Research Paper

Aftereffects of frontoparietal theta tACS on verbal working memory: Behavioral and neurophysiological analysis

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A B S T R A C T

Verbal working memory is supported by a left-lateralized frontoparietal theta oscillatory (4–8 Hz) network. We tested whether stimulating the left frontoparietal network at theta frequency during verbal working memory can produce observable after-stimulation effects in behavior and neurophysiology. Weak theta-band alternating electric currents were delivered via two 4 × 1 HD electrode arrays centered at F3 and P3. Three stimulation configurations, including in-phase, anti-phase, or sham, were tested on three different days in a cross-over (within-subject) design. On each test day, the subject underwent three experimental sessions: pre-, during- and post-stimulation sessions. In all sessions, the subject performed a Sternberg verbal working memory task with three levels of memory load (load 2, 4 and 6), imposing three levels of cognitive demand. Analyzing behavioral and EEG data from the post-stimulation session, we report two main observations. First, in-phase stimulation improved task performance in subjects with higher working memory capacity (WMC) under higher memory load (load 6). Second, in-phase stimulation enhanced frontoparietal theta synchrony during working memory retention in subjects with higher WMC under higher memory loads (load 4 and load 6), and the enhanced frontoparietal theta synchronization is mainly driven by enhanced frontal→parietal theta Granger causality. These observations suggest that (1) in-phase theta transcranial alternating current stimulation (tACS) during verbal working memory can result in observable behavioral and neurophysiological consequences post stimulation, (2) the short-term plasticity effects are state- and individual-dependent, and (3) enhanced executive control underlies improved behavioral performance.

Introduction

Working memory (WM) is a cognitive system where information is held online temporally in service of behavioral goals. As a fundamental cognitive faculty, WM is known to underlie such diverse cognitive functions as planning, learning, reading comprehension, and problem solving (Adams and Hitch, 1997; Barrett et al., 2004; Cantor and Engle, 1993; Daneman and Carpenter, 1980). In neurological and psychiatric disorders, working memory is one of the first cognitive functions to become impaired (Baddeley et al., 1991; Jaeggi et al., 2014; Lee and Park, 2005; Owen et al., 1990; Pincham, 2014). Thus, understanding working memory and its neural mechanisms, as well as developing effective methods (e.g., neurostimulation) for achieving lasting improvement of working memory, have both basic and clinical neuroscience significance.

Neuroimaging and lesion studies have provided ample evidence that

WM is supported by regions in frontal and parietal cortices (Chein and Schneider, 2005; Jonides et al., 2008; Owen et al., 2005). In particular, the central executive component of WM is linked to the frontal cortex (D'Esposito et al., 1995; Kane and Engle, 2002), whereas the storage component is associated with the parietal cortex (Champod and Petrides, 2010; Olson and Berryhill, 2009; Postle et al., 2006). In verbal working memory (VWM), in which the information being remembered is language-related, there is further evidence suggesting a left-hemisphere dominance in these cognitive operations (D'Esposito et al., 1998; Smith et al., 1996).

During WM, the frontal and parietal regions interact, and this interaction is thought to be mediated by theta (4 – 8 Hz) oscillations (Buzsáki, 1996; Rutishauser et al., 2010; Sarnthein et al., 1998). WM-related theta oscillatory activity is state-dependent with increased frontoparietal long-range theta synchrony accompanying increased cognitive demands (e.g., higher working memory load) (Jensen and

Tesche, 2002; Payne and Kounios, 2009; Sauseng et al., 2005). WM-related theta activity is also individual-dependent; it is higher and more strongly modulated by experimental conditions in individuals with stronger executive functions (Zakrzewska and Brzezicka, 2014). It can probably be said that WM is a cognitive function most hypothesized to be based on sustained neural oscillations and synchrony because of the need to maintain internal representations of information.

The neuronal communication via neuronal coherence (NCNC) hypothesis (Fries, 2005) has provided guidance for studies applying neurostimulation to modulate WM. According to the NCNC hypothesis, the theta phase difference between frontal and parietal sites is functionally significant, with the phase difference close to 0 degree (in-phase) or close to 180 degree (anti-phase) associated with facilitation or hindrance of neuronal communications, respectively. Consistent with this idea, Polanía et al. (2012) applied 6 Hz transcranial alternating current stimulation (tACS) over left prefrontal and parietal regions with either 0 degree relative phase (in-phase condition) or 180 degree relative phase (anti-phase condition) in a delayed letter discrimination task, and found that exogenously induced frontoparietal theta synchronization (in-phase stimulation) or desynchronization (anti-phase stimulation) significantly improved or degraded visual memory-matching performance as compared to sham stimulation. More recently, using a change detection task with images of real-world objects, Reinhart and Nguyen (2019) applied in-phase tACS to prefrontal and temporal regions simultaneously in older adults, and found that it can bias frontotemporal functional connectivity and enhance working-memory performance.

