# Alpha oscillatory activity causally linked to working memory retention: insights from online phase-locking closed-loop transcranial alternating current stimulation (tACS)

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## ABSTRACT

Although previous studies have reported correlations between alpha oscillations and the "retention" sub-process of working memory (WM), no direct causal evidence has been established in human neuroscience. Here, we developed an online phase-locking closed-loop transcranial alternating current stimulation (tACS) system capable of precisely controlling the phase difference between tACS and concurrent endogenous oscillations. This system permits both up- and down-regulation of brain oscillations at the target stimulation frequency, and is here applied to empirically demonstrate that parietal alpha oscillations causally relate to WM retention. Our experimental design included both in-phase and anti-phase alpha-tACS applied to 39 participants during the retention intervals of a modified Sternberg paradigm. Compared to in-phase alpha-tACS, anti-phase alpha-tACS decreased both WM performance and alpha activity. Moreover, the in-phase tACS-induced changes in WM performance were positively correlated with alpha oscillatory activity. These findings strongly support a causal link between alpha oscillations and WM retention, and illustrate the broad application prospects of phase-locking tACS.

## INTRODUCTION

Working memory (WM) is considered to be foundational for a broad range of cognitive functions (*e.g.*, the capacity for general intelligence, categorization, retrieving selected long-term memories, language learning, etc.) (*1*). WM enables the maintenance, manipulation, and retrieval of mental representations, as well as the use

of this information in goal-directed behaviors (*1*). Because of its essential role in human cognition, investigating the neural mechanisms underlying WM has been a focus of neuroscience research for decades (*2, 3*).

WM can be subdivided into three fundamental sub-processes: encoding, retention, and retrieval (*4*). Neural oscillations in the alpha frequency band (8-13Hz) have been associated with WM retention (*5*): alpha activity in the occipitoparietal areas increases during memory retention (*6-10*). Previous studies reported that alpha power increased parametrically with memory load during the retention interval (*6, 11*). However, as these previous studies only used MEG or EEG, their inferences are correlational in nature; there have not been direct experimental investigations which successfully demonstrated a causal role for alpha oscillations in WM retention in humans.

Transcranial alternating current stimulation (tACS) may provide us with the opportunity to experimentally investigate a causal role for alpha oscillations in WM retention, owing to its ability to entrain naturally occurring neural oscillations based on externally-applied, sinusoidal electric fields at a targeted frequency (*12-15*). It should be noted that results from previous alpha-tACS studies are at the center of a controversial debate in which some studies failed to replicate successful entrainment effects of alpha-tACS (*16*). This discrepancy across alpha-tACS studies may be related to the fact that previous efforts have (to our understanding) not accounted for phase differences between an applied sinusoidal waveform and the concurrent, endogenous oscillations occurring in the targeted brain regions (*17*). Although direct evidence based on monitoring oscillatory neural activity is absent, recent computational (*18*) and

indirect experimental evidence (*19*) suggests that the phase differences between tACS and concurrent brain oscillations are impactful for determining the efficacy of tACS (also for determining the robustness of effects and replicability). Moreover, contrary to the retention sub-process, decreased (rather than increased) alpha oscillations during the encoding and retrieval sub-processes was reported to be beneficial for WM performance (*20, 21*). Thus, tACS investigating the causal role of alpha oscillations in WM retention should be time-locked to the specific WM sub-process of interest (i.e., retention but not encoding or retrieval), to avoid the offset effects of tACS applied during the other two sub-processes.

Given this background, there are two major reasons that conventional tACS methods do not support experimental investigations about the function(s) of alpha oscillations in WM retention: conventional tACS methods cannot adjust for phase differences between tACS and endogenous brain oscillations in real-time ("online"), and conventional tACS methods do not support tACS stimulation that can be time-locked to the specific sub-process of WM. These ideas motivated our desire for a tACS technology with the following capabilities: 1) a capacity for online monitoring of endogenous alpha oscillatory activity, to support real-time phase-locking between externally applied tACS and concurrent endogenous alpha oscillations, and 2) a capacity to induce short-duration (within seconds) tACS in a time-locked manner which can be matched to the short-duration retention interval in each trial.

The present study successfully developed a trial-by-trial closed-loop tACS-EEG design that is capable of selectively aligning an applied alpha-tACS phase to the phase

of concurrent endogenous oscillations (**Fig. 1 & 2**), specifically during the retention interval of a Sternberg WM task. Importantly, this technique also enables recording of alpha oscillations in real-time, thus supporting analyses of the online effects of tACS on alpha oscillations. This allowed us to directly investigate whether alpha oscillations exert any causal impacts on WM retention. Our experimental investigations included alpha-tACS with three different phase differences to the online monitored endogenous alpha oscillations (in-phase tACS, anti-phase tACS, and random-phase tACS, **Fig. 3C**) applied specifically during the WM retention interval, as well as a control theta-tACS experiment to exclude any general effects of tACS (*i.e.*, independent of frequency) (*22*). Fascinatingly, we found that compared to in-phase alpha-tACS, anti-phase alpha-tACS suppressed WM performance, parietal alpha power, and frontoparietal alpha synchronization, and noted that changes in WM performance induced by in-phase alpha-tACS were positively correlated with changes in endogenous alpha oscillatory activity. Ultimately, beyond experimentally demonstrating a causal role for parietal alpha oscillations in WM retention, our results clearly illustrate how phase differences between tACS and concurrent endogenous brain oscillations determine the efficacy and replicability of tACS effects.

## Results

**No systematic differences between in-phase tACS and anti-phase tACS at pre-test.** We have developed an online phase-locking closed-loop tACS system that is able to measure brain oscillations by an EEG instrument, analyze the raw EEG data online

using the computer to extract the phase of an underlying brain rhythm, and control the timing of tACS based on the phase of the underlying oscillations, and closing the loop, affect the brain oscillations (**Fig. 1**). Then, we used the online phase-locking closed-loop tACS system to modulate alpha oscillations during the retention interval of WM (**Fig. 2**; Methods; Supplementary material: 1.1. The details of online phase-locking closed-loop tACS) while participants performed a Sternberg paradigm (**Fig. 3A**; Methods) (*23*). The Sternberg task is chosen because of its suitability for separating the three different processes of working memory: encoding, retention, retrieval, so that we can apply tACS specifically during the retention interval on a trial-by-trial basis. TACS with 2-mA peak-to-peak amplitude at individual alpha frequency (IAF, see Supplementary material: 1.2. IAF and threshold determination) was applied to the central parietal-occipital brain areas in in-phase (with 0° relative phase to the endogenous alpha oscillations), anti-phase (with 180° relative phase to the endogenous alpha oscillations), and random-phase (the phase difference between the tACS stimulation and the endogenous alpha oscillations was different across trials) conditions (**Fig. 3C**; Methods). To test the accuracy of the correction for the phase difference, we analyzed the phase alignment between unstimulated EEG signal and artificial tACS waveform predicted offline, and the phase alignment is as expected (see Supplementary material: 1.3. Analysis of the phase alignment between EEG signal and tACS waveform for detail information).

Each participant performed a session of each tACS condition (in-phase tACS, anti-phase tACS, and random-phase tACS) separated by at least 3 days in a pseudo-

randomized cross-over design. Within each session, spontaneous EEG was recorded during the retention interval of Sternberg paradigm in the first block (pre-test) before tACS to calculate IAF as the stimulation frequency for the subsequent tACS. Then, participants performed 3 tACS-EEG blocks (During1) while performing Sternberg task. EEG data during the retention interval of each trial was monitored and the online phase-locking closed-loop tACS was triggered and lasted for 0.8 s at each trial once the EEG signal met requirements. Some previous studies reported that the effects of transcranial electrical stimulation do not necessarily accumulate over time (*24*), and sometimes the effect of stimulation with longer duration was even opposed to the stimulation with shorter duration (*25*). Therefore, to explore the potential duration-dependent effect of our online phase-locking closed-loop tACS, our experiments included a second "During" that followed During1. After a rest period of about 2 min, another 3 tACS-EEG blocks (During2) were performed with the design and tACS parameters same as During1. Finally, after stimulation, participants completed a final 8$^{th}$ block of the Sternberg paradigm (post-test) while recording EEG (**Fig. 3B**; Methods).

To measure the behavioral performance of the Sternberg WM task, we calculated the rate correct score (RCS, the number of correct responses per second, see Methods for details on RCS), accuracy (defined as the percentage of correct responses), and mean reaction times (RT, only correct RTs were included in the analysis). Power spectra were computed via a fast Fourier transform (FFT) for every trial, and then averaged across trials and the alpha-bands (8-13Hz) as the absolute power in alpha band. The relative alpha power was finally defined as the absolute power in alpha band divided

by the absolute total power across the frequency band of 1-45 Hz (see Methods for detail information). The mean phase lag index (PLI) between the alpha activity (8-13Hz) of the 7 frontal electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8) and Pz electrode was used to measure the frontoparietal alpha synchronization (see Methods for detail information).

