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Individually tuned theta HD-tACS improves spatial performance

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A R T I C L E I N F O

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Background: Using transcranial alternating current stimulation (tACS) to improve visuospatial working memory (vsWM) has received considerable attention over the past few years. However, fundamental issues remain, such as the optimal frequency, the generality of behavioral effects, and the anatomical specificity of stimulation.

Objectives: Here we examined the effects of two theory-driven tACS protocols for improving vsWM on behavioral and electroencephalogram (EEG) measures.

Methods: Twenty adults each completed 3 HD-tACS conditions (Tuned, Slow, and Sham) on two separate days. The Tuned condition refers to a situation in which the frequency of tACS is tuned to individual theta peak measured during a vsWM task. By contrast, the frequency was fixed to 4 Hz in the Slow condition. A high-definition tACS was deployed to target smaller frontal and parietal regions for increasing their phase-locking values. During each tACS condition, participants performed vsWM, mental rotation (MR), and arithmetic tasks. Resting-state EEG (rs-EEG) was recorded before and after each condition.

Results: Compared with Sham, Tuned but not Slow improved both vsWM and MR but not arithmetics. The rs-EEG recording showed an increased fronto-parietal synchrony for Tuned, and this increase in synchronicity was correlated with the behavioral improvement. A follow-up study showed no behavioral improvement in Tuned with an anti-phase setting.

Conclusion: We provide the first evidence that simulating right fronto-parietal network with the tuned frequency increases the interregional synchronicity and improves performance on two spatial tasks. The results provide insight into the structure of spatial abilities as well as suggestions for stimulating the fronto-parietal network.

1. Introduction

Visuo-spatial working memory (vsWM) — our ability to store and manipulate visuo-spatial information — depends on activity in frontal and parietal lobes, and their connectivity [10,51]. Behavioral and imaging studies show correlation between vsWM and other cognitive functions, including mental rotation and mathematics [12,14,21,25,38,40,47]. Neural oscillations, including increased amplitude and synchronicity in the theta (4–8 Hz), alpha (9–13 Hz) and gamma (above 30 Hz) band, has been linked to vsWM maintenance [13,15,36,44,49].

In recent years, there has been growing interest in using the transcranial alternating current stimulation (tACS) in vsWM research. tACS is a non-invasive brain stimulation that can modulate brain intraregional and interregional rhythmic activity [5,6,45]. While the online effect of tACS may be explained by neural entrainment [33], its offline effect may refer to plastic changes [59,60]. tACS has attracted interest in vsWM research for at least two reasons. Following research that indicates the electrophysiological correlates of vsWM, tACS contributes to inferring the causal relationship between neural oscillations and vsWM performance [3]. Furthermore, tACS appears to be a promising tool for enhancing vsWM performance in clinical and aging populations with impaired vsWM [19,48].

Despite the evidence that vsWM can be modulated with tACS, some issues should be addressed further. Firstly, it is still not known whether the observed effect of theta fronto-parietal stimulation is task-specific or whether it generalizes to other tasks too. The tACS

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protocol for vsWM often involves theta stimulation on the right fronto-parietal network (e.g. Refs. [43,50], as right fronto-patietal theta activity is critical for vsWM processing [13,15]. Two studies intervening WM with right theta fronto-parietal tACS reported task-specific improvements [24,58], for example, that the protocol improved WM measured by the 2-back task but not general alertness and response speed measured by the choice-reaction task [58]. However, the task-specific improvement may result from task selection. It is possible that research involving the tasks more closely related to WM can observe the task-general effect. In terms of vsWM, mental rotation and mathematics can be used to examine if the effect is task-general – stimulating right fronto-parietal theta network improves cognitive abilities in addition to vsWM – given they are often associated with vsWM in behavioral and neural imaging studies [12,14,21,25,38,40,47].

Selecting the temporal parameter of tACS also requires consideration for improving vsWM. Theta usually refers to the frequency range of 4 Hz–8 Hz. There are two theory-driven approaches to determining the exact theta frequency for stimulating right fronto-parietal network. The cross-frequency coupling theory suggests that the cycle of theta oscillation works as a slot for vsWM to embed encoded sensory information which is coded in higher frequencies, such as gamma [32]. A slower theta implies a wider slot that permits more high-frequency wavelengths and thus more sensory information to be encoded at the same time, which is supported by empirical research (e.g. Ref. [4]. Following this perspective, tACS can improve vsWM by slowing down the right fronto-parietal theta activity. Previous tACS studies (e.g. Ref. [62] have used 4 Hz, the lower boundary of the conventional theta band, to slow down regional theta activity. Alternatively, improving vsWM may be achieved by adjusting the temporal parameter of tACS to the individual theta frequency, which is often defined as a peak frequency hovering between 4 Hz and 8 Hz during the delay phase of WM tasks [3,19]. This is based on the theory of system resonance and suggests that tACS can induce a larger electrical response when it matches the brain's intrinsic oscillations [37]. Individually tuned fronto-temporal theta tACS has been used to improve object WM performance [48]. Based on the existing literature, both 4 Hz and individually tuned theta can modulate the fronto-parietal network and improve vsWM; however, empirical evidence is needed.