TACS studies guided by the NCNC hypothesis have mainly focused on stimulation effects on behavior during stimulation. To what extent these effects persist post stimulation remains to be better understood. It has been suggested that the aftereffect of tACS is achieved by synaptic changes induced during stimulation via spike-timing-dependent plasticity (Vossen et al., 2015; Wischnewski et al., 2019; Zaehle et al., 2010; Fröhlich and McCormick, 2010; Johnson et al., 2020; Krause et al., 2019). In this regard, in-phase stimulation, according to the NCNC hypothesis, enhances neural synchrony, which in turn promotes synaptic plasticity (Bergmann and Born, 2018; Fell and Axmacher, 2011; Gregoriou et al., 2009; Wang, 2010). In addition, according to the theta tagging hypothesis, stronger theta oscillations represent a stronger depolarizing influence on NMDA receptor-containing cells, which enables stronger facilitation of LTP, one of the main mechanisms of short-term synaptic plasticity (Vertes, 2005). These considerations suggest that the aftereffects following in-phase theta tACS would be state- and individual-dependent with stronger effects observable in individuals with higher executive functions under more demanding cognitive conditions. No such effects should be observed for anti-phase and sham stimulations.

In the present study, in-phase, anti-phase, and sham stimulation protocols were implemented via two 4 × 1 HD electrode placed at F3 and P3 to modulate frontoparietal theta in subjects performing a verbal WM task with three levels of WM load. Behavioral, EEG, and pupillometry data were collected in the post-stimulation session. Individual differences in executive functioning were assessed using working memory capacity (WMC) in a separate experiment. We tested whether in-phase theta tACS would enhance post-stimulation behavioral performance in a state-and individual-dependent manner and characterized the potential neurophysiological underpinnings by analyzing frontoparietal theta synchrony and Granger causality. Using the pupil size as an index of arousal, we further examined whether the possible aftereffects on behavior and neural synchrony are driven by differences in the brain's arousal levels.

Materials and methods

Participants

The experimental protocol was approved by the University of Florida Institutional Review Board. Twenty-five healthy college students (14 females, 23 ± 3 years of age) gave written informed consent and participated in the study. All subjects reported having no implanted electronic devices, no metal implants in the head, and no history of psychiatric or neurological disorders; they were also not current users of psychoactive medication, were not pregnant, and had normal or corrected-to-normal vision. Five participants did not complete all the study sessions and were therefore excluded from further analysis. The data from the remaining 20 subjects (11 females, 24 ± 3 years of age) were analyzed and reported here. We note that, although n = 20 was a moderate sample size, it is in the same range as recent related studies using the cross-over or within-subject design (Biel et al., 2022; Polanía et al., 2012). The cross-over design has the benefit of helping to reduce variance associated with having different cohorts participate in different stimulation sessions. Nevertheless, we stress that the results reported here should be considered preliminary.

Experimental procedure

Study Design. As illustrated in Fig. 1A, the experiment employed a single-blind, cross-over (within-subject), and sham-controlled design. It consisted of four study sessions with 1 week between successive sessions. During the first study session (baseline), participants took the OSPAN test online, which yielded working memory capacity (WMC). The OSPAN test was administered by Millisecond (https://www.millisecond.com/). The participant logged into Millisecond's website and completed the test online. The WMC obtained from the OSPAN test measures the ability to maintain information in the focus of attention in the presence of distraction. In light of the fact that working memory is a significant component of executive function, WMC is viewed as an index of the brain's executive function (Engle, 2002; Engle et al., 1999; Kane and Engle, 2002). A 12-minute resting state EEG, comprising 6-minutes eyes-open rest and 6-minutes eyes-closed rest, were then recorded. Subsequently, the subjects performed the WM task for 30 min while their EEG and pupil data were recorded. For each of the following three study sessions, participants started with a pre-stimulation EEG and pupil data recording (30 min) in which they performed the verbal WM task (pre-stimulation session). Then, they performed the WM tasks for 30 min (during-stimulation session) while receiving in-phase, anti-phase, or
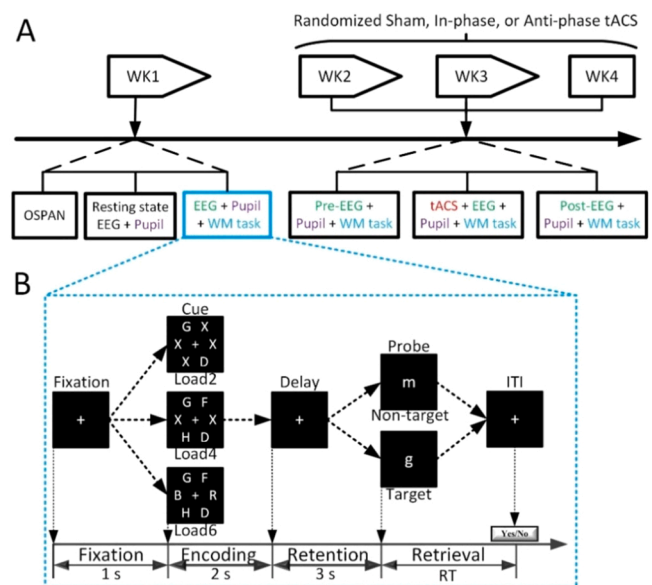