In order to test the main hypothesis that tACS effects depend on the phase difference between tACS and endogenous brain oscillations, the lower or even opposite effects of anti-phase tACS compared to in-phase tACS were tested first considering previous simulation results suggest that anti-phase tACS suppresses while in-phase tACS entrains targeted brain oscillations in a short time (*18*). To ensure that there were no systematic differences between in-phase and anti-phase sessions before tACS, two-tailed paired *t*-tests were conducted for pre-test data. There were no significant differences in WM performance between the two stimulation sessions (permuted paired *t*-test, accuracy: $t(38) = 0.039$, $p = 0.956$; RT: $t(38) = -0.135$, $p = 0.894$; RCS: $t(38) = -0.002$, $p = 0.998$). Further, there were no significant differences between the two sessions in pre-test alpha power or frontoparietal alpha synchronization values (permuted paired *t*-test, alpha power: $t(38) = -0.316$, $p = 0.743$; PLI: $t(38) = -1.658$, $p = 0.104$).

**Anti-phase tACS impaired working memory performance compared to in-phase tACS**. Due to the suggested different mechanisms underlying online tACS effects (*18, 26*) (effects observed during stimulation: During1 and During2) and offline tACS effects (*27*) (aftereffects beyond stimulation: post-test), we analyzed the online tACS

effects firstly by comparing the online behavioral and EEG effects induced by in-phase and anti-phase tACS, then the offline tACS effects. We first investigated whether anti-phase tACS down-regulated WM performance compared to in-phase tACS as hypothesized, measured by RCS, accuracy, and RT. RCS was used to integrate measurements of RT and accuracy into a single measure to avoid contradictory findings in these two important aspects of performance (*28*). This score can be interpreted as the number of correct responses per second of activity. At During1, anti-phase tACS significantly induced RCS reduction compared to in-phase tACS (permuted paired *t*-test, $t(38) = -2.209$, $p_{corrected} = 0.036$, Cohen's $d = 0.354$, one-tailed), which reflected fewer correct responses per second of activity in anti-phase tACS (**Fig. 4A**). At During2, anti-phase tACS did not impair WM performance compared to in-phase tACS, without RCS reduction (permuted paired *t*-test, $t(38) = -0.607$, $p_{corrected} = 0.556$, one-tailed) (**Fig. 4A**). No significant differences in accuracy and RT were observed between in-phase and anti-phase tACS at both During1 and During2 (**fig. S2**), probably due to their weaker reliability than RCS which integrated the two important aspects of performance into a single measure (*46*) (See more details in Supplementary material: 2. The online comparison of accuracy and RT between in-phase tACS and anti-phase tACS). The different effects on WM performance between in-phase tACS and anti-phase tACS indicate the phase differences between tACS and concurrent endogenous brain oscillations affect tACS-induced behavioral effects.

**Anti-phase tACS suppressed alpha power compared to in-phase tACS**. We next

assessed whether in-phase tACS and anti-phase tACS modulated the targeted parietal alpha oscillations during stimulation. Alpha power (8-13Hz) at Pz electrode was analyzed because its signal served as the trigger for tACS and it was near to the stimulation electrode. As hypothesized, anti-phase tACS significantly suppressed the alpha power at Pz electrode compared to in-phase tACS at both During1 (permuted paired *t*-test, $t(38) = -2.257$, $p_{corrected} = 0.027$, Cohen's $d = 0.361$, one-tailed) and During2 ( permuted paired *t*-test, $t(38) = -2.185$, $p_{corrected} = 0.039$, Cohen's $d = 0.350$, one-tailed) (**Fig. 4B**). This finding suggests the modulation effects of our closed-loop tACS system on the targeted brain activity and the influence of phase on tACS electrophysiological effects. To support the frequency-specific effects of our online phase-locking closed-loop system, we next tested the influences of alpha tACS on the full physiological frequency band of the EEG (1-45 Hz). No significant differences between in-phase tACS and anti-phase tACS were found in all of the frequency bands except alpha band at both During1 and During2 (For more details, see Supplementary material: 3. The comparison of power in full frequency band between in-phase tACS and anti-phase tACS). This result indicates the frequency-specific modulation of our alpha tACS on endogenous brain oscillations as previous tACS studies have shown (*12, 27*) (**fig. S3**). Therefore, it is reasonable to attribute the behavioral effects of tACS to the modulation on alpha oscillations at the retention interval, supporting the causal link between alpha oscillations and WM retention.

**Anti-phase tACS disturbed frontoparietal alpha synchronization compared to in-**

**phase tACS.** Previous studies have reported the potential impact of tACS on functional connectivity (*29, 30*) and found increases in the strength of alpha synchronization with increasing memory load among the frontoparietal regions known to underlie executive and attentional functions during WM maintenance (*31, 32*), so online tACS effects in frontoparietal alpha synchronization were also assessed. The phase lag index (PLI) was used to describe frontoparietal alpha synchronization considering its reliable estimates of phase synchronization against the presence of volume conduction (*33*).

Anti-phase tACS induced a significant decrease in frontoparietal alpha synchronization compared with in-phase tACS at During1 (permuted paired *t*-test, t(38) =-2.067, $p_{corrected}$ = 0.044, Cohen's d = 0.331, one-tailed), but not significant at During2 (permuted paired *t*-test, t(38) =-1.715, $p_{corrected}$ = 0.096, one-tailed) (**Fig. 4C**). To further illustrate the reliability of our results, we also used weighted phase lag index (WPLI) to measure frontoparietal alpha synchronization, which may be more sensitive to unrelated noise resources (*34*). As expected, compared to in-phase tACS, anti-phase tACS disturbed frontoparietal alpha synchronization, measured by WPLI, at During1 and During2 (**fig. S4)**. These results suggest that our online phase-locking closed-loop tACS not only affect the brain activity of the targeted region, but also modulate the connectivity of distributed brain regions.

Besides, there were no significant differences between tACS conditions (in-phase and anti-phase) for tACS-induced side effects (for more details of the analysis, see the Supplementary material: 1.4. Analysis of tACS questionnaire), so it rules out that the above reported different behavioral and electrophysiological effects between in-phase

tACS and anti-phase tACS were caused by tACS-related sensations.

**Compared to pre-test, anti-phase tACS significantly down-regulated RCS of WM performance and alpha power at an early stimulation period.** Above results showed that compared to in-phase tACS, anti-phase tACS induced significant online down-regulation in RCS, alpha power, as well as frontoparietal alpha synchronization at During1. To further investigate whether the down-regulation at During1 was due to in-phase tACS-induced improvement or anti-phase tACS-induced suppression, we analyzed the changes from pre-test to During1 within in-phase tACS and anti-phase tACS. RCS and alpha power at Pz electrode in anti-phase tACS reduced significantly at During1 (permuted paired *t*-test, RCS: $t(38) = -2.117$, $p_{corrercted} = 0.038$, Cohen's $d = 0.339$, one-tailed; alpha power: $t(38) = -2.171$, $p_{corrected} = 0.04$, Cohen's $d = 0.348$, one-tailed). No anti-phase tACS-induced disturbance in frontoparietal alpha synchronization was found at During1 (permuted paired *t*-test, $t(38) = -1.926$, $p_{corrected} = 0.058$, one-tailed) (**fig. S5**). But in in-phase tACS, no improvement was observed from pre-test to During1 for all the three metrics (**fig. S5**). These results indicated that the different effects between in-phase tACS and anti-phase tACS at During1 was mainly due to the suppression effects of anti-phase tACS rather than the improvement induced by in-phase tACS. For more details about the changes from pre-test to subsequent time points within in-phase tACS and anti-phase tACS, see supplementary material (supplementary material: 5. The changes from pre-test to During1, During2 and post within in-phase tACS and anti-phase tACS).

**The correlations between tACS-induced changes in alpha activity and RCS were significantly positive for in-phase condition, which was different from anti-phase condition.** As anti-phase tACS not only impaired RCS, but also suppressed alpha power and frontoparietal alpha synchronization compared to in-phase tACS at During1, we further investigated whether the modulation of alpha activity related to WM performance at During1. We performed permuted Pearson' s correlations between the stimulation-induced changes (from pre-test to During1) in RCS and EEG metrics. The correlation between in-phase tACS-induced change in alpha power and RCS was significant at During1 (permuted Pearson's correlation, $r = 0.348$, $p = 0.030$). This finding indicated that across participants increased alpha power related to larger RCS, further supporting the causal role of alpha oscillations for WM retention. No correlation was found between anti-phase tACS-induced change in alpha power and RCS (permuted Pearson's correlation, $r = -0.244$, $p = 0.138$). More importantly, there was a significant difference between the correlation coefficients in in-phase tACS and anti-phase tACS at During1 ($Z = 2.599$, $p = 0.009$) (**Fig. 5A**).