Due to the lack of high-definition tACS (HD-tACS) in earlier studies, there is a degree of spatial uncertainty surrounding the effects observed in fronto-parietal theta stimulation. tACS commonly generates widespread effects outside the targeted brain region, which consequently poses a spatial uncertainty when interpreting the neural substrates of an observed behavioral improvement [7,45]. A partial solution to this problem is the high-definition tACS which can elicit more focal stimulation to increase the confidence of spatial inference by surrounding the stimulation electrodes with oppositely polarized return electrodes [2,7,52].

To address the three questions, we examined the effects of two theta protocols - a slow theta protocol and a tuned theta protocol - on three cognitive tasks, namely vsWM, mental rotation, and arithmetic, with a high-definition tACS deployed over the right fronto-parietal region. The stimulating frequency in the slow theta protocol was fixed at 4 Hz, whereas in the tuned protocol the frequency was determined by the intrinsic theta of each participant measured during performance of a vsWM task. The involvement of mental rotation and arithmetic helps to address the question of the task-specific effect versus to the task-general effect on non-vsWM tasks that are still expected to rely on the same, or similar, fronto-parietal regions. To address the spatial uncertainty, two sets of 2-by-1 electrodes were used to deploy our fronto-parietal tACS. Our hypothesis was that both fronto-parietal theta tACS protocols would have the task-generate effect on performance compared to sham. In addition, resting-state EEG (rs-EEG) over fronto-parietal regions was recorded before and after stimulation to examine the mechanism underlying each theta stimulation.

2. Material and methods

2.1. Participants

Medium or large effects of tACS on modulating different types of WM have been reported [48,58,62]. The current study used a conservative medium effect size – Cohen's f – to estimate the sample size. For a within-subject design, a minimum of 17 participants is required (power 0.8 and significance 0.05). Twenty participants were recruited in Stockholm. The sample included 13 females, had a mean age of 27.5 (range from 22 to 36, SD = 4.0), and was all right-handed but 1. All participants met the inclusion criteria: (1) no history of psychiatric or neurological diagnosis, (2) no history of substance abuse, (3) no history of migraine, (4) no metallic implants in the head, (5) no peacemakers, and (6) normal color vision and visual acuity. All participants gave written informed consent. The study was approved by the Swedish Ethics committee.

2.2. Procedure

Each participant completed 3 conditions – sham stimulation (Sham), 4-Hz slow stimulation (Slow), and in-phase tuned stimulation (Tuned) – on two consecutive days. Participants were unaware of the stimulation order. The 2-day testing was scheduled as Fig. 1.

On the first day, we firstly measured the maximum vsWM capacity of each participant (please see section 2.3.1 below for details). Then, participants were guided to complete 20 trials of vsWM at their maximum levels while EEG was recorded. This process was for quantifying individual fronto-parietal theta connectivity peaks that would later be used for the tuned stimulation. After this, a short tACS on the target brain regions with 1000 μA was delivered to check if participants experienced any phosphene. Experimental sessions were conducted after a self-paced break. Sham stimulation was firstly delivered, followed by Slow or Tuned stimulation after a 20-mins break at least. On the second day, Tuned or Slow stimulation was firstly delivered, followed by a second sham stimulation. The subjects were blinded to the order of the stimulation. The order of Slow and Tuned was counterbalanced between participants. The results of two sham stimulations were averaged to obtain Sham to control potential order or practice effects. During each simulation, participants were guided to complete arithmetic, MR, and vsWM tasks in a fixed order. To counterbalance potential order effects, each task contained two blocks, and all blocks were scheduled as Fig. 1.

2.3. Tasks

2.3.1. vsWM

vsWM capacity of each participant was estimated with the spatial forward span task. Participants were instructed to repeat the sequence of the dots which were randomly presented in a 5-by-5 grid. Each dot was presented for 1000 ms, and a 3000 ms delay phase followed after all dots were presented. The subjects then responded by pointing at all the positions where cues had appeared, in the correct order. The task started with 4 cues. Participants had 2 attempts at each level. If at least one of the attempts was successful, the load was increased with 1 cue, and the test would stop until 2 failures at the same level. Practice trials were given before formal testing. The formal testing consisted of 2 runs.

