

# Frontal-midline theta frequency and probabilistic learning: A transcranial alternating current stimulation study

Zsófia Zavecz<sup>a,b,c</sup>, Kata Horváth<sup>a,b,c</sup>, Péter Solymosi<sup>b</sup>, Karolina Janacsek<sup>b,c,e,1</sup>,  
Dezso Nemeth<sup>b,c,d,1,\*</sup>

<sup>a</sup> Doctoral School of Psychology, ELTE Eötvös Loránd University, Budapest, Hungary

<sup>b</sup> Institute of Psychology, ELTE Eötvös Loránd University, Budapest, Hungary

<sup>c</sup> Brain, Memory and Language Research Group, Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

<sup>d</sup> Lyon Neuroscience Research Center (CRNL), INSERM, CNRS, Université Claude Bernard Lyon 1, Lyon, France

<sup>e</sup> Centre of Thinking and Learning, Institute for Lifecourse Development, School of Human Sciences, University of Greenwich, London, United Kingdom

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## ABSTRACT

Probabilistic learning is a fundamental cognitive ability that extracts and represents regularities of our environment enabling predictive processing during perception and acquisition of perceptual, motor, cognitive, and social skills. Previous studies show competition between neural networks related to executive function/working memory vs. probabilistic learning. Theta synchronization has been associated with the former while desynchronization with the latter in correlational studies. In the present paper our aim was to test causal relationship between fronto-parietal midline theta synchronization and probabilistic learning with non-invasive transcranial alternating current (tACS) stimulation. We hypothesize that theta synchronization disrupts probabilistic learning performance by modulating the competitive relationship. Twenty-six young adults performed the Alternating Serial Reaction Time (ASRT) task to assess probabilistic learning in two sessions that took place one week apart. Stimulation was applied in a double-blind cross-over within-subject design with an active theta tACS and a sham stimulation in a counter-balanced order between participants. Sinusoidal current was administered with 1 mA peak-to-peak intensity throughout the task (approximately 20 min) for the active stimulation and 30 s for the sham. We did not find an effect of fronto-parietal midline theta tACS on probabilistic learning comparing performance during active and sham stimulation. To influence probabilistic learning, we suggest applying higher current intensity and stimulation parameters more precisely aligned to endogenous brain activity for future studies.

## 1. Introduction

Probabilistic learning (often referred to as statistical learning as well) is a fundamental cognitive ability that underlies automatic behaviors and skills, such as motor, linguistic or social skills and habits [1–7]. It facilitates the extraction of statistical regularities from the environment and enables predictions of environmental events. Several studies discussed the neural background of probabilistic learning using functional magnetic resonance imaging (fMRI) [8–10], magnetoencephalography (MEG) [11], electroencephalography (EEG) [12,13] or neuropsychology [14–17]. However, these studies used correlational methods only. In the present paper our aim was to test the causal relationship between brain activity and probabilistic learning by directly

manipulating oscillatory activity with non-invasive electric brain stimulation.

Oscillatory synchronization is a fundamental mechanism for information transmission between neural populations and for forming larger networks [18–20]. For instance, theta (4–7 Hz) activity was consistently observed particularly within the fronto-midline areas during working memory and declarative memory tasks [21–28]. Tóth et al. [13] showed in an EEG study that theta activity was correlated with probabilistic learning as well: weaker phase synchronization in theta frequency was associated with better learning performance. Thus, in contrast to declarative and working memory, in theta frequency, desynchronization, and not synchronization seems to be beneficial for probabilistic learning. This is in line with the competition framework in

\* Corresponding author at: CRNL - Neurocampus, 95 boulevard Pinel, 69675 Bron Cedex, France.

E-mail address: [dezso.nemeth@univ-lyon1.fr](mailto:dezso.nemeth@univ-lyon1.fr) (D. Nemeth).

<sup>1</sup> Senior authors.

which there is an antagonistic relationship between fronto-hippocampal and striatal networks and related functions such as working and declarative memory vs. probabilistic and sequence learning [29–33].

A possible method to test causal relationships between brain networks and cognitive performance is brain stimulation. Transcranial alternating current stimulation (tACS) is a suitable method to influence oscillatory brain activity [34,35]. Based on the above presented evidence for the role of theta frequency in prefrontal-dependent processes (including working memory) and the antagonistic relationship of these processes with probabilistic learning [36–40], we hypothesized that induced theta synchronization is detrimental for probabilistic learning. Thus, in the present paper, we used a frontal-midline theta frequency tACS stimulation to disrupt probabilistic learning.