Similar to the correlations between the changes in alpha power and RCS, we also found a positive correlation between in-phase tACS-induced change in frontoparietal alpha synchronization and RCS at During1 (permuted Pearson's correlation, $r = 0.415$, $p = 0.007$), but no correlation was found in anti-phase tACS (permuted Pearson's correlation, $r = -0.242$, $p = 0.14$). A significant difference between the correlation coefficients in in-phase tACS and anti-phase tACS was also observed at During1 ($Z =$

2.921, p = 0.003) (**Fig. 5B**). The observed inverse correlation trends for in-phase tACS and anti-phase tACS further illustrate the different effects of in-phase tACS and anti-phase tACS on WM.

**Rebound following anti-phase tACS-induced suppression on working memory and brain activity.** Above results show that anti-phase tACS impaired WM performance at During1 when compared to in-phase tACS, but the impairment disappeared at During2. To explore the reason, we compared the RCS and EEG metrics between During1 and During2 within in-phase tACS and anti-phase tACS. In anti-phase tACS, the decreased RCS and parietal alpha power of During1 were improved significantly at During2, and frontoparietal alpha synchronization also exhibited an increase tendency although not reaching statistical significance (permuted paired *t*-test, RCS: t(38) = 2.508, $p_{corrected}$ = 0.029, Cohen's d = 0.402; alpha power: t(38) = 3.083, $p_{corrected}$ = 0.008, Cohen's d = 0.494; PLI: t(38) = 1.614, $p_{corrected}$ = 0.236) (**Fig. 6**). During in-phase tACS, there was no significant difference in RCS and frontoparietal alpha synchronization between During2 and During1 (permuted paired *t*-test, RCS: t(38) = 0.517, $p_{corrected}$ = 1.000; PLI: t(38) = 1.209, $p_{corrected}$ = 0.450). Although in the in-phase condition, the alpha power of the Pz electrode at During2 increased compared with During1 (permuted paired *t*-test, alpha power: t(38) = 3.842, $p_{corrected}$ < 0.001, Cohen's d = 0.615) , it might be more like the accumulation of in-phase tACS effect considering the enhancement effect of in-phase tACS at the early stimulation period (During1) (**Fig. 6**). These results suggest the null difference on WM performance between in-phase

tACS and anti-phase tACS at During2 may be due to the rebound following anti-phase tACS-induced suppression of parietal alpha oscillations and WM performance.

Besides the observed rebound of anti-phase tACS-induced suppression in RCS and parietal alpha power, the correlation between anti-phase tACS-induced change in alpha power and RCS at During2 was significantly different from that at During1 (During2 vs During1: $Z = 1.970$, $p = 0.049$), changing from a negative trend to a positive trend (**Fig. 5D**). The correlation results further support that the rebound following anti-phase tACS-induced suppression effects occurred at During2. In in-phase tACS, the correlations between in-phase tACS-induced changes in alpha power and RCS were always in a positive trend (permuted Pearson's correlation, During1: $r = 0.348$, $p = 0.030$; During2: $r = 0.31$, $p = 0.055$), without difference between During1 and During2 (During2 vs During1: $Z = -0.182$, $p = 0.856$) (**Fig. 5C**). This result suggests that the increase in parietal alpha power at the later stimulation period might be the accumulation of in-phase tACS effect. Moreover, the rebound following anti-phase tACS-induced suppression effects was not induced by the change of tACS parameters (including the number of tACS and the start time of tACS within WM retention interval), because no difference of tACS parameters between During2 and During1 was found (see supplementary material: 6. The comparison of tACS parameters between During2 and During1 for detail information).

**The effects of random-phase tACS and the offline tACS effects.** To explore the effects of the control condition, here we compared the effects of random-phase tACS

with both in-phase and anti-phase tACS. At both During1 and During2, random-phase tACS-induced changes in RCS (**fig. S6A**), the alpha power at Pz electrode (**fig. S7A**) and frontoparietal alpha synchronization (**fig. S7B**) were all between in-phase tACS and anti-phase tACS. At both During1 and During2, the correlations between random-phase tACS induced changes in alpha activity and RCS were not significant (**fig. S8**), between in-phase tACS and anti-phase tACS (See the detailed results for random-phase tACS in Supplementary material: 7. Complementary analysis of the behavior and EEG data in the random-phase tACS). These results indicate the effects of random-phase tACS were between in-phase tACS and anti-phase tACS, further supporting the influence of phase on tACS effects.

Next, we explored the offline tACS effects (tACS effects beyond the stimulation period: post-test) on WM performance and brain activity. Anti-phase tACS significantly reduced alpha power at Pz electrode compared to in-phase tACS (permuted paired *t*-test, $t(38) = -1.704$, $p = 0.047$, Cohen's $d = 0.273$, one-tailed) (**fig. S11**). But we observed no difference in WM performance and frontoparietal alpha synchronization between in-phase tACS and anti-phase tACS at post-test. The absence of offline effects in WM performance and frontoparietal alpha synchronization could be attributed to the rebound of anti-phase tACS-induced suppression at During2 (For more details about offline effects, see Supplementary material: 8. The offline comparison between in-phase tACS effects and anti-phase tACS effects).

**Frequency-specific effects of tACS on WM performance.** To provide additional

support to the conclusion that the observed changes in WM performance can be attributed to changes in parietal alpha oscillations during the WM retention interval, rather than to some general effects from tACS (independent of frequency) (*22*), we conducted a control experiment in which both in-phase tACS and anti-phase tACS were delivered in the theta frequency band (3-8Hz) to 21 participants (For more details about theta-tACS experiment, see Supplementary material: 9. In-phase and anti-phase tACS at the theta frequency). No suppression effects from the anti-phase theta-tACS were observed on WM performance (RCS) at any time point (During1, During2) as compared to the in-phase theta-tACS (permuted paired *t*-test, During1: $t(20) = 0.391$, $p_{corrected} = 1$, one-tailed; During2: $t(20) = 0.563$, $p_{corrected} = 1$, one-tailed) (**Fig. 4D**). This lack of any detected impact from theta-tACS supports that modulation of alpha oscillations—rather than some general impacts from electrical stimulation—can explain the observed effects of alpha-tACS on WM performance.

## Discussion

In this study, we used an online phase-locking closed-loop tACS system to modulate alpha oscillations specifically during the WM retention interval on a trial-by-trial level, with a predetermined phase difference between the tACS and the concurrent endogenous oscillatory activity. Compared to in-phase tACS, anti-phase tACS decreased working memory performance, and these decreases were paired with corresponding decreases in alpha power and in frontoparietal alpha synchronization at the earlier of two stimulation periods. Notably, the detected changes in alpha power and

frontoparietal alpha synchronization induced by in-phase tACS were both positively correlated to behavioral changes. Our study therefore provides direct causal evidence of a specific functional impact of alpha oscillatory activity in human WM retention. Our work also illustrates that phase differences between tACS and the target brain oscillations represent a decisive factor for determining the effects of tACS, both on neural modulation and on behavioral performances.

Our behavioral and electrophysiological findings strongly support a causal link between parietal alpha oscillations and WM retention. Alpha oscillations during the memory retention interval have been suggested to be instrumental in transiently protecting the encoded memory information by filtering task-irrelevant input and preventing further sensory processing that could interfere with the stored information (*5, 7, 35, 36*). However, no direct causal evidence supporting this functional role of alpha oscillatory activity has been established in human neuroscience to date. A few previous studies attempted to investigate alpha oscillations and WM retention in humans using non-invasive brain stimulation (NIBS) to induce neural entrainment (*37-39*), but multiple aspects of these studies prevent causal inferences. One alpha-tACS study applied continuous tACS throughout the three sub-processes of WM rather than specifically during retention interval, so it was not sufficient to establish a functional role of alpha oscillations in WM retention *per se* (*37*). rTMS studies tried to modulate alpha oscillations during the retention interval, but did not measure rTMS-induced changes in endogenous alpha activity, so the attribution of the observed behavioral effects on WM performance to the modulation of alpha oscillations remains speculative

(*38, 39*). Addressing these aspects, we used our online phase-locking closed-loop tACS system to modulate parietal alpha oscillations specifically during WM retention, while also recording EEG during intervals without tACS artifacts to investigate the influence of external modulation on alpha oscillations. We found that anti-phase tACS in our study induced both WM performance impairment and parietal alpha inhibition. We also observed a positive association between in-phase tACS-induced changes in parietal alpha power and the changes in working memory performance, further supporting the causal link between alpha activity and WM retention.