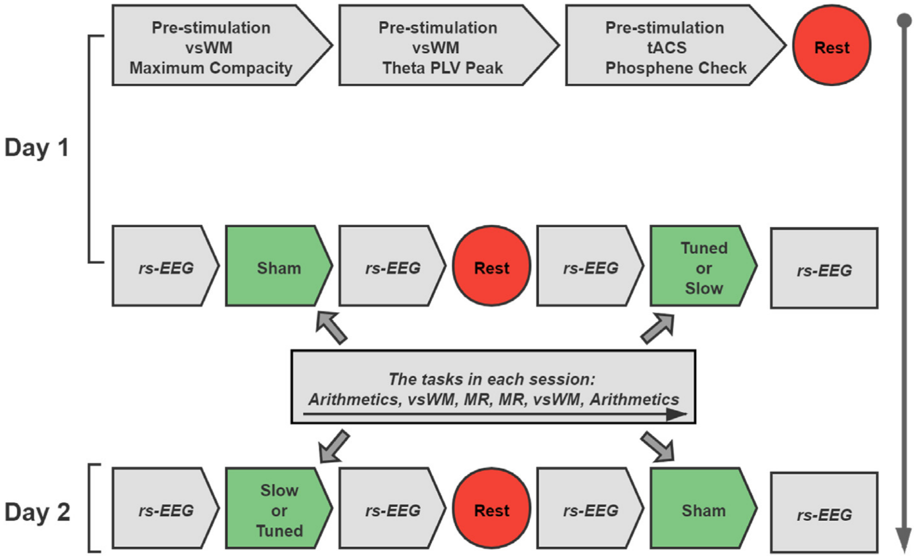

Fig. 1. The schema of the experimental procedure. vsWM, visuo-spatial working memory. PLV, phase-locking value. rs-EEG, resting-state EEG. MR, mental rotation.

The highest level achieved in the 2 runs was selected as the maximum vsWM capacity. To make sure that the highest level was not achieved by chance, 4 more trials at the highest level were conducted. If there was at least 1 correct trial, the highest level was regarded as appropriate to represent the maximum vsWM capacity. All participants passed this double-check procedure.

For the subsequent testing involving vsWM, only the highest level was used. Twenty trials were given in the pre-stimulation vsWM for probing theta peak and in each stimulation for examining the effects of HD-tACS, respectively.

2.3.2. MR

Our MR task was based on the task by Ref. [53]; but with a larger set of stimuli, as reported by Ref. [16]. A 3D object is presented to the left of the screen and a target object to the right. Participants were required to determine if the two objects were identical, or mirrored versions of each other, which requires mentally rotating the target object. The two objects were always rotated at 100 or 150° relative to each other, and each session included 40 pairs (20 of which were identical and 20 which were mirrored). Participants were encouraged to respond as quickly and accurately as possible.

2.3.3. Arithmetic

Arithmetic problems were based on previously published tests [9,17]. The problems consisted of addition and subtraction operations and were divided into two types: simple problems and procedure problems. Zero, one, or tie-related problems (e.g. $5 + 0$, $7-1$, and $28 + 28$) were excluded. The simple problems included one-digit addition (e.g. $3 + 6$) and subtraction (e.g. $8-3$) problems with sums or differences between 2 and 10. The procedure problems included two-digit addition and subtraction problems that required carrying or borrowing with outcomes between 11 and 99. Each problem was generated in 4 similar versions (e.g. $54 + 27$, $55 + 28$, $58 + 24$, and $56 + 25$), which were then assigned to each of the 4 sessions to counterbalance the task difficulty. There were practice trials before testing, and the problems used in the practice trials were excluded from testing. Each session included 20 simple problems and 20 procedure problems. Participants answered by pointing on a pad with numbers on the screen and were encouraged to answer as quickly and accurately as possible.

2.4. HD-tACS and rs-EEG

HD-tACS and the rs-EEG recording were conducted by StarStim 8 (Neuroelectics). Its head cap followed a subset of the 10-10 system. The NG Pistim electrodes – made by Ag/AgCl and having a 1 cm radius filled with conductive gel – were used in this study. Six electrodes were placed on fronto-parietal regions, and one electrode was placed on the outer canthus of the left eye to record EOG while recording EEG. All electrodes were online referenced to the Common Mode Sense and the Driven Right Leg attached to the right earlobe. Only when their impedances were below 10 kΩ did we start HD-tACS or EEG recording. EEG was amplified with a bandpass of 0–125 Hz and was digitized online at a sampling rate of 500 Hz.

2.4.1. HD-tACS protocols

tACS started 3 min before behavioral tests and ended after all tests were completed. Each stimulation session lasted for approximately 25 min. We used an approach similar to Ref. [48] for increasing interregional neural synchrony. Two 2-in-1 montages shown in Fig. 2A were deployed to stimulate right MFG and right IPS, and the simulation of its electric field shown in Fig. 2B was done in SimNIBS 3.1 [55]. The electrodes at F4 and P4 received a sinusoidal alternating current with 1000 μA in-phase. Return electrodes were placed at Fp2, Fz, Pz, and O2. Each of them received 500 μA with a 180° phase difference relative to F4 and P4. In Tuned, the current was delivered at the frequency where participants showed the strongest theta connectivity in the pre-stimulation vsWM task, whereas the frequency of Slow condition was fixed at 4 Hz. In Sham, the frequency was fixed at 6-Hz, and the current was delivered at 90 s at the beginning and the end of the behavioral testing. We asked the first eight participants to guess if they had received real or sham stimulation at the end of each session. The participants were unaware of the condition since the guess was below the level of chance. Besides, all participants were encouraged to report any discomfort they experienced during and after stimulation sessions. Some mild adverse events, such as tingling and itching, were reported during stimulation. None of the participants experienced phosphenes during the experiment.