## 2. Methods

### 2.1. Participants

Twenty-six young adults (19 females) were selected from a large pool of undergraduate students from the Eötvös Loránd University in Budapest ( $M_{\text{Age}} = 21.38$  years,  $SD = 1.52$  years;  $M_{\text{Years of education}} = 14.46$  years,  $SD = 1.45$  years). Participants had no previous history of neurological, psychiatric or cardiovascular disorders, brain injuries and they had no metal implants in the head or neck area. They reported not taking any substances that affect the nervous system. All participants completed all sessions: two sessions with different stimulation conditions (sham vs. active stimulation) during the probabilistic learning task and an additional session for other neuropsychological tests. They were naïve regarding the exact purpose of the study and did not know in which session they were assigned to receive active or sham stimulation. Participants gave written and verbal informed consent before participating and received course credits for taking part in the experiment. The experiment was in accordance with the guidelines of the Declaration of Helsinki, and was approved by the ethics committee of the Eötvös Loránd University, Budapest, Hungary (identifier: 2016/120).

### 2.2. Experimental design

This study utilized a within-subject, cross-over design consisting of two stimulation sessions: 1 mA active tACS stimulation and sham stimulation (Fig. 1). These sessions took place one week apart from each other, starting at the same time of the day to eliminate time-of-day effects. The order of the sessions was counterbalanced across participants, and the stimulation was double-blinded. Therefore, neither the main investigator nor the participant was aware of the current stimulation condition. A second investigator who was not involved in the interaction with participants was responsible for setting the stimulation only. The stimulation was administered simultaneously with the probabilistic learning task (Alternating Serial Reaction Time, ASRT task). In the two sessions, participants learned two different, partly overlapping sequences. The overlap was controlled across participants (see Probabilistic learning section in Tasks for details).

### 2.3. Tasks

**Probabilistic learning** - The Alternating Serial Reaction Time (ASRT) task [41,42] was used to measure probabilistic sequence learning. In this task, a stimulus (a dog's head) appeared in one of the four empty circles on the screen, and participants had to press the corresponding button as fast and as accurately as possible (Fig. 1A). The target remained on the screen until the participant pressed the correct button. The response-to-stimulus interval (RSI) was 120 ms. The computer was equipped with a special keyboard with four marked keys (Z, C, B and M on a QWERTY keyboard), each corresponding to one of the horizontally aligned circles. The ASRT task consisted of 20 blocks, with

85 trials per block. The first five stimuli were random for practice purposes, then an eight-element alternating sequence was repeated ten times. The alternating sequence was composed of fixed sequence (pattern) and random elements (e.g., 2-R-4-R-3-R-1-R, where each number represents one of the four circles on the screen and “R” represents a randomly selected circle out of the four possible ones). As one block took 1–1.5 min, the whole task took approximately 20–25 min.

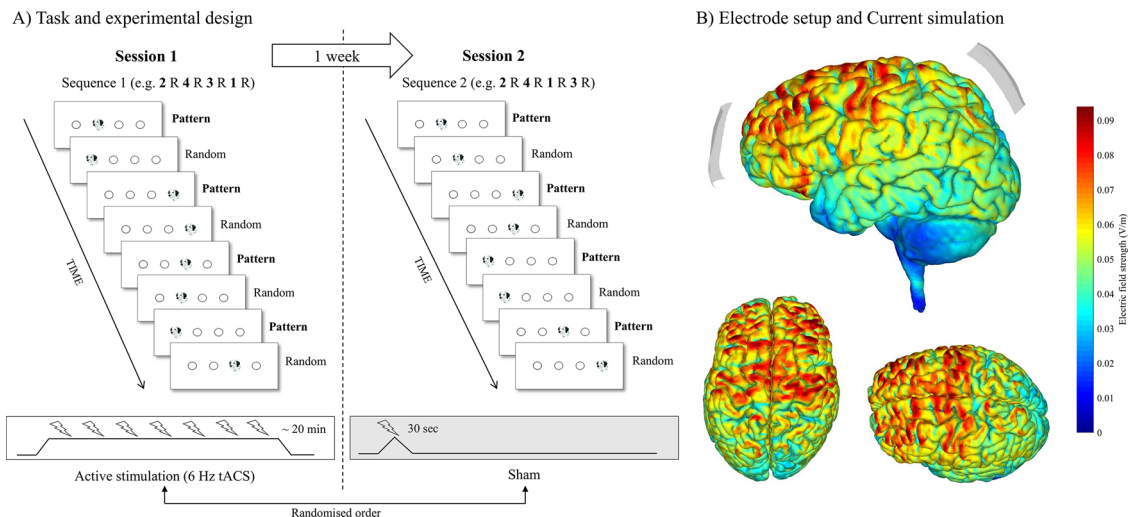
Due to the alternating sequence in the ASRT task, some triplets or runs of three consecutive events are more probable (*high-probability triplets*) than others (*low-probability triplets*). For example, in the abovementioned sequence (2-R-4-R-3-R-1-R), 2-X-4 is a high-probability triplet (where X denotes to any of the four possible positions), since the first and the third elements can either be a pattern or a random stimulus. However, 2-X-1, 2-X-2, and 2-X-3 are low-probability triplets, since the first and the third elements can only be a random stimulus. Therefore, for analyzing the data we determined whether each trial was the last element of a high-probability or a low-probability triplet. Note that in this way, we determine the probability of each triplet throughout the task in a sliding window manner (i.e., one stimulus is the last element of a triplet, but also the middle and the first element of the consecutive triplets). The high-probability triplets are five times more predictable than the low-probability triplets. Therefore, the last element of a triplet is more predictable in high-probability triplets compared to low-probability ones. Previous studies have shown that as people practice the ASRT task, they come to respond more quickly and more accurately to the high-probability triplets compared to low-probability triplets, revealing probabilistic learning [36,41,42,45].