Fig. 1. Overall experimental design and verbal working memory task. (A) The randomized, single-blind, cross-over, and sham-controlled design. (B) Timeline of the verbal working memory task with three levels of memory load (load 2, load 4, and load 6).

sham theta tACS stimulation with the order of stimulation schemes randomized and counterbalanced across subjects. The stimulation session was followed by another 30 min of the WM task while EEG and pupil data were collected (post-stimulation session).

Verbal WM Task. In each of the four study sessions, participants performed a Sternberg WM task (Fig. 1B). In this task, each trial started with a fixation cross presented at the center of the screen for 1 s, followed by a 2 s presentation of the memory set, which contained two, four, or six uppercase consonant letters placed with equal probability in any of six positions arranged in a circle centered on the fixation cross. When the memory set consisted of less than 6 letters, filler symbols (X) were added as a placeholder to make the sensory input for the three memory-load conditions comparable. The memory set varied randomly from trial to trial. The offset of the memory set was followed by a 3-second delay (retention), after which a lower-case letter, the probe, was shown at the center of the screen for 1 s. Subjects responded via a button press to indicate whether this character was part of the previously presented memory set. On half of the trials, the probe letter was part of the memory set, and on the other half, it was not. The filler symbol x was never used as a probe. Subjects were encouraged to respond as quickly and accurately as possible. The entire task consisted of three blocks with 72 trials in each block. The three memory loads were equally likely to occur. Breaks were given between blocks. Participants received a practice session prior to the experiment to familiarize with the task.

Administration of tACS. The tACS was administered using a Soterix Medical 1 × 1 HD-tES stimulator and two 4 × 1 HD-tES splitters. A schematic illustration of the electrode configuration was shown in Fig. 2A. Five sintered Ag/AgCl electrodes attached to plastic holders filled with conductive gel were embedded in the Biosemi EEG cap to form each of the two 4 × 1 stimulation arrays. The center electrode of each array was placed at F3 and P3 with the surround electrodes being placed at AF3, F5, F1, and FC3 for the frontal array and at CP3, P5, P1, and PO3 for the parietal array. The goal of the stimulation was to modulate synchronized neural oscillations in the theta band between the left frontal and left parietal cortex during verbal WM. Fig. 2B shows the simulated electric field distribution (Soterix Medical HDExplore Software) associated with the in-phase HD-tACS protocols. It can be seen the left frontal and left parietal cortex are maximally stimulated.

Sinusoidal alternating current of 1 mA in magnitude was administered at each individual participant's frontal peak theta frequency (PTF) for 30 min. The PTF, defined with a 0.5 Hz frequency resolution in the theta range (4–8 Hz), was determined from the resting state EEG data recorded during Week 1 (WK1). (Since no resting state EEG data were collected in Weeks 2–4, the reproducibility of the PTF was not tested.) Individual PTFs ranged from 4.50 to 6.50 Hz (M = 5.45, S.D. = 0.40 Hz) in the sample with a resolution of 0.5 Hz. All participants were familiarized with tACS-induced skin sensations with random noise stimulation of 30 s in duration (Clancy et al., 2018). During tACS, the current ramped up to 1 mA over a time period of 30 s. In the in-phase condition, stimulation was delivered with 0 degree relative phase difference between two arrays, whereas in the antiphase condition, stimulation was delivered with a 180 degree relative phase difference. The sham stimulation condition followed the same procedure as the active condition, but stimulation only lasted 30 s, ramping up and down at the beginning and at the end of the 30-minute period, simulating the tingling sensation that subjects typically experience and then quickly habituate to during