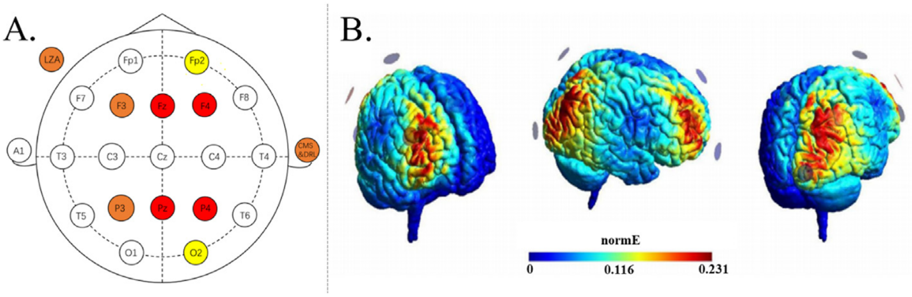

Fig. 2. (A) Electrode set-up. The red electrodes were used for both stimulation and rs-EEG recording, while the yellow electrodes were used only for stimulation, and the orange electrodes were used only for rs-EEG recording. LZA stands for Left Zygomatic Arch, the electrode placed on the left eye. CMS & DRL stands for Common Mode Sense & Driven Right Leg, the reference system. (B) Simulation of the electrical field generated by the in-phase fronto-parietal tACS. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.4.2. EEG recording and preprocessing

EEG recording was conducted for two purposes. To probe the individual feature of right fronto-parietal theta connectivity, EEG was recorded on the F4 and P4 electrodes before tACS while participants completed 20 trials of vsWM at their maximum capacity levels. Moreover, to probe the effects of the tACS protocols on brain fronto-parietal connectivity, 3-min of rs-EEG was recorded on fronto-parietal regions – F4, Fz, F3, P4, Pz, and P3 – immediately before and after each tACS session. EEG recording sessions are outlined in Fig. 1.

EEG data were pre-processed in EEGLAB [11]. All channels were re-sampled at 250 Hz and bandpass filtered from 1 to 70 Hz with a 50 Hz notch filter. To exclude artifacts, we firstly applied the Artifact Subspace Reconstruction [28] as well as through visual inspection. Eyes artifacts were rejected by Gratton's correction [18]. For vsWM-EEG, data were segmented time-locked to the onset of the vsWM delay phase which lasted for 3 s. For rs-EEG, continuous EEG data were segmented into 2-s epochs. For both the recordings, epochs containing activity larger than ±150 μv were excluded.

2.4.3. EEG measures

EEG measures were quantified with Fieldtrip [42]. Time-frequency decomposition was conducted with Morlet wavelets. To increase the frequency resolution, the number of cycles in wavelets was set as 7, which resulted in, at a given frequency f, the spectral bandwidth of 2f/7 and the wavelet duration of 7π/f. Fronto-parietal connectivity was measured by phase-locking value (PLV, [29]. For vsWM-EEG, to avoid edge artifacts, the segments from 500 to 3000 ms after the onset of the delay phase were analyzed. Right fronto-parietal (F4–P4) PLV was thus calculated. To probe the individual stimulation frequency, we followed [48]. The 2500 ms segments were firstly zero-padded to 10000 ms to achieve a 0.1 frequency resolution. The frequency within the theta range (4 Hz–8 Hz) showing the maximum PLV was extracted. The frequency was then rounded up from 0.3 Hz, as the stimulation device had a frequency solution of 0.5 Hz. For example, participants with a maximum PLV at 5.2 Hz rounded down to 5 Hz whereas participants with a maximum PLV at 5.3 Hz rounded up to 5.5 Hz. The rounded frequency was set as the individual tuned frequency (See Fig. 3 for its distribution among participants). For rs-EEG, PLV was calculated from 3 pairs of the electrodes, F4–P4, Fz-Pz, and F3–P4. The same time-frequency decomposition was applied but without zero paddings. Hence, with the 2-s rs-EEG epochs, it has a frequency resolution of 0.5 Hz. Given that theta online stimulation can affect resting alpha range activity [26], both theta band and alpha band (8.5 Hz–13 Hz) were analyzed with PLV to examine stimulation effects. The band PLV was calculated by averaging PLVs within their bands. Besides, the theta peak PLV, defined as the PLV at the individual theta peak frequency, was used for further analysis.