The ASRT task was performed in two sessions during the experiment, with 20 blocks in each session. For this, pairs of sequences were created, where the two sequences shared two position orders out of the four (e.g., 2-R-4-R-3-R-1-R and 2-R-4-R-1-R-3-R, see Fig. 1A) which results in a 25% overlap in high-probability triplets between the sequences. One of these pairs of sequences was randomly assigned to each participant to keep constant the overlap in the two sequences amongst participants.

Finally, it is important to note that participants were unaware of the underlying alternating sequence structure, thus they acquired the probabilistic regularities incidentally and that knowledge remained implicit throughout the task. This was confirmed using a short questionnaire [42,45] after the second stimulation session. The questionnaire included the following two increasingly specific questions: “Have you noticed anything special regarding the task?”, “Have you noticed some regularity in the sequence of stimuli?”. The experimenter rated subjects' answers on a 5-point scale where 1 denoted “Nothing noticed” and 5 denoted “Total awareness”. None of the participants reported noticing regularities in the ASRT task.

### 2.4. Transcranial alternating current stimulation (tACS)

A commercial, battery driven constant current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) delivered a sinusoidal alternating current stimulation to the participant's scalp via two 5 cm × 5 cm electrodes. The electrodes were covered with a thin layer of electrode gel and were placed over positions Fpz and Pz according to the international 10–20 system (Fig. 1B). This frontal-midline electrode montage choice was based on a previously reported stimulation design [46]. Impedances were kept below 30 kΩ (average impedance was  $8.25 \pm 3.83$  kΩ). TACS was applied at a peak-to-peak current intensity of 1 mA oscillating at 6 Hz. While recent papers suggest using higher current intensity [47], these intensities can cause intense discomfort. In our study, to ensure that all participants complete both sessions and to maintain blindness of the participants to the stimulation settings, we decided to use a smaller current intensity that was proven successful in previous studies [48–50]. To avoid possible discomfort during the onset of tACS, the stimulation current was gradually ramped up from 0 to 1.0 mA over a period of 30 s. After the 30 s ramp up, the stimulation intensity was maintained for the length of the task (approximately 20



**Fig. 1. Overview of the experimental design and stimulation parameters.** **A) Task and experimental design.** The stimulation was carried out in a double-blind, placebo-controlled crossover design. Healthy young adults participated in two sessions (one week apart) during which they received 1 mA active theta frequency tACS stimulation, or sham stimulation in a counterbalanced order. Active tACS stimulation was administered throughout the task (approximately 20 min), while sham stimulation lasted only 30 s. In both cases there were 30 s ramp up and ramp down periods. Participants completed the Alternating Serial Reaction Time (ASRT) task both times to assess probabilistic learning performance. In this task, pattern elements alternate with random ones, constituting a probabilistic sequence, in which some runs of three consecutive trials (“triplets”) occur more frequently than others. We refer to probabilistic learning as a performance difference between high-probability compared to low-probability triplets. Participants learned two different probabilistic sequences during the two sessions. **B) Electrode setup and current simulation.** A battery driven constant current stimulator delivered a sinusoidal alternating current stimulation to the participant’s scalp via two 5 cm × 5 cm electrodes placed over positions Fpz and Pz according to the international 10–20 system. TACS was applied at a peak-to-peak current intensity of 1 mA oscillating at 6 Hz. To model tACS, we performed a simulation on a template head model by using a free software package called Simulation of Non-invasive Brain Stimulation (for details, see section ‘Transcranial Alternating Current Stimulation (tACS)’ in the main text). The spatial distribution of the absolute electric field magnitudes in the gray-matter compartment is in mV/mm. We used a robust maximum (99.9th percentile) of the absolute values for the scale limit. Lateral (top), top (bottom left) and superior lateral (bottom right) views are presented. The mean and maximal electric field strength of the robust maximum in the frontal, paracentral (pre- and post-central and central gyri and sulci) and parietal (superior gyri and sulci) regions were 0.088, 0.096, 0.083, 0.093, 0.072, 0.074 V/m respectively.

min) in case of the active stimulation condition. To control for tACS-unspecific effects (such as fatigue and beliefs of the participant), there was a sham (placebo) stimulation condition, consisting of 30 s of stimulation following the 30 s ramp up. In both conditions there was a 30 s ramp down period after the stimulation.