Our findings also suggest that frontoparietal alpha synchronization contributes to WM. Anti-phase tACS induced a significant decrease in frontoparietal alpha synchronization. This decrease may be attributed to disturbance of the parietal alpha phase by anti-phase tACS, which likely impairs its coupling with frontal brain areas. A few studies have found that synchrony is strengthened with increasing memory load in frontoparietal regions previously shown to mediate attentional functions during memory retention (*31, 32*). In line with these studies, we observed a positive association between in-phase tACS-induced changes in frontoparietal alpha synchronization and altered RCS values, which demonstrates that increased frontoparietal alpha synchronization during WM retention also contributes to the observed improvement in WM performance.

In agreement with previous computational modelling predictions (*18*), we found that anti-phase tACS significantly inhibited parietal alpha activity, frontoparietal alpha synchronization, and working memory performance compared to in-phase tACS.

Therefore, we provide the direct experimental evidence that the phase of endogenous brain oscillations relative to tACS is impactful for determining the direction and magnitude of tACS. This evidence also calls for the consideration of brain states during tACS (e.g., the phase differences between the tACS waveform and the endogenous alpha oscillations) to overcome the unwelcome inconsistent effects of conventional open-loop tACS (*17*). Some rTMS studies previously investigated the effects of high-frequency rTMS synchronized to the phase of the ongoing brain oscillations, for example delivering rTMS at the peaks or throughs of the monitored brain oscillations (*40, 41*). The thinking underlying this phase-locking rTMS is that different phases of brain oscillations may represent different states of cortical excitability (*42*), thus affecting the efficacy of rTMS synchronized to that phase. However, the reported null effects of phase-locking rTMS on the monitored brain oscillations (*40*) might indicate that phase-locking rTMS does not directly modulate the monitored brain oscillations. Unlike phase-locking rTMS, our phase-locking closed-loop tACS can directly increase or suppress the targeted brain oscillations. The suppression effects of anti-phase tACS compared to in-phase tACS also suggest that tACS can act by the modulation of cortical neurons, rather than through peripheral nerve stimulation in the scalp (*43*). Effects from in-phase tACS vs. anti-phase tACS should not differ if they result only from transcutaneous stimulation (*43*) based on electrodes positioned at the head and the right shoulder.

The capacity to induce or suppress target oscillatory activities within a relatively short period of time (within several seconds) is a unique and highly valuable feature of

our online phase-locking closed-loop tACS system as compared to other currently available tACS techniques. Conventional long-term continuous tACS and recently published brain signal based closed-loop tACS set-ups have to date only reported unidirectional modulation of brain oscillations at the stimulation frequency (*24, 44-46*). This inflexibility regarding directionality is problematic for many functions and applications. Consider for example that previous studies of the Sternberg paradigm have indicated that alpha activity tends to increase during the retention interval but to decrease during the encoding and retrieval intervals (*20, 21*). If conventional long-term (several to dozens of minutes) continuous tACS was performed throughout the entire task process, the effects in different intervals might cancel each other out. We selectively regulated alpha oscillations during the retention interval; future studies could try to up-regulate alpha activity during the retention interval while also—and in the same trial—down-regulating oscillatory alpha activity or even manipulating related oscillations at other frequencies during the encoding and retrieval intervals. This method, or many variations thereof, may prove to be an even more effective way of modulating cognitive processes, e.g., working memory. It is further plausible to speculate that our closed-loop tACS system may broaden the clinical therapy applications of tACS, with its expanding capacity to suppress brain oscillations. Our results show that anti-phase tACS can significantly inhibit oscillatory activity, which illustrates the possibility of using tACS to down-regulate abnormal high brain rhythms, potentially as a treatment option for various neurological and psychiatric disorders (e.g., attention deficit hyperactivity disorder (ADHD) (*47, 48*), somatoform pain disorder

(SPD) (*49*), and addiction (*50*), among many others).

One striking aspect of the anti-phase tACS-induced effects was the opposite pattern of effects seen early (During1) as opposed to later (During2) during stimulation, which might be due to both excessive practice of the Sternberg paradigm and plasticity of the human cortex. Parietal alpha power and frontoparietal alpha synchronization tended to be increased over time in both in-phase tACS and anti-phase tACS. The increases in alpha oscillations over time for all tACS conditions may reflect the increased effort needed to suppress irrelevant information after a long time of task performance (*51*). But it's hard for practice effects to explain why the correlations between changes in alpha power and WM performance increased over time in anti-phase tACS (**Fig. 5D**), while didn't change in in-phase tACS (**Fig. 5C**). Therefore, practice effects are not sufficient to fully explain the phenomenon of the rebound of anti-phase tACS effects. Actually, similar to our study, time-dependent rebound or reversal of effects have also been reported and discussed in previous NIBS studies (*25, 52*). One study reported that 5 min gamma tACS applied at the human motor cortex (M1) induced a decrease in resting-state $GABA_A$ inhibition, but a rebound of this effect was found when tACS lasted for 15 min (*25*). Likewise, previous TMS studies also found that when prolonging the duration of stimulation (thereby creating a later stimulation duration, as with our present study), TMS effects on cortex excitability also reversed. (*52*). These findings all support adaptation of brain activity to external electromagnetic stimulation, apparently illustrating plasticity of the human brain. However, exploring the reasons for the rebound of anti-phase tACS effects is not the focus of our study, and further

studies with more direct evidence are needed.

In summary, our study provides empirical evidence for a causal link between parietal alpha oscillations and the retention interval of working memory. To establish this link, we here pioneered an online phase-locking closed-loop tACS system that offers the possibility of directly up- and down-regulating oscillatory brain activity at a predetermined stimulation frequency, and we show that this system does induce effects on both brain rhythms and behavior.

## Methods

**Participants**. We calculated the needed sample size using G*Power 3.1 software (*53*). The main purposes of the present study were to investigate whether anti-phase tACS suppressed WM performance and alpha activity compared to in-phase tACS, and whether tACS-induced changes in WM performance were associated with changes in alpha activity. Assuming a Cohen's d of medium effect size (Cohen's d = 0.5) for one-tailed t-tests and a statistical power of 80%, a sample size of 27 participants were needed. Assuming a correlation coefficient of medium effect size (r = 0.4) and a statistical power of 80%, a sample of 44 participants were needed. A total of 48 healthy participants between the ages of 18 and 40 participated in the experiment. All participants were right-handed according to the Edinburgh Handedness Inventory, reported no brain implants, no implanted electronic devices, no history of neurological problems or head injury, no current use of psychoactive medication, no history of craniotomy, no skin sensitivity, were nonpregnant, and had normal or corrected-to-

normal visual acuity. Participants who failed to follow directions or did not understand instructions were removed from the study. All participants were recruited in Hefei, China through online advertisements or posters. Eight participants were excluded from the analyses due to poor EEG signal or equipment failure to trigger electrical stimulation as intended. One participant whose alpha activity was too weak for detection and who therefore had to repeat the task 3 times per session was also excluded. The remaining 39 participants (19 females, mean age ± SD：21.1 ± 2.2 years, mean education ± SD： 14.7 ± 1.8 years) were tested and their data submitted to behavioral and EEG analyses, which meets the sample size requirement.

The study was approved by the Human Ethics Committee of the University of Science and Technology of China (IRB No.: 2020KY161) and performed according the Helsinki Declaration. All participants provided written informed consent prior to the study and were paid 300 RMB after completing three experimental visits.

**Experimental procedures.** The procedure is illustrated in **Fig. 3B**. Each participant underwent 3 experimental sessions separated by at least 3 days at approximately the same time of the day. Using a within-subject design, participants received in-phase tACS, anti-phase tACS, or random-phase tACS in each session, with the order of sessions counterbalanced across participants.

At the start of each session, preparing the closed-loop tACS-EEG setup required about 30 minutes per participant. Following this preparation phase, participants first completed a practice block of the Sternberg working memory paradigm consisting of

32 trials. The Sternberg task is suited to separate the three different processes of working memory: encoding, retention, and retrieval. Participants practiced the Sternberg task until their performance stabilized at an accuracy level of at least 75% correct. Following this practice, participants performed 8 blocks of the Sternberg paradigm. Within each block, there were 30 positive trials (probe part of the memory list) and 30 negative trials (probe not part of the memory list).