2.5. Statistical analysis

We used trial-level and block-level analyses for identifying outliers. The trial-level analysis was conducted for the tasks involving RT – MR and arithmetic. Trials with RT exceeding 3 standard deviations from the mean were excluded. The block-level analysis was conducted for all tasks using Cook's distance. The cutoff value was set based on the cumulative probability exceeding 0.5 in the F distribution with df = (k + 1, n - k - 1) where k is the number of predictors and n is the number of participants [1]. One participant's procedure arithmetic performance failed to meet this criterion and was excluded. Thus, the analysis of arithmetic was derived from the remaining 19 participants.

As both RT and accuracy were recorded for the simple arithmetic, procedure arithmetic, and MR task, to avoid potential speed-accuracy tradeoffs, the current study combined RT and accuracy with the approach called the linearly integrated speed-accuracy score (LISAS; [56,57], defined as:

$$\mathrm{LISAS} = \mathrm{RT}_j + \left(\mathrm{PE}_j \times \frac{\mathrm{SD}_{rt}}{\mathrm{SD}_{pe}}\right)$$

where RT is the participant's mean RT_j in condition j, PE_j is the corresponding proportion of errors in the condition, and SD_{rt} and SD_{pe} are the corresponding standard deviation, respectively.

The test-retest reliability of each task measure was examined by the intra-class coefficient (ICC). The measures of each task over Protocol (Tuned, Slow, and Sham) entered into a two-way mixed ICC model. The average measure ICCs were reported, together with their 95% confidence intervals. Values less than 0.5 indicate poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability [27].

Before analyzing the effects of two theta protocols on tasks performance, the EEG data recorded during vsWM were used to examine the relationship between the maximum vsWM level and theta features including right fronto-parietal theta band PLV, right fronto-parietal theta peak PLV, and the peak frequency. The bias-corrected and accelerated bootstrap 95% confidence intervals are reported together with Pearson's r, estimated from 1000 iterations.

The effects of the tACS protocols on the behavioral measures were examined by the one-factor repeated ANOVA. The Greenhouse-Geisser correction was applied if the sphericity assumption was violated. The main effects were followed by the planned contrasts: Tuned versus Sham and Slow versus Sham. It

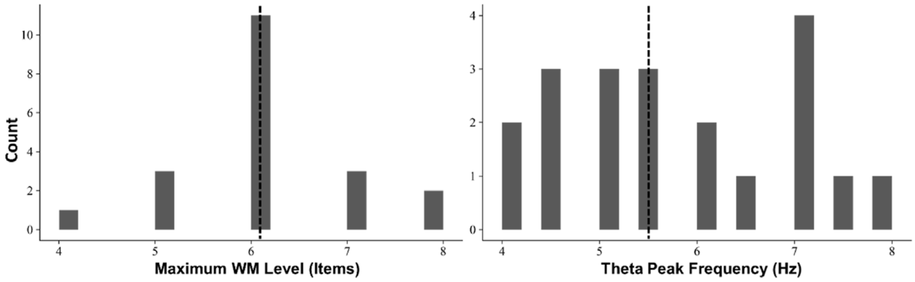

Fig. 3. The histograms of individual maximum WM level and the theta peak frequency. The dashed line represents the median.

should be noted that the performance of Sham was derived by averaging the outcomes of two sham sessions. Effect sizes were calculated by partial eta squared.

The three-factor repeated ANOVA was used for examining the effects on rs-EEG with three factors: Protocol (Sham, Tuned, and Slow), Time (Pre-stimulation and post-stimulation), and Location (F4–P4, Fz-Pz, and F3–P4). Specifically, the current study focused on Protocol × Time interaction and Protocol × Time × Location interaction. In the case of the three-way interaction, the two-way interaction – Protocol × Time – was analyzed at each level of Location. The planned contrasts were conducted for disentangling Protocol effects: Tuned versus Sham and Slow versus Sham. Effect sizes were calculated by partial eta squared.

3. Results

3.1. Task test-retest reliability

For vsWM, the ICC value was 0.957 $\mathrm{CI}_{95\%}$ [0.909–0.982], F (19, 38) = 23.171, $p < 0.001$. For MR, the ICC value was 0.933 with a $\mathrm{CI}_{95\%}$ [0.857–0.972], F (19, 38) = 16.957, $p < 0.001$. For simple arithmetic, the ICC value was 0.897 with a $\mathrm{CI}_{95\%}$ [0.794–0.955], F (18, 36) = 26.992, $p < 0.001$. For procedure arithmetic, the ICC value was 0.914 with a $\mathrm{CI}_{95\%}$[0.827–0.963], F (18, 36) = 32.945, $p < 0.001$. The ICC values indicated that the behavioral measures had a good to excellent reliability [27].

3.2. vsWM performance and task-related theta

The maximum WM level and the theta peak frequency in our sample are shown in Fig. 3. The maximum WM level was significantly correlated with the theta band PLV ($r = 0.509$, 95% Bca CI [0.28, 0.75] and the theta peak PLV ($r = 0.435$, 95% Bca CI [0.21, 0.65], but not with the theta peak frequency ($r = -0.23$, 95% Bca CI [−0.55, 0.19].

