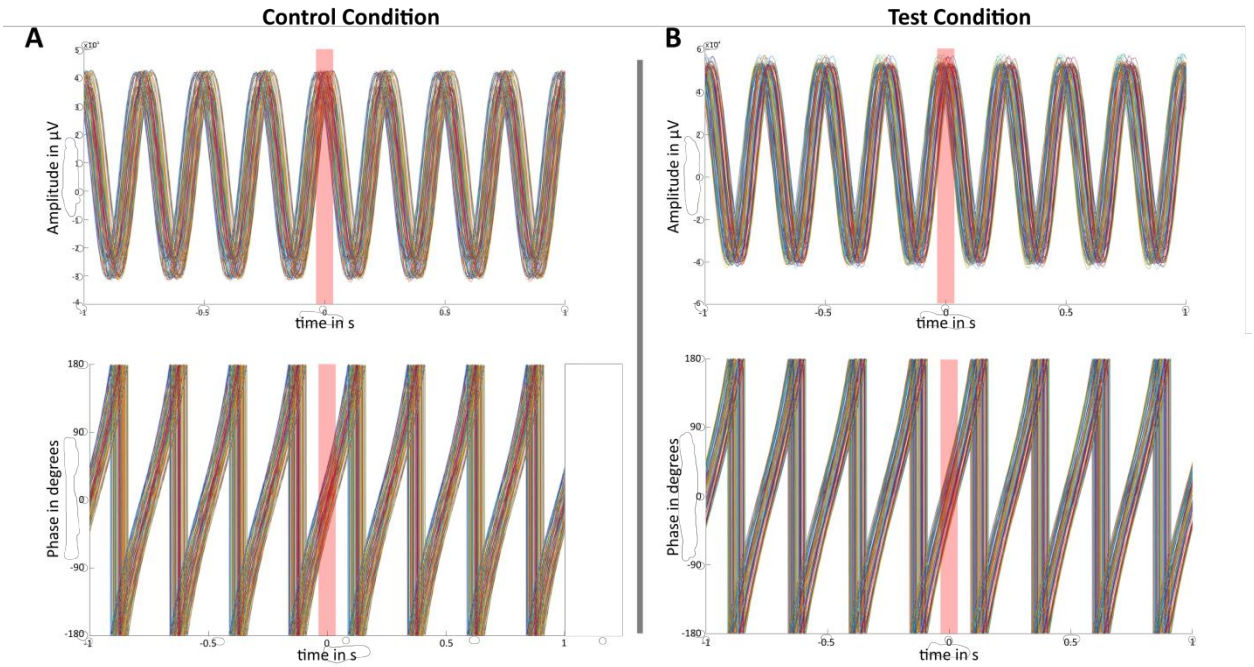

Figure 3: Figure demonstrating the RSS quality for the two different montages. This example only contains data from trials that were categorized of having phase angles between -45° and 45° (equivalent to the 0 phase condition in the actual experiment). The red bar indicates the time point 0 in all the figures. The upper figures always show the unfiltered raw signal while the lower figures demonstrate their corresponding phase-values **A**) Data collected with the control montage. **B**) Data from recordings with the experimental test montage. Note that the signal is so much stronger than native ongoing neural activity that barely any variance exists in the recorded signal amplitude. The stimulation artefact dominates the signal despite no filtering being applied.