In the first block (pre-test), spontaneous EEG was recorded for 2.5s in each trial once the retention interval of Sternberg task began. Subsequently, the EEG data at the pre-test block was analyzed to determine the IAF to be used for the subsequent electrical stimulation of this session, and the threshold for subsequent triggering of tACS (see more details in IAF and threshold determination section in Supplementary material: 1.2. IAF and threshold determination).

Then, participants performed 3 tACS-EEG blocks (During1) while performing the Sternberg task. EEG data was monitored once the retention interval began. The closed-loop stimulation intervention was triggered and lasted for 0.8 s when the EEG signal met requirements (for detailed description of the tACS intervention, see Online phase-locking closed-loop tACS section, below). The 0.8s duration of stimulation was a balance of total effective stimulation time and the consistency of the phase relationship between brain signal and tACS, which has been shown to decrease as the length of the tACS time window increases (*54*). Once stimulation started, the EEG recording stopped to avoid tACS artifacts until the onset of the next trial.

After a rest period of about 2 min, in order to explore any duration-dependent

effect(s) of our online phase-locking closed-loop tACS, another 3 tACS-EEG blocks (During2) were performed, with the design and tACS parameters the same as During1. After During2 completed, participants filled out a tACS questionnaire to assess tACS-induced discomfort (see more details in tACS questionnaire section). Finally, after the six tACS-EEG blocks, participants completed a final 8$^{th}$ block of the Sternberg paradigm (post-test) while recording EEG.

**Sternberg task**. Participants performed a previously published modified version of the Sternberg WM task (*6, 8*) (**Fig. 3A**). Participants had to remember a horizontally arranged list of seven consonants that was presented simultaneously at the center of the computer monitor for 2 s. After a 3 s retention interval (blank screen with fixation cross on the middle position of the monitor), the probe stimuli were presented and displayed for the duration of the recognition interval (1 s). All responses were made with the right hand, by pressing the left arrow with the index finger and the right arrow with the middle finger. Participants had to press a button (left arrow or right arrow) if the probe matched one of the consonants in the memory set, and press the other button if the probe did not match. Instructions stressed both speed and accuracy, but underscored the importance of high accuracy. The inter-trial interval (ITI) was a uniformly distributed random number between 1.5s and 2.5s. A fixation cross was presented during the ITI. During the trial, the letters and the fixation cross were presented in black on a gray background. After the button press, a green fixation cross was shown, indicating the participant responded successfully and was allowed to make eye blinks. One second

before the start of the next trial the fixation cross turned black again. Participants were instructed not to blink from this moment until they had pressed a button.

**EEG data collection**. EEG data was recorded using a UEA-16BZ amplifier (SYMTOP, Beijing, China). Thirteen Ag/AgCl electrodes were placed on the scalp at specific locations according to the international 10-20 system (Fp1, Fp2, F7, F3, Fz, F4, F8, C3, Cz, C4, P3, Pz, P4). In addition, the electrical activities were recorded over left and right mastoids. The average of bilateral mastoids was used as reference, because previous studies reporting the relationship between alpha oscillations and WM retention usually used mastoids as reference (*6, 21*). Besides, the sources accounting for alpha activity in WM is relatively large, extending from parietal region to occipital region (*8*), so it may be not suitable to imitate closed-loop EEG-triggered rTMS studies (*41*), which used the average of surrounding electrodes around the Pz electrode as reference. Impedance between the reference electrode and any recording electrode was kept under 5 kΩ. All signals were sampled at 1000 Hz during data collection. A low-pass filter with a cut-off frequency of 45Hz and a 50Hz notch filtered were applied on-line.

**Online phase-locking closed-loop transcranial alternating current stimulation (tACS)**. In order to be able of stimulating specifically at the retention interval in each trial with a predetermined difference between the applied stimulation phase and the endogenous oscillatory phase at the target brain region, we here introduced an online phase-locking closed-loop tACS setup pioneered by our group. As shown in **Fig. 2**, our

online phase-locking closed-loop tACS system enabled us to administer 0.8 s sinusoidal current stimulation of 2mA (peak-to-peak) amplitude with a certain phase difference relative to the target brain oscillations (for more details about how to realize the certain phase difference, see the Supplementary material: 1.1. The details of online phase-locking closed-loop tACS).

The stimulation electrode (4×6 cm, rubber electrode covered with conductive paste) was placed over central parietal-occipital cortex (between Pz and Oz, according to the international 10-20 system), with the upper edge about 2 cm away and the center about 4 cm away from Pz electrode, to prevent tACS-induced artifacts from contaminating EEG data at Pz electrode for online analysis during tACS. Although the stimulation electrode was not placed precisely over Pz electrode, the position of it in our study is suitable due to the high coherencies of EEG signal at about 5 cm electrode separations (*55*) and the relatively large sources accounting for alpha activity in WM, extending from parietal region to occipital region (*8*). The return electrode (6×9 cm, rubber electrode covered with conductive paste) was over the right shoulder. Impedances were kept below 10 kΩ using a conductive paste (Nuprep, Weaver and Company, Aurora, CO, USA), which also held the electrodes in place.

During stimulation, the recorded EEG from Pz electrode was analyzed in the computation module to trigger the stimulation module at the right time point. Then, the stimulation was set to provide a sinusoidal current stimulation at the individual alpha frequency (IAF) (IAF was calculated using the EEG signal of the first block (pre-test), see more details in Supplementary material: 1.2. IAF and threshold determination). The

onset phase of the sine-wave stimulation was 0 in all conditions. The closed-loop stimulation was monitored and applied for about 1 h during the 3 blocks of During1 and the 3 blocks of During2, no stimulation was delivered during the first (pre-test) or last block (post-test).

In order to explore the role of alpha oscillations in WM retention, the closed-loop tACS system only modulated alpha power during the retention interval of each trial. Once stimulation started, the EEG recording stopped to avoid amplifier saturation until the onset of the next trial. In each trial, in order to avoid the duration of electrical stimulation beyond the retention interval of working memory, monitoring for triggering the electrical stimulation was limited to 1.8 s after the start of the memory retention interval. If the electrical stimulation was not triggered within 1.8 s, the system stopped the monitoring and continued recording the EEG data to 2.5s. Due to the probably unstable phase of the EEG signal, we analyzed the phase alignment between EEG signal recorded at pre-test and tACS waveform generated offline using the same method as online stimulation to test the phase alignment of our system (see Supplementary material: 1.3. Analysis of the phase alignment between EEG signal and tACS waveform for detailed information).

**TACS sensations**. Before the During period of each session, all participants were exposed to tACS stimulation for a short period of time (no more than 5 times, 0.8 s each time) to make sure that they were comfortable to complete the session. Participants were blinded to the stimulation condition they received. At the end of the During period

of each session, participants completed a questionnaire to assess ten possible side-effects of the tACS stimulation by rating from 0 (none) to 4 (strong) the intensity of: itching, pain, burning, warmth/heat, pinching, metallic/iron taste, fatigue, dizziness, nausea, phosphenes, or any other side-effects perceived. If a certain side-effect was present, participants were asked to evaluate the possibility that it was related to tACS by rating from 0 (none) to 4 (definite).

Analysis of the tACS questionnaires indicated that there were no significant differences between tACS conditions (in-phase, random-phase, and anti-phase) for tACS-induced side effects (for more details of the analysis, see the Supplementary material: 1.4. Analysis of tACS questionnaire).

**Blinding.** Participants were blinded to the stimulation conditions they received. Participants invariably received verum stimulation in all of the three tACS conditions (*i.e.*, without sham stimulation). Moreover, self-reported questionnaires for sensations elicited by tACS didn't differ between conditions (for more details of the analysis, see the Supplementary material: 1.4. Analysis of tACS questionnaire), indicating that participants cannot distinguish between different stimulation conditions.

**Offline EEG analysis.** Analysis of the EEG data was performed using custom-built scripts implemented in MATLAB 2016a (MathWorks, Natick/USA) using the EEGLAB Toolbox (version 13.5.4b) (*56*).

*Pre-processing.* The EEG data obtained from the 3 blocks of During1 and the 3 blocks of During2 were processed first. For every block, epochs were extracted from the EEG according to trial. Since the time to trigger electrical stimulation in the retention interval was different across trials, the length of epochs for different trials were also different. Then we deleted the epochs corresponding to the trials with wrong responses in the Sternberg task, and only the epochs for correct trials remained. Next, the detrending was performed to remove DC offsets and slow drifts (<1 Hz). Eye blink contaminations were then eliminated using an independent component analysis approach (implemented in EEGLAB). Epochs with residual eye movements or other artifacts were removed through visual inspection of the data. Because the trial numbers and the duration of trials obtained from the three stimulation conditions were quite different, it was necessary to balance the trial numbers and trial lengths of the three stimulation conditions. For each participant and each block, we deleted the shortest trials in the two conditions with more trials so that the trial numbers were the same as the condition with the minimum number of trials. Next, the epochs in each condition were sorted by the length of epochs from short to long. Then we made the three epochs with the same order among the three stimulation conditions have the same length by discarding the tail EEG data of the two longer epochs.