To model tACS, we performed a simulation on a template head model by using a free software package called Simulation of Non-invasive Brain Stimulation (SimNIBS; version 2.1.2, Fig. 1B). SimNIBS generates anatomically realistic, multi-compartment head models from structural magnetic resonance imaging by using the finite element method. The head mesh entailed ca. 3,500,000 tetrahedral elements and five compartments. We used standard, isotropic conductivity values for the compartments, all values are expressed in S/m: white matter = 0.126; gray matter = 0.275; cerebrospinal fluid = 1.654; bone = 0.01; scalp = 0.465; eyes = 0.5; silicon rubber electrode = 29.4; conductive medium = 1.0. The physical dimensions of both electrodes were 50 × 50 mm and 4 mm thick. The thickness of the conductive medium was set to 2 mm. The electric field was modeled by using 0.5 mA peak to baseline intensities. To quantify the strength of the induced electric field in particular brain areas, we used the parcellation of human cortical gyri and sulci proposed by Destrieux, Fischl, Dale and Halgren [51]. We computed the mean and maximal electric field strength of the robust maximum (99.9th percentile) in the following regions of interest (ROIs): frontal (superior, middle and orbital gyri and sulci), paracentral (pre- and post-central and central gyri and sulci), and parietal (superior gyri and sulci). The electric field strength was  $Mean_{max} = 0.088$  V/m,  $Max_{max} = 0.096$  V/m in the frontal,  $Mean_{max} = 0.083$  V/m,  $Max_{max} = 0.093$  V/m in the paracentral and  $Mean_{max} = 0.072$  V/m,  $Max_{max} = 0.074$  V/m in the parietal regions.

## 2.5. Statistical analysis

Statistical analyses were carried out with the Statistical Package for

the Social Sciences version 22.0 (SPSS, IBM) and JASP Version 0.11.1 [52]. To facilitate data processing, the blocks of ASRT were organized into four epochs of five blocks in each session. The first epoch contained blocks 1–5, the second epoch contained blocks 6–10, etc. We calculated mean accuracy scores (ACCs) for all responses and median reaction times (RTs) for correct responses only, separately for high- and low-probability triplets and for each subject and each epoch. Note that for each trial we defined whether it was the last element of a high- or a low-probability triplet. Two kinds of low-probability triplets were eliminated from the analysis: repetitions (e.g., 222 and 333) and trills (e.g., 212 and 343), as people often showed pre-existing response tendencies to them [41,42,45].

Overall RTs significantly differed between the two sessions (as revealed by the significant main effect of SESSION in the repeated-measures ANOVA on RTs with SESSION (First vs. Second), EPOCH (1–4) and TRIPLET TYPE (High vs. Low) as within-subject factors:  $F(1, 25) = 39.510$ ,  $p < .0001$ ,  $\eta^2_p = .612$ ): participants were faster when completing the task for the second time ( $M_{RT} = 369.70$ ,  $SEM = 5.31$ ,  $M_{RT} = 336.20$ ,  $SEM = 5.29$  for the first and the second session, respectively). Therefore, we calculated z-scores within each subject in each session to eliminate the effects of different baseline speeds when comparing performance between the two sessions. A similar ANOVA computed on accuracy data revealed no significant difference between the two sessions (main effect of SESSION:  $F(1, 25) = 0.376$ ,  $p = .545$ ,  $\eta^2_p = .015$ ).

For each epoch, we calculated learning scores both for RT and ACC data. For RT, the learning score was calculated as the difference between the z-transformed RTs for low-probability triplets minus the z-transformed RTs for high-probability triplets. For ACC, the learning score was calculated as the raw ACCs for high-probability triplets minus the raw ACCs for low-probability triplets. In both cases, higher learning scores indicated better learning. To evaluate changes in probabilistic learning as a function of stimulation, we conducted mixed-design

analyses of variance (ANOVAs) separately for the RT and ACC learning scores with STIMULATION (Sham vs. Active) and EPOCH (1–4) as within-subject factors and ORDER (Sham first vs. Stimulation first) as a between-subject factor. We included the ORDER between-subject factor to ensure that the order in which participants received sham and active stimulation did not influence the effects of stimulation. Greenhouse–Geisser epsilon ( $\epsilon$ ) correction was used when necessary. Original df values and corrected p-values (if applicable) are reported together with partial eta-squared ( $\eta^2_p$ ) as the measure of effect size.

Furthermore, as suggested by Biel and Friedrich [53] we conducted the same mixed-design ANOVAs separately for the RT and ACC learning scores with STIMULATION and EPOCH as within-subject factors and ORDER as a between-subject factor with a Bayesian approach as well. The Bayesian ANOVA contrasts the predictive performance of competing models instead of F-tests of main effects and interactions [54]. Models were compared using  $BF_{10}$ , which quantifies the evidence in favor of each model relative to the best model in the respective comparison. To summarize the importance of the within-subject factors across all models, we also performed model averaging, which provides us with evidence for inclusion for main effects and interactions ( $BF_{inclusion}$ ). The inclusion Bayes factor quantifies the change from prior inclusion odds to posterior inclusion odds and can be interpreted as the evidence in the data for including a predictor.