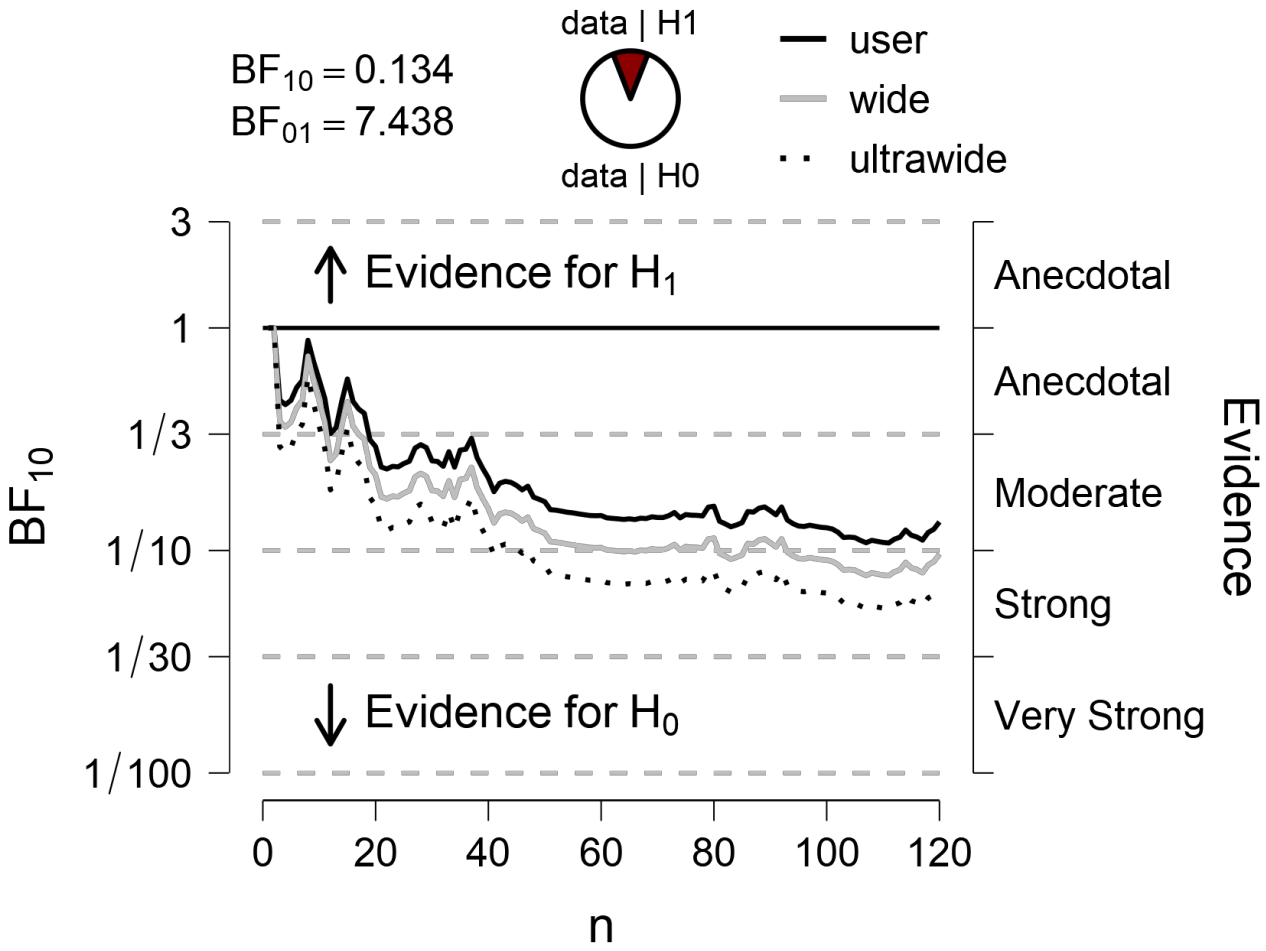

Figure 4: Figure demonstrating the development of the Bayes Factor with every additionally added subject. The solid line indicates the analysis with the prior specified in the methods section. The other grey and dotted lines represent the same evolution with more conservative priors (assuming smaller effect sizes).