For pre-test and post-test EEG data, at first we matched the data lengths of the pre-test and post-test epochs to that of the epochs during tACS. For every participant and every condition, since the duration of all pre-test and post-test epochs was 2.5 s, we randomly assigned the length of all epochs from the 6 blocks of During1 and During2

to the epochs of pre-test and post-test, and deleted the tail EEG data of each epoch at pre-test and post-test based on the length assigned. Then the preprocessing of the EEG data from pre-test and post-test was the same as that of the EEG data recorded in During1 and During2.

*Analysis of alpha power*. For each epoch, the absolute spectrum was calculated using the Matlab function *pwelch*. Because of the different duration of different epochs, each epoch was first divided into several small segments with a length of 500 ms. And there was an overlap of 400 ms from segment to segment. A fast Fourier transform (FFT) was calculated for each segment using a Hamming window and zero-padding to 2.048s. Then the spectra of the segments for every epoch were averaged to obtain the spectrum of the epoch. The resulting spectra of each block were averaged across epochs as well as across the alpha-bands (8-13Hz) per tACS-condition as the power in alpha band. Finally, for subsequent statistical analysis, the relative alpha power of each block was calculated as the power in alpha band divided by the power across the frequency band of 1-45 Hz.

*Phase synchronization analysis*. The phase lag index (PLI) can obtain reliable estimates of phase synchronization against the presence of volume conduction. Therefore, phase synchronization between Pz electrode and other 12 electrodes was estimated using the PLI method as described by Stam (*33*).

To obtain phase information, preprocessed data were bandpass-filtered in alpha-

bands (8-13Hz) and Hilbert-transformed. The instantaneous phase could be extracted from the resulting complex values. The phase lag index (PLI) between Pz electrode and one of the other 12 electrodes was then computed for every epoch. Within every block, PLIs were then averaged across epochs in each tACS-condition. Finally, the average PLI between the alpha activity of the 7 frontal electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8) and Pz electrode was used as an indicator to describe the frontoparietal alpha synchronization.

**Statistical analysis**. The rate correct score (RCS) is defined as

$$\mathrm{RCS} = \frac{c}{\sum \mathrm{RT}} \qquad (1)$$

where c is the number of correct responses, and the denominator refers to the sum of all RTs in the set of trials under consideration.

RCS was used to integrate measurements of RT and accuracy into a single measure to avoid contradictory findings in these two important aspects of performance (*28*); RCS has been reported as being more reliable than single measures of RT and accuracy (*57*).

Due to the suggested different mechanisms underlying online tACS effects (*18, 19*) (effects observed during stimulation) and offline tACS effects (*27*) (aftereffects beyond stimulation), we analyzed the online tACS effects firstly by comparing the online behavioral and EEG effects induced by in-phase and anti-phase tACS , then the offline tACS effects. To exclude the influences of pre-test, we normalized all metrics by subtracting the corresponding pre-test values.

The statistical analysis was performed using MATLAB 2016a. For statistical significance analysis, we used nonparametric permutation test throughout the manuscript to avoid the assumption of normality. For comparisons with two paired groups, we used the permuted paired *t*-test statistic wherein we randomly mixed values from the two groups 5000 times to create a distribution of paired *t* values and computed an empirical p value from this distribution. Note that *t*-test statistics and degree of freedom are provided for reference only, and the reported p-values may differ from those expected from the *t*-distribution. Bonferroni correction was used to correct for multiple comparisons. Corrected p-values were reported in this case. All corrected p-values larger than 1 were reported as p = 1. The effect size (Cohen's d-value) was calculated via G*Power 3.1 software (*53*). Correlation analysis between two variables was calculated using permutation test based on Pearson's linear correlation coefficient (r-value, two-tailed). We used the permuted Pearson's correlation coefficients wherein we hold one variable constant and randomly permuted the other variable 5000 times to create a distribution of r values and computed an empirical p value from this distribution. To test for differences between two correlations, the obtained correlation coefficients were converted into z-values with Fisher's r-to-z transformation so that the z scores can be analyzed for statistical significance.

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## Author contributions

X.Z. conceived the experiments; X.C., R.M., Q.W., and A.Y. performed the experiments; X.C., R.M., and W.Z. analyzed the data; R.M., X.X., and J.C. designed and realized the online phase-locking closed-loop tACS system; X.C., R.M., and X.Z.

wrote the manuscript; A.T.S., M.M.L., N.X.Y, S.W., Z.D., Q.Z., and J.B. helped to polish the writing.

## Competing interests

The authors declare that they have no competing interests.

## Data and materials availability

The original data that support the findings of this study and the custom code used to generate the figures and statistics are available from the corresponding author (X.Z.) upon reasonable request.

## Materials & Correspondence

Correspondence and requests for materials should be addressed to X.Z.

# Figures

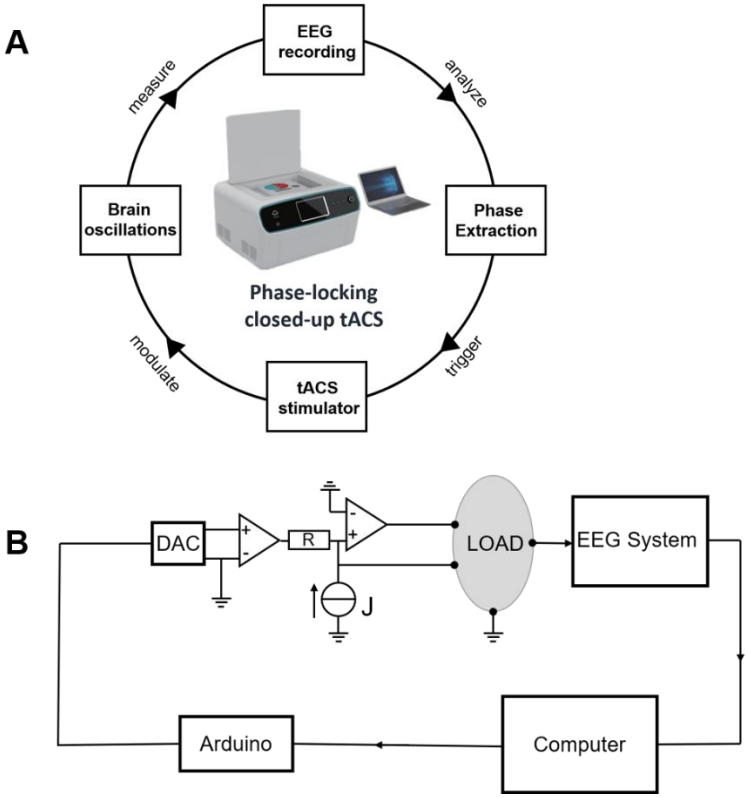


**Fig. 1. The components of the online phase-locking closed-loop tACS system and the design of the tACS stimulator.** (**A**) The online phase-locking closed-loop tACS system consists of an EEG instrument measuring brain oscillations, a computer extracting the online phase of brain oscillations to decide the timing of tACS stimulator, and a custom designed tACS stimulator which communicates with the computer to regulate the application of tACS to the human brain. (**B**) The design of the tACS stimulator. The major components of the the tACS stimulator include an Arduino Uno microcontroller board, a digital-to-analog converter (DAC), a constant-voltage source (J), and two operational amplifiers. The timing of next tACS (calculated from the computer) is communicated to the Arduino board. The triangle represents the

operational amplifier; R represents the resistance.