To ensure that the partially overlapping sequence in the task between the two sessions did not distort the effects of the stimulation, we recomputed learning scores excluding the responses (RT and ACC) to those triplets that were high-probability in both sessions and ran frequentist and Bayesian repeated-measures ANOVAs on these modified RT and ACC learning scores over time and stimulation (see section ‘Does the partial overlap between the sequences practiced during the two stimulation sessions influence the effects of the stimulation?’ and Fig. S1 in the Supplementary results). Importantly, the results after the elimination of the overlapping high-probability triplets are identical to the results without the elimination of these triplets and are not discussed further in the main text.

Lastly, as a post-hoc analysis we investigated the effects of baseline performance on the stimulation. We ran four additional mixed-design ANOVAs (both frequentist and Bayesian) including a between-subject factor for good vs. poor initial/baseline performance in four measures of ASRT (average reaction times, reaction time learning scores, average accuracy, accuracy learning scores) on the learning scores over time and stimulation (see section ‘Does baseline performance influence the effects of the stimulation?’ in the Supplementary results). We did not find a differential effect of the stimulation in good vs. poor performers based on initial speed, accuracy, RT or ACC probabilistic learning.

### 3. Results

#### 3.1. Do RT learning scores differ between stimulation conditions?

The frequentist mixed-design ANOVA on the z-transformed RT learning scores revealed a significant Intercept ( $F(1, 24) = 66.277, p < .001, \eta^2_p = .734$ ), suggesting that learning occurred in the ASRT task. The main effect of EPOCH was also significant ( $F(3, 72) = 6.663, p < .001, \eta^2_p = .217$ ), indicating that the learning scores increased throughout the task, independent of the stimulation condition (Fig. 2A). However, we did not find any significant differences between the active stimulation and sham conditions either in overall learning (main effect of STIMULATION:  $F(1, 24) = 0.093, p = .763, \eta^2_p = .004$ ) or in the time course of learning (STIMULATION \* EPOCH interaction:  $F(3, 72) = 0.637, p = .593, \eta^2_p = .026$ ). The order of the stimulation sessions did not seem to affect the overall learning scores (main effect of ORDER:  $F(1, 24) = 2.345, p = .139, \eta^2_p = .089$ ), the trajectory of the learning scores (ORDER x EPOCH interaction:  $F(3, 72) = 0.048, p = .986, \eta^2_p = .002$ ), the effect of stimulation (ORDER x STIMULATION interaction:  $F(1, 24) = 0.974, p = .333, \eta^2_p = .039$ ) or the trajectory of

the learning scores during the two stimulation conditions (ORDER x EPOCH x STIMULATION interaction:  $F(3, 72) = 0.627, p = .600, \eta^2_p = .025$ ).

The analysis of effects (model-averaged results) of the Bayesian mixed-design ANOVA on the z-transformed RT learning scores showed that the main effect of Epoch should be included in the model ( $BF_{inclusion} = 74.684$ ), while the effects related to the Stimulation and the Session order should not (all  $BF_{inclusion} < 1$ , Table 1). Thus, based on the Bayesian analysis of effects, the learning scores changed throughout the task, but they were independent of the stimulation condition or the order of the stimulation.

As our primary interest was the effect of the stimulation on probabilistic learning and the number of models was too high with the ORDER between-subject factor, as well as there was no evidence to include that factor, we recomputed the Bayesian ANOVA with only the STIMULATION and EPOCH as within-subject factors. Based on this Bayesian ANOVA, the best model for our data was with only the main effect of Epoch (Table 2). This model with the main effect of Epoch was ~6.5 times more likely than any model including the effect of the Stimulation. Altogether the Bayesian ANOVA for the RT learning scores provides evidence for the model with only the main effect EPOCH to explain best our data. This suggests that while the learning scores changed during the task, this was independent of the stimulation condition and the order of the stimulation condition.