3.3. Effects on behavioral outcomes

The results were summarized in Fig. 4. Concerning vsWM, the Greenhouse–Geisser estimates of sphericity showed a substantial deviation ($\varepsilon = 0.768$). The conditions of tACS had a significant effect on vsWM, F (1.536, 29.175) = 3.684, $p < 0.05$, $\eta_p^2 = 0.162$). The follow-up pair-wise comparison showed that, compared to Sham, Tune improved vsWM performance (F (1, 19) = 5.103, $p < 0.05$, $\eta_p^2 = 0.212$) whereas Slow did not (F (1, 19) = 0.089, $p > 0.05$, $\eta_p^2 = 0.005$).

There was a significant effect of Condition on MR, F (2, 38) = 3.758, $p < 0.05$, $\eta_p^2 = 0.165$. Compared to Sham, Tune improved on the MR performance (F (1, 19) = 5.332, $p < 0.05$, $\eta_p^2 = 0.219$) whereas Non-tuned did not (F (1, 19) = 0.330, $p > 0.05$, $\eta_p^2 = 0.017$).

In contrast, there were no effects of Condition on the two mathematical outcomes: simple arithmetics (F (1.460, 26.273) = 1.014, $p > 0.05$, $\eta_p^2 = 0.053$) or procedural arithmetics (F (2, 36) = 1.969, $p > 0.05$, $\eta_p^2 = 0.099$).

3.4. Effects on EEG connectivity

For the alpha-based PLV, Protocol × Time was not significant (F (2, 38) = 2.998, $p > 0.05$, $\eta_p^2 = 0.136$) but there was a significant Protocol × Time × Location (F (4, 76) = 5.000, $p = 0.001$, $\eta_p^2 = 0.208$). Thus, Protocol × Time was further examined at each pair through the planned contrasts. The results are summarized in Fig. 5. For F4–P4, the two-way interaction was significant (F (2, 38) = 3.257, $p < 0.05$, $\eta_p^2 = 0.146$), and compared to Sham, Tune showed a larger Session effect, with significant increase in synchronicity (F (1, 19) = 7.832, $p < 0.05$, $\eta_p^2 = 0.292$) whereas Non-Tune did not show such an effect (F (1, 19) = 0.835, $p > 0.05$, $\eta_p^2 = 0.042$). A similar effect was observed for Fz-Pz. There was a significant two-way interaction (F (2, 38) = 5.011, $p < 0.05$, $\eta_p^2 = 0.209$). Compared to Sham, Tune showed a larger Session effect (F (1, 19) = 8.688, $p < 0.001$, $\eta_p^2 = 0.314$) whereas Non-Tune did not show such an effect (F (1, 19) = 0.192, $p > 0.05$, $\eta_p^2 = 0.010$). In contrast, F3–P3 did not show the two-way interaction (F (2, 38) = 1.478, $p > 0.05$, $\eta_p^2 = 0.072$).

The same analysis was conducted for the theta band PLV and the theta peak PLV. Neither Protocol × Time nor Protocol × Time × Location reached the significant level for any of the conditions (all $p > 0.05$).

3.5. rs-EEG change correlated to behavioral change

We further examined the relationship between the behavioral change and the rs-EEG change. The difference in performance between Sham and Tuned was calculated for vsWM and MR, respectively. The two differences were then transformed into z-scores. Before averaging these two z-scores to obtain the behavioral composite change, considering that a larger LISAS of MR indicates worse performance, the z-score of MR was thus multiplied by −1. To calculate the rs-EEG change for F4–P4 and Fz-Pz, we subtracted the pre-post difference of Sham from that of Tune. The two rs-EEG differences were then transformed to z-scores and averaged to obtain the rs-EEG composite change. A bivariate correlation was conducted between the behavioral composite change and the rs-EEG composite change. We found that the two composite

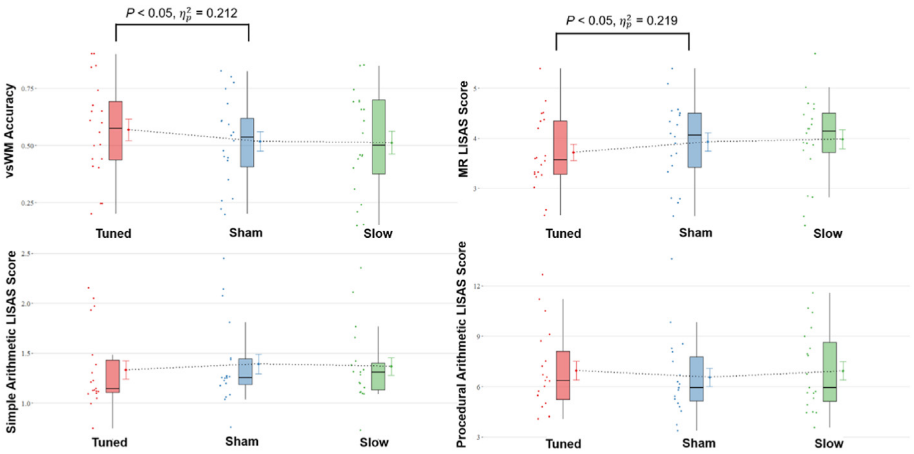

Fig. 4. The Protocol effect on each behavioral task. The scatted dots left to box plots represent the performance of each participant. The single dots right to box plots represent the means.

changes were significantly correlated ($r = 0.46$, 95% Bca CI [0.09, 0.72]) (Fig. 6).