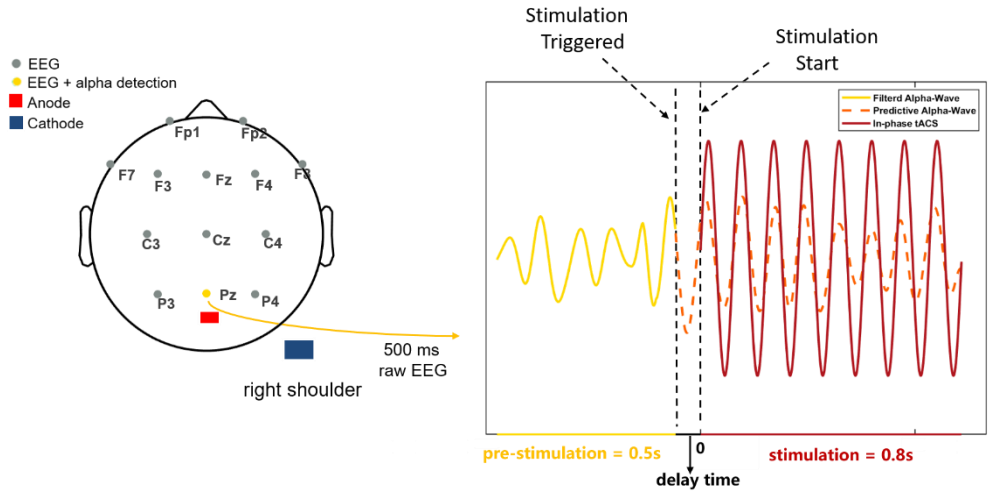


**Fig. 2. Illustration of the online phase-locking closed-loop tACS system.** The left panel shows a standard 10-20 electrode system used in the experiment; the right panel shows an example of alpha-wave detection and the application of in-phase tACS. The stimulation electrode (shown with the red rectangle in the left panel) was placed over the central parietal-occipital cortex (between the Pz electrode and Oz electrode), with the return electrode over the right shoulder (shown with the blue rectangle in the left panel). The raw EEG data at the retention interval of the Sternberg task, as measured from the Pz electrode, was stored in a moving time window of 500ms with a 10ms step. This signal was filtered within the frequency range of IAF±2Hz (shown with the dark-yellow solid line) in real time. When two consecutive peaks exceeding the threshold were detected (note that the threshold was determined using pre-test EEG data), stimulation was triggered; subsequently after a specific delay time, in-phase tACS (shown in dark red) was initiated and lasted for 0.8 seconds. The dark-orange dashed line shows the alpha wave after the triggering of stimulation (please note that it is not

recorded due to tACS artifacts). The illustrative waveform in the figure does not represent the actual size.

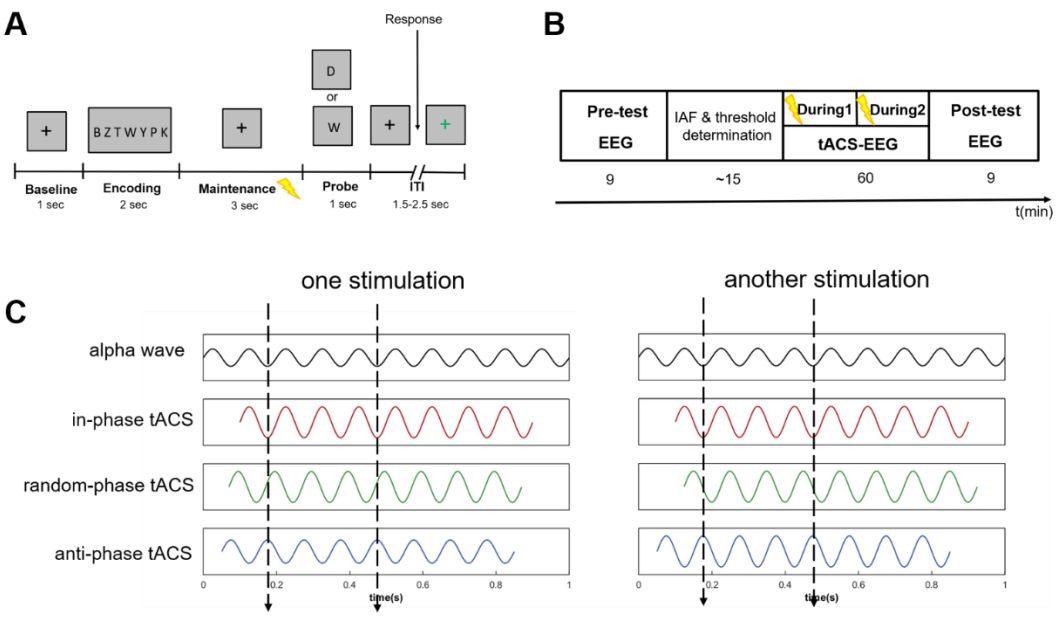


**Fig. 3. Sternberg task, experimental procedures, and stimulation conditions.** (**A**) Schematic representation of the modified Sternberg paradigm used in this study. For each trial, participants were shown a list of 7 consonants and were asked to indicate by button press whether the probe was part of the memory list. (**B**) Experimental procedure for each session. First, participants completed a pre-test block of the Sternberg task with EEG recorded during the retention interval. Subsequently, the individual alpha frequency (IAF) of this session was determined using the pre-test EEG data. Then, 3 tACS-EEG blocks (During1) of the Sternberg task were performed with tACS delivered specifically during the retention interval, followed by another 3 tACS-EEG blocks (During2). After that, participants completed a final block of the Sternberg paradigm (post-test) while recording EEG signals. (**C**) Three tACS conditions: TACS was applied

at the IAF with 0° relative phase to the endogenous alpha oscillations in the in-phase condition and with 180° relative phase to the endogenous alpha oscillations in the anti-phase condition; in the random-phase condition, no phase alignment between the delivered tACS and the detected endogenous alpha oscillations were used, with the phase differences changing from 0° to 360° across trials. For all conditions, the onset phase of the sine-wave tACS was invariably 0°. The illustrative waveforms in the figure do not represent the actual sizes.