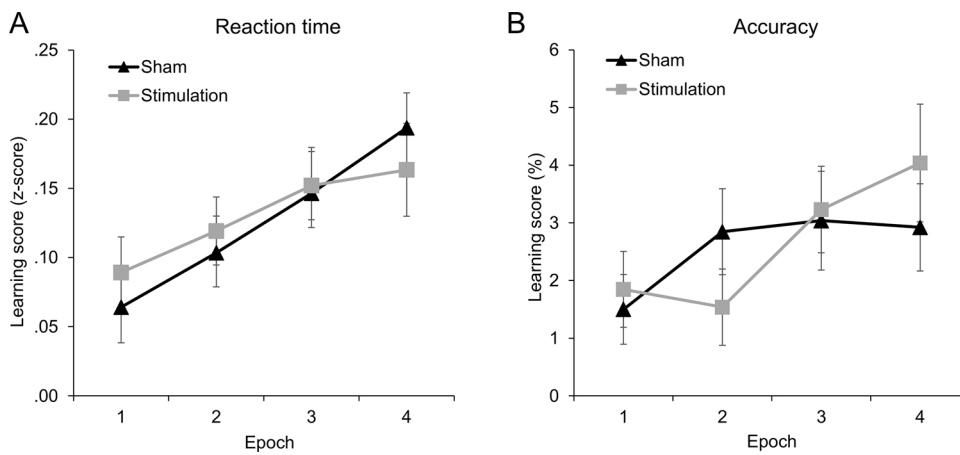
#### 3.2. Do ACC learning scores differ between stimulation conditions?

The frequentist mixed-design ANOVA on the ACC learning scores revealed a significant Intercept ( $F(1, 24) = 62.307, p < .001, \eta^2_p = .722$ ), suggesting that learning occurred in the ASRT task. The main effect of EPOCH showed a trend ( $F(3, 72) = 2.237, p = .091, \eta^2_p = .085$ ), indicating that the learning scores increased throughout the task, independent of the stimulation condition (Fig. 2B). We did not find significant differences between the active stimulation and sham conditions either in overall learning (main effect of STIMULATION:  $F(1, 24) = 0.054, p = .819, \eta^2_p = .002$ ) or in the time course of learning (STIMULATION \* EPOCH interaction:  $F(3, 72) = 1.065, p = .359, \eta^2_p = .042$ ). The order of the stimulation sessions did not seem to affect the overall learning scores (main effect of ORDER:  $F(1, 24) = 1.874, p = .184, \eta^2_p = .072$ ), the trajectory of the learning scores (ORDER x EPOCH interaction:  $F(3, 72) = 0.249, p = .862, \eta^2_p = .010$ ), the stimulation (ORDER x STIMULATION interaction:  $F(1, 24) = 1.831, p = .189, \eta^2_p = .071$ ) or the trajectory of the learning scores during the two different stimulation condition (ORDER x EPOCH x STIMULATION interaction:  $F(3, 72) = 1.731, p = .182, \eta^2_p = .067$ ).

The analysis of effects (model-averaged results) of the Bayesian mixed-design ANOVA on the ACC learning scores showed that none of the effects related to Epoch, Stimulation or Session order should be included in the model (all  $BF_{inclusion} < 1$ , Table 3). Thus, based on the Bayesian analysis of effects, the learning scores were stable throughout the task and they were independent of the stimulation condition or the order of the stimulation.

Again, as our primary interest was the effect of the stimulation on probabilistic learning and the number of models was too high with the ORDER between-subject factor, as well as there was no evidence to include that factor, we recomputed the ANOVA with only the STIMULATION and EPOCH within-subject factors. This Bayesian ANOVA showed that the best model for our data is the Null model (Table 4). This Null model is ~6 times more likely than any model including the Stimulation factor. Altogether the Bayesian ANOVA for the ACC learning scores provides evidence for the Null model to explain best our data. This suggests that learning scores were stable throughout the task and were independent of epochs, the stimulation condition and the order of the stimulation condition.

To reveal possible patterns in the stimulation effects, we visualized individual learning score trajectories for both stimulation conditions



**Fig. 2.** Probabilistic learning in terms of reaction times (A) and accuracy (B) in the active stimulation vs. sham conditions across the four epochs of the ASRT task. There was no significant difference between the active stimulation in theta frequency (grey squares) and sham (black triangles) conditions either in overall learning or in the time course of learning. Error bars indicate the Standard Error of Mean (SEM).

**Table 1**  
Model-averaged results of Bayesian ANOVA for RT learning scores.

Effects	P(incl)	P(incl data)	BF <sub>inclusion</sub>
Stimulation	0.737	0.170	0.073
Epoch	0.737	0.995	74.684
Order	0.737	0.437	0.278
Stimulation x Epoch	0.316	0.014	0.030
Stimulation x Order	0.316	0.030	0.067
Epoch x Order	0.316	0.023	0.051
Stimulation x Epoch x Order	0.053	1.564e -5	2.816e -4

Note: The Effects column denotes predictors of interest, the column P(incl) shows the prior inclusion probability, P(incl | D) shows the posterior inclusion probability, and BF<sub>inclusion</sub> shows the inclusion Bayes factor.