3.6. Follow-up study

A possible explanation of the effect observed in Tuned is that the behavioral benefit was just caused by the local stimulations, for example, individualized theta tACS on F4 or P4 alone but not specifically related to the synchronization of the stimulation. A follow-up study was conducted to clarify the importance of phase synchronization in the effects of the in-phase tuned condition. Ten participants accepted our invitation for revisiting our lab. The testing consisted of an anti-phase tuned stimulation and a sham stimulation. The anti-phase stimulation followed the same procedure as the in-phase tuned stimulation but that F4 had a 180° phase difference relative to P4. The order of two stimulations was counterbalanced between participants. The same statistical analyses were applied but the tACS protocols only included an anti-phase stimulation and a sham stimulation.

The anti-phase tuned condition did not differ from the sham condition in vsWM ($F\,(1, 9) = 0.783$, $p > 0.05$, $\eta_p^2 = 0.080$) or MR ($F\,(1, 9) = 0.016$, $p > 0.05$, $\eta_p^2 = 0.002$. Similarly, the alpha-based PLV did not show the significant Protocol × Time interaction ($F\,(1, 9) = 0.108$, $p > 0.05$, $\eta_p^2 = 0.012$) or the Protocol × Time × Location interaction ($F\,(2, 18) = 0.338$, $p > 0.05$, $\eta_p^2 = 0.036$).

4. Discussion

Here we examined the effects of two fronto-parietal theta protocols with HD-tACS on three cognitive tasks and rs-EEG. We found that the in-phase, individually tuned protocol of theta tACS improved vsWM and MR and increased fronto-parietal phase synchronization. In contrast, the fixed, slow (4 Hz) theta protocol did not show any behavioral or electrophysiological effects.

Mental rotation and vsWM are similar in that they both require creation and retention of an internal spatial representation [34]. Although mental rotation also requires additional manipulation of the spatial representation, performance on vsWM and MR tend to be correlated (e.g. Refs. [30,38]. Neuroimaging studies furthermore suggests that this could be related to common neural basis in fronto-parietal networks [46].

There is an ongoing debate of whether there is only one spatial abilitiy, or several, with some large studies favoring a single [35], while others suggest that there might be at least 2 during childhood [38]. Although we did not include enough tasks to perform a latent variable analysis of spatial ability, the effect on two spatial tasks could suggest that a general spatial ability was improved, and it

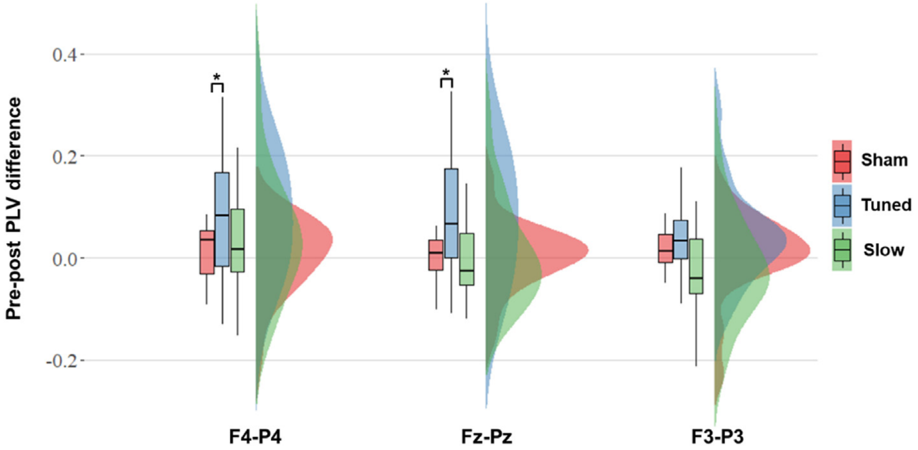

Fig. 5. The effect of Protocol on the rs-EEG pre-post difference in the alpha band. PLV, phase-locking value. * represents $p < 0.05$.