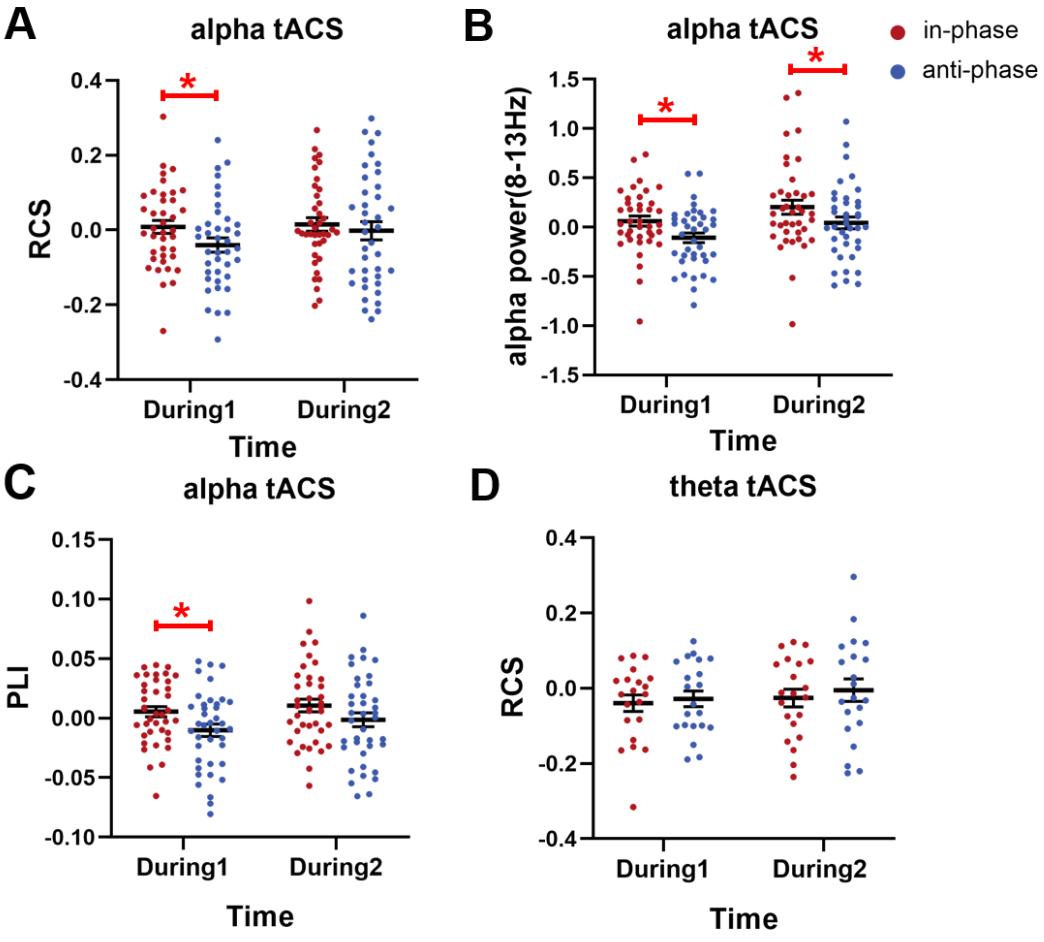


**Fig. 4. Compared to in-phase tACS, anti-phase tACS at the alpha frequency band**

**decreased alpha activity and WM performance.** (**A**)Effects of anti-phase tACS delivered at the IAF on the rate correct score (RCS, the number of correct responses per second). At an early stimulation period (During1), anti-phase tACS significantly decreased the RCS as compared to in-phase tACS (permuted paired *t*-test: t(38) = -2.209, $p_{corrected}$ = 0.036); this decrease was not significant at a later stimulation period (During2). (**B**) Anti-phase tACS at the IAF significantly suppressed parietal alpha power (8-13 Hz) as compared to in-phase tACS at both During1 (permuted paired *t*-test: t(38) =-2.257, $p_{corrected}$ = 0.027) and During2 (permuted paired *t*-test: t(38) = -2.185, $p_{corrected}$ = 0.039). (**C**) As compared to in-phase tACS, frontoparietal alpha synchronization in anti-phase tACS (indexed by PLI) decreased significantly at During1 (permuted paired *t*-test: t(38) =-2.067, $p_{corrected}$ = 0.044) but not at During2. (**D**) For tACS at the theta frequency band, no suppression effects on WM performance were detected between the anti-phase tACS and in-phase tACS. Note that all instances of the RCS, alpha power, and frontoparietal alpha synchronization values are given relative to the pre-test data (*i.e.*, values after subtracting the corresponding pre-test values). Within-group comparisons used one-tailed permuted paired t-tests and evaluated the hypothesis that an effect (*e.g.*, the RCS value, the alpha power value, etc.) for anti-phase tACS was weaker than for in-phase tACS. Bonferroni correction was used to correct for multiple comparisons. Error bars represent the SEM; *significant at $p_{corrected}$ <0.05 (one-tailed permuted paired *t*-tests), ** significant at $p_{corrected}$ <0.01 (one-tailed permuted paired *t*-tests).

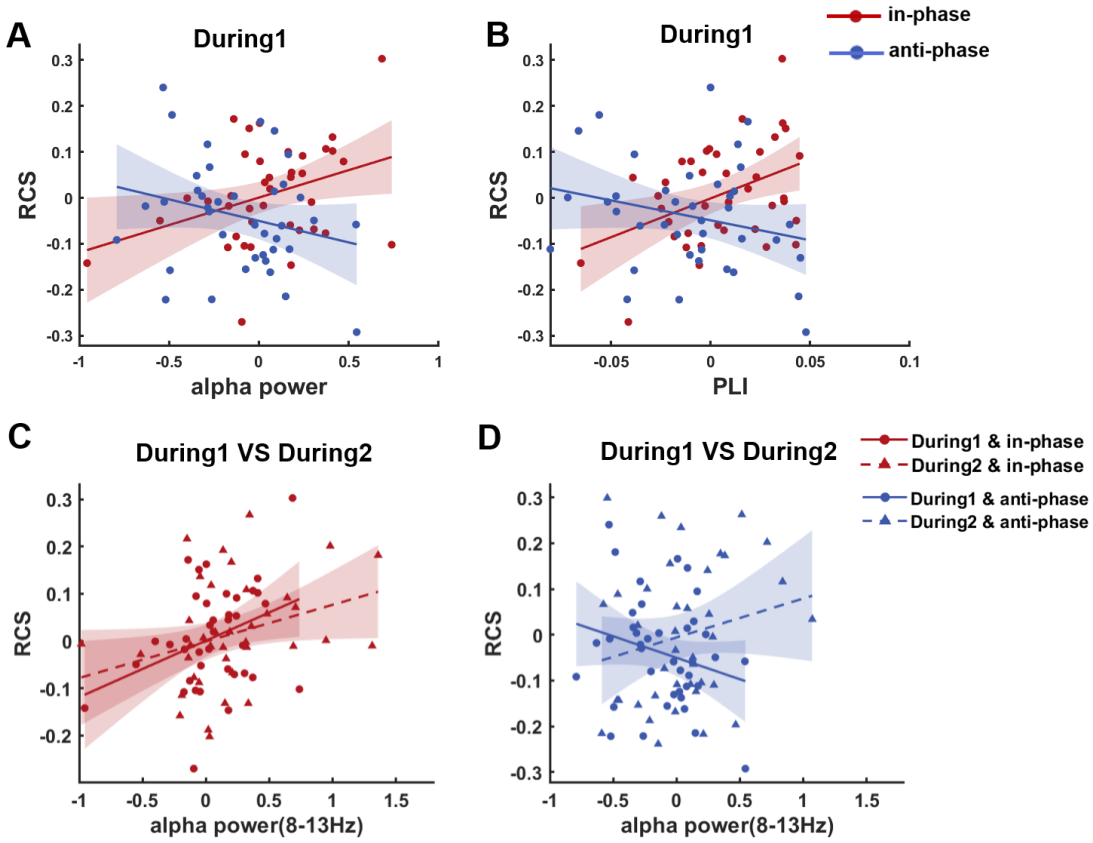


**Fig. 5. Correlations between tACS-induced changes in RCS and EEG metrics differed between in-phase tACS and anti-phase tACS.** (**A**) Permuted Pearson's correlation (two-tailed) analysis was used to assess the potential relationship between RCS and parietal alpha power at During1: the in-phase tACS-induced change in RCS was positively correlated with the induced change in alpha power (permuted Pearson' correlation: r = 0.348, p = 0.030); no correlation was found for anti-phase tACS (permuted Pearson' correlation: r = -0.244, p = 0.138). There was a significant difference between the correlation coefficients in in-phase tACS and anti-phase tACS at During1 (Z = 2.599, p = 0.009). (**B**) Similar assessment of the relationship between tACS-induced change in RCS and induced frontoparietal alpha synchronization at During1. A positive correlation between the tACS-induced change in frontoparietal

alpha synchronization and RCS was found for in-phase tACS (permuted Pearson' correlation: r = 0.415, p = 0.007) but not for anti-phase tACS (permuted Pearson' correlation: r = -0.242, p = 0.140). These two correlation coefficients differed significantly between in-phase tACS and anti-phase tACS (Z = 2.921, p = 0.003). (**C**) The correlations between in-phase tACS-induced change in alpha power and induced change in RCS did not change from During1 to During2 (Z = -0.182, p = 0.856). (**D**) The correlations between anti-phase tACS-induced change in alpha power and induced change in RCS at During2 were significantly stronger than the corresponding values at During1 (Z = 1.970, p = 0.049). The RCS, alpha power, and frontoparietal alpha synchronization values are given relative to the pre-test values (*i.e.*, after subtracting corresponding pre-test values). The obtained permuted Pearson's correlation coefficients were converted into z-values using Fisher z-transformation to support comparison of two correlations.

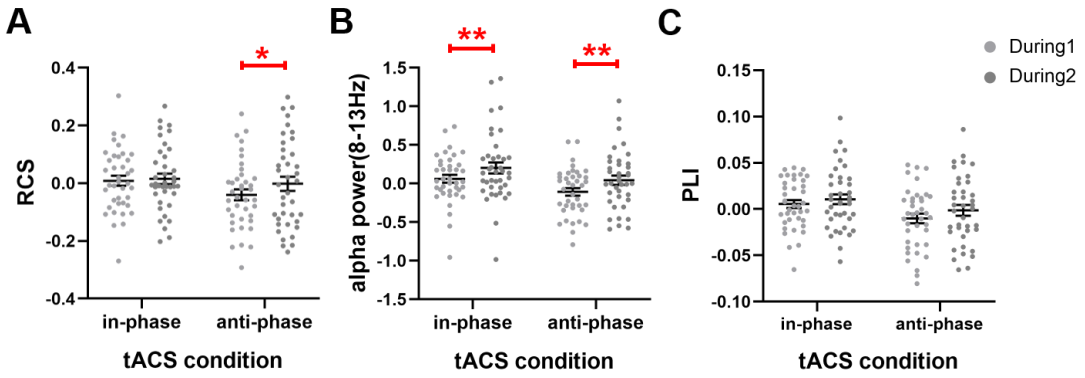


**Fig. 6. The suppression effects of anti-phase tACS at During1 rebounded at During2.** (**A**) The anti-phase tACS-induced impairment in the RCS value at During1

rebounded at During2 (permuted paired *t*-test: t(38) = 2.508, $p_{corrected}$ = 0.029); no such rebound was detected upon in-phase tACS. (**B**) Alpha power increased upon in-phase tACS (permuted paired *t*-test: t(38) = 3.842, $p_{corrected}$ < 0.001) and rebounded in anti-phase tACS (permuted paired *t*-test: t(38) = 3.083, $p_{corrected}$ = 0.008). (**C**) Frontoparietal alpha synchronization changes, indexed by PLI, did not differ between During1 and During2 for either in-phase or anti-phase tACS. Bonferroni correction was used for multiple comparison corrections. Error bars represent the SEM; *significant at $p_{corrected}$ <0.05 (two-tailed permuted paired *t*-tests), ** significant at $p_{corrected}$ <0.01 (two-tailed permuted paired *t*-tests).