**Table 2**  
Bayesian model comparisons for RT learning scores.

Models	P(M)	P(M data)	BF <sub>M</sub>	BF <sub>10</sub>	error %
Epoch	0.200	0.853	23.178	1.000	
Stimulation + Epoch	0.200	0.129	0.595	0.152	1.559
Null model	0.200	0.013	0.052	0.015	2.618
Stimulation + Epoch + Stim. x Epoch	0.200	0.004	0.017	0.005	0.610
Stimulation	0.200	0.0006	0.003	0.0008	0.978

Note: All models include Subject. The Model column shows the predictors included in each model, the P(M) column the prior model probability, the P(M | D) column the posterior model probability, the BF<sub>M</sub> column the posterior model odds, and the BF<sub>10</sub> column the Bayes factors of all models compared to the best model. The final column, 'error' is an estimate of the numerical error in the computation of the Bayes factor. All models are compared to the best model and are sorted from highest Bayes factor to lowest.

**Table 3**  
Model-averaged results of Bayesian ANOVA for ACC learning scores.

Effects	P(incl)	P(incl data)	BF <sub>inclusion</sub>
Stimulation	0.737	0.089	0.035
Epoch	0.737	0.220	0.101
Order	0.737	0.618	0.578
Stimulation x Epoch	0.316	0.005	0.011
Stimulation x Order	0.316	0.014	0.031
Epoch x Order	0.316	0.005	0.012
Stimulation x Epoch x Order	0.053	2.526e -5	4.547e -4

Note: The Effects column denotes predictors of interest, the column P(incl) shows the prior inclusion probability, P(incl | D) shows the posterior inclusion probability, and BF<sub>inclusion</sub> shows the inclusion Bayes factor.

**Table 4**  
Bayesian model comparisons for ACC learning scores.

Models	P(M)	P(M data)	BF <sub>M</sub>	BF <sub>10</sub>	error %
Null model	0.200	0.533	4.566	1.000	
Epoch	0.200	0.328	1.956	0.616	0.523
Stimulation	0.200	0.081	0.353	0.152	1.680
Stimulation + Epoch	0.200	0.050	0.210	0.093	2.373
Stimulation + Epoch + Stim. x Epoch	0.200	0.008	0.031	0.014	1.860

Note: All models include Subject. The Model column shows the predictors included in each model, the P(M) column the prior model probability, the P(M | D) column the posterior model probability, the BF<sub>M</sub> column the posterior model odds, and the BF<sub>10</sub> column the Bayes factors of all models compared to the best model. The final column, 'error' is an estimate of the numerical error in the computation of the Bayes factor. All models are compared to the best model and are sorted from highest Bayes factor to lowest.

separately for RT and ACC learning scores (see section 'Are there any obvious patterns in the stimulation effects for different individuals?' and Fig. S2-S3 in Supplementary results). Furthermore, to explore visually whether the order of the conditions influenced the effect of stimulation, we grouped the participants based on whether they completed the sham condition (Fig. S2A and S3A), or the active stimulation condition first (Fig. S2B and S3B). Altogether, the plots did not unravel obvious subgroups based on the difference between the active stimulation and sham conditions either in overall learning or in the time course of learning. Furthermore, the order of the stimulation did not seem to interact with the effects of the stimulation, further supporting the findings reported above.

#### 4. Discussion

In the current study, our aim was to alter probabilistic learning by applying theta tACS during learning in a double-blinded cross-over within-subject design. We did not find differences either in overall learning performance or the time course of learning between the active stimulation and sham conditions. Moreover, Bayesian model comparisons provided evidence for no effect of stimulation on the learning performance.

Contrary to our expectations, we did not find an effect of the tACS on probabilistic learning. It is possible that the chosen parameters for the tACS stimulation, such as the fronto-parietal midline montage, the relatively weak (1 mA) current intensity, and/or the chosen theta frequency were not appropriate to influence probabilistic learning. Importantly, however, previous studies successfully influenced other

cognitive functions (such as short term and working memory, or decision making) with stimulation parameters similar to ours [46,55–57], suggesting that these stimulation parameters might be effective for altering some cognitive functions but not others. Specifically, these studies aimed to influence prefrontal-network dependent, expectation/hypothesis-driven (top-down) cognitive processes. It is possible that stimulus-driven, bottom-up processes such as probabilistic learning can be successfully influenced by different frequency and/or electrode positions. Previous studies using similar, bottom-up tasks with deterministic sequential regularities (Serial Reaction Time Task, SRTT) reported alpha and beta frequencies to be successful for stimulation [34,58]. Antal, Boros, Poreisz, Chaieb, Terney and Paulus [34] showed that alpha frequency tACS specifically improved motor sequence learning in contrast to beta or gamma frequencies over the primary motor cortex. Pollok, Boysen and Krause [58] successfully applied both alpha and beta frequency tACS over the left primary motor cortex to improve motor sequence learning. Note that while these studies tested multiple frequencies to influence sequence learning, neither of them applied theta frequency. Importantly, these tasks were deterministic sequence learning tasks, which potentially rely more on motor representations as opposed to the ASRT task that we used in the current study, therefore, we did not rely on these results when determining our stimulation parameters. To the best of our knowledge, our study was the first to test if probabilistic learning can be influenced by tACS and we chose theta frequency stimulation as it has been proven successful in several studies investigating working memory and it has not been studied in tasks with acquiring regularities of stimuli. Future studies are needed to investigate whether different frequency bands (in particular alpha or beta) or different electrode montages (targeting motor cortex, or frontal or parietal areas selectively) are more suitable to influence probabilistic learning.