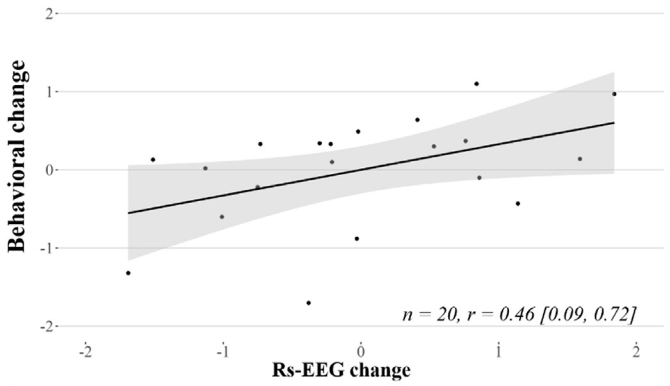

Fig. 6. The correlation between the rs-EEG change and the behavioral change. The Bootstrap 95% confidence intervals are reported in the square brackets.

could relate two the common aspects of the tasks, i.e. representation and retention of spatial information, but not manipulation. To our knowledge, this is the first study demonstrating the effect of right fronto-parietal tACS on a more general spatial ability. In future studies, it would be of interest to evaluate the effect on a wider range of spatial abilities.

Spatial ability is also correlated with performance on a wide range of STEM-related tasks [8,21,39,41,61,63]. Training-induced enhancement of vsWM also improves math performance [25]. Based on these associations, we also included a pre-post test of mathematics but did not observe any significant improvement. Since the correlation between spatial abilities and mathematics is weaker than between MR-vsWM, the lack of significance could be a power issue. But it should also be kept in mind that mathematics is a wide concept, and the results could differ depending on the type of mathematical content, and to what extent performance it is determined by prior long-term memory based knowledge rather than on-line processing. We can therefore not exclude that another type of mathematical task would give another result. Future studies may examine whether the tuned protocol presented here can help facilitate STEM learning, particularly for those who have difficulty learning STEM subjects.

The positive effects were only found after tuning tACS to the individual theta frequency measured during vsWM, but not by using a fixed slow, 4 Hz theta stimulation. The effect of the tuned protocol is consistent with a recent study in which individually tuned fronto-temporal theta tACS improved WM for objects [48]. The present study extends these findings by showing an improvement also for vsWM and transfer to a task not used for tuning.

In contrast to some prior studies [20,62], we did not observe the positive effect of the slow-theta protocol. One reason for this might be differences in the strength of stimulation. While we used a 1 mA current, which is well tolerated by most individuals [19,62] used 2 mA and [20] used a 1.5 mA current. The none effect of the slow protocol on rs-EEG gives some indication that 1 mA may not be sufficient to slow down the fronto-parietal network. Although 1 mA can be intense enough to enhance the intrinsic oscillation, i.e. entrainment [22], more intense stimulation might be necessary when the frequency of tACS does not match the intrinsic oscillation [22,37].

Another possible explanation is that only the strength of the fronto-parietal connection, rather than its speed, is relevant to the spatial ability involved here. The finding that slower theta leads to better vsWM is based on regional electrophysiological recordings, such as intracranial EEG recording in human hippocampus [4]. The slower theta provides more slots to embed stimuli encoded by local faster oscillations (e.g. gamma); however, this does not necessarily mean that slower interregional theta synchrony is better for vsWM. In our sample, only the strength of the fronto-parietal theta PLV, but not the frequency at which the PLV is strongest, was correlated with vsWM performance. Future studies may examine whether only the synchrony degree matters by comparing the condition where the fronto-parietal network has been successfully slowed down (possibly by using a larger-than-1 mA tACS) with the individually tuned condition.

The underlying mechanism of tACS might be to enhance synchronization of brain regions for more efficient information processing [19]. In line with this, we found increased fronto-parietal phase synchronization after tuned stimulation with offline EEG, and the synchronization increase was significantly correlated with the improvement in spatial ability. However, the enhancement was evident in the alpha-frequency but not in the stimulated frequency. The finding that offline effects can differ in frequency from online stimulation has been reported before [26]. The studies that have found increased rs-EEG changes matching the stimulation has most often found these after alpha-stimulation, which is also the dominant frequency during rest [59].

A possible explanation for a mismatch in frequency is that stimulation both results in entrainment and enhances synaptic plasticity [59]. It may be that fronto-parietal synchronization of theta is increased when tACS is being delivered, but the plasticity results in increased synchronization in the alpha range in the offline recording as alpha is the dominant neural oscillation in the resting state. Future studies may address this by adopting both online and offline EEG recording.

5. Conclusions

Overall, this study demonstrates the effect of the tuned fronto-parietal theta stimulation on the general spatial ability in healthy adults. Also, we reduce the temporal-spatial uncertainty in this area by comparing the tuned protocol with a slow protocol and by using HD-tACS. Our findings contribute to a better understanding of the structure of spatial abilities and provide suggestions for stimulating the fronto-parietal network.

CRediT authorship contribution statement

Da-Wei Zhang: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Alexandros Moraidis:** Software, Investigation, Resources, Validation, Project administration. **Torkel Klingberg:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

none.

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