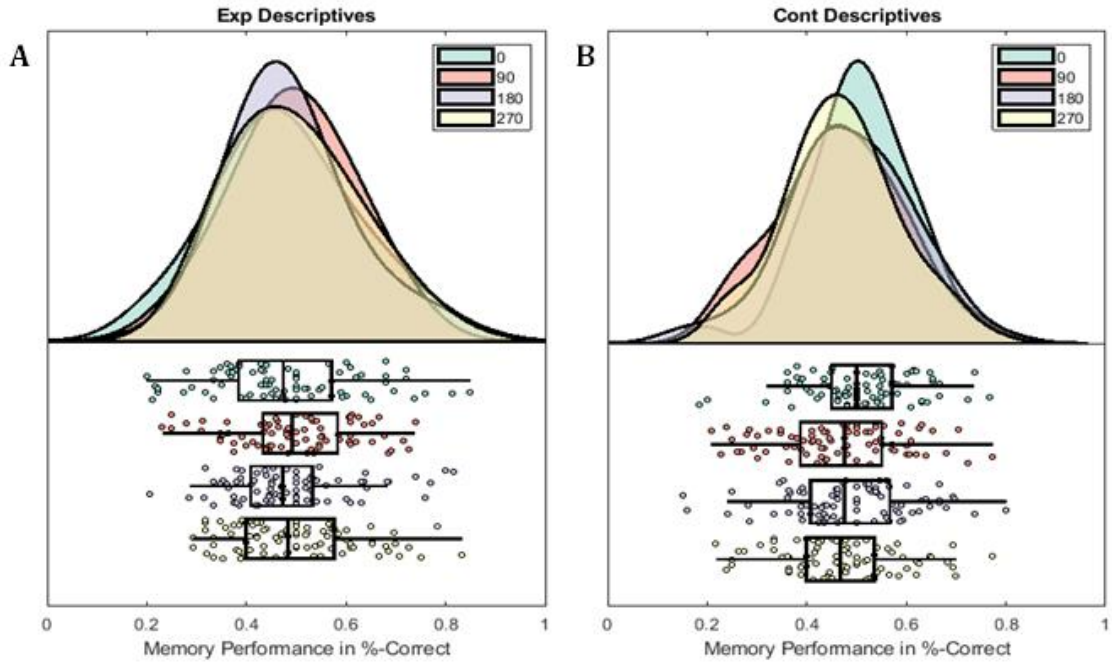

Figure 5: Figure demonstrating the distribution of average memory performances for each subject per phase bin for the Experimental (A) and the Control Condition (B) .

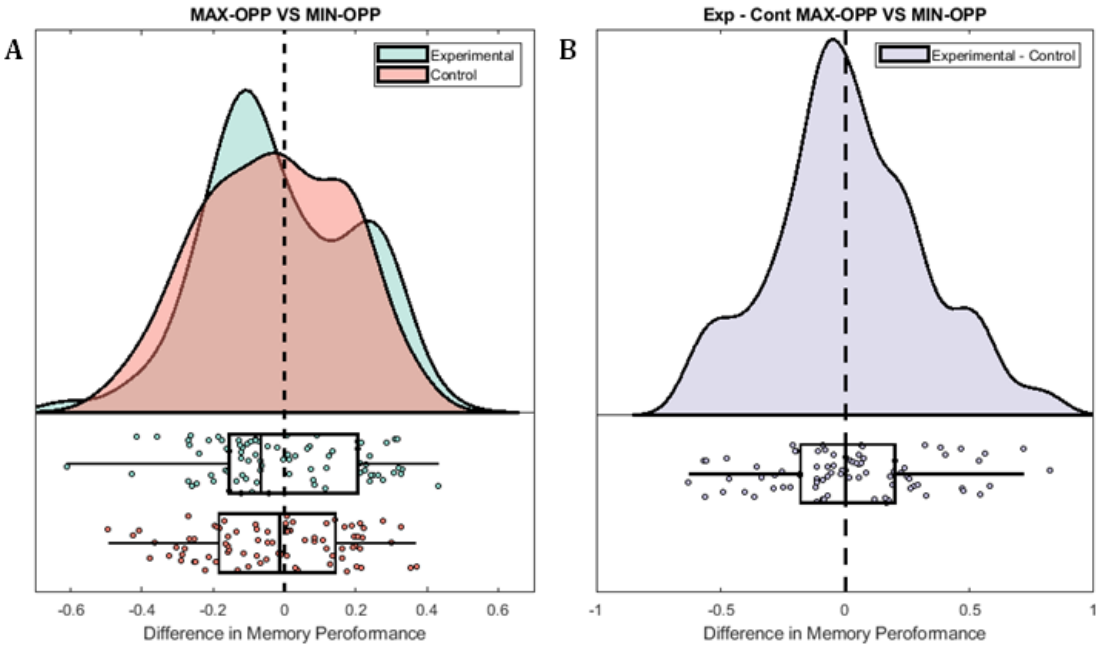

Figure 6: A) Distributions resulting from the Max-OPP-Min-OPP analysis. B) Distribution of the difference between the experimental and control Max-OPP-Min-OPP

Table 1: Descriptives of trial-numbers as resulting from backsorting for each condition.

Experimental		Control	
Mean	Standard Deviation	Mean	Standard Deviation
23.7027	4.479437	24.1982	3.865511
24.59459	4.888536	23.96396	4.201575
24.00901	4.691376	23.76577	3.995349
23.69369	4.03348	24.07207	4.170702