It is also possible that desynchronization instead of synchronization with the same parameters would have a bigger impact on probabilistic learning (although opposite effect). In support of this, Alekseichuk, Pabel, Antal and Paulus [59] found that fronto-parietal synchronization induced by 0° tACS did not significantly influence brain connectivity (measured via EEG) and working memory performance. In contrast, fronto-parietal desynchronization induced by 180° tACS affected both connectivity and performance. We did not have the appropriate equipment to induce desynchronization in the current study, but based on the finding of Tóth et al. [13], that desynchronization in theta frequency is associated with better probabilistic learning, it would be worth testing this stimulation design in case of a probabilistic learning paradigm (see for example the design in [57]).

Picking the appropriate stimulation parameters enables electrical stimulation to induce changes in brain activity and, therefore, possibly behavior. Thut, Schyns and Gross [60] claim that the entrainment of endogenous brain oscillations by tACS is possible if there is phase-alignment between the stimulation and internal oscillators. For this, an internal oscillator is needed, namely entrainment can occur only if there is a neural population that exhibits oscillations at the stimulation frequency under natural conditions. Moreover, the closer the external rhythm is to the internal one, the smaller the force needed to entrain endogenous oscillations [61]. Antal and Herrmann [62] showed that the electrical current intensity with the standard stimulation strengths of 1–2 mA can be sufficient to induce changes in the brain activity but the induced voltage gradients in the brain are small. Based on our simulation, the induced electric field was up to 0.1 V/m, in particular in frontal and paracentral brain regions in our study. Altogether, tACS with 1 mA stimulation strength (as in our study) will likely influence brain activity only if the chosen stimulation frequency and stimulated brain areas match the patterns of naturally occurring brain activity during the given task. Thut et al. [63] suggested several approaches to increase the alignment between the brain stimulation and the ongoing endogenous activity, for example, setting the stimulation parameters by obtaining instantaneous phase or power of oscillatory brain activity

from simultaneous EEG/MEG recording, or using EEG/MEG recordings prior to interventions to detect the individual frequency of the oscillation of interest. Further studies with more precise alignment could clarify if fronto-parietal theta entrainment can influence probabilistic learning.

Beyond the stimulation parameters, other factors could also influence the effects of the stimulation. We studied healthy young adults who generally perform well in cognitive tasks [64,65] and therefore their performance may be less susceptible to the effect of the stimulation. However, this is unlikely the case in our study as we also tested the effects of baseline performance on stimulation (see section ‘Does baseline performance influence the effects of the stimulation?’ in Supplementary results) and did not find differential effects of the stimulation in participants performing worse at the beginning of the task. Nevertheless, the effect of theta tACS stimulation on probabilistic learning in a population with poorer cognitive performance remains to be explored.

#### 4.1. Limitations

Similarly to most of the previous tACS studies, we did not monitor the brain activity during the stimulation, therefore there is no evidence that the stimulation induced changes in the endogenous activity. Furthermore, offline monitoring of brain activity preceding the stimulation is also lacking. This design would have enabled us to pick an individual theta frequency for each participant. Stimulating with the frequency matching the participant’s dominant frequency could promote stronger stimulation effects [62]. However, previous studies used similar tACS stimulation successfully to alter behavior. Lastly, as our stimulation parameters relied on previous studies that targeted working memory performance, a working memory control task could have been used to validate these parameters within the current sample. However, as our aim was not replication but to test the effect of simulation on probabilistic learning, we decided not to include other tasks in the stimulation conditions.

#### 4.2. Conclusions

To the best of our knowledge, our study was the first to apply tACS to influence probabilistic learning. We did not find statistically significant effects of fronto-parietal midline theta tACS (with ~0.1 V/m electrical field strength) on probabilistic learning comparing behavior during active and sham stimulation. Our results draw attention to possible methodological flaws in electrical stimulation experiments. It is possible that with greater current intensity and/or with stimulation parameters more precisely aligned to endogenous brain activity during probabilistic learning, stimulation effects could be observed.

#### Data availability

The datasets analysed during the current study are available in the Open Science Framework repository, <https://osf.io/kqp28/>.

#### CRediT authorship contribution statement

**Zsófia Zavecz:** Methodology, Project administration, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Kata Horváth:** Methodology, Project administration, Investigation, Formal analysis, Writing - review & editing. **Péter Solymosi:** Investigation, Formal analysis. **Karolina Janacsek:** Conceptualization, Methodology, Software, Resources, Writing - review & editing. **Dezso Nemeth:** Conceptualization, Methodology, Resources, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bbr.2020.112733>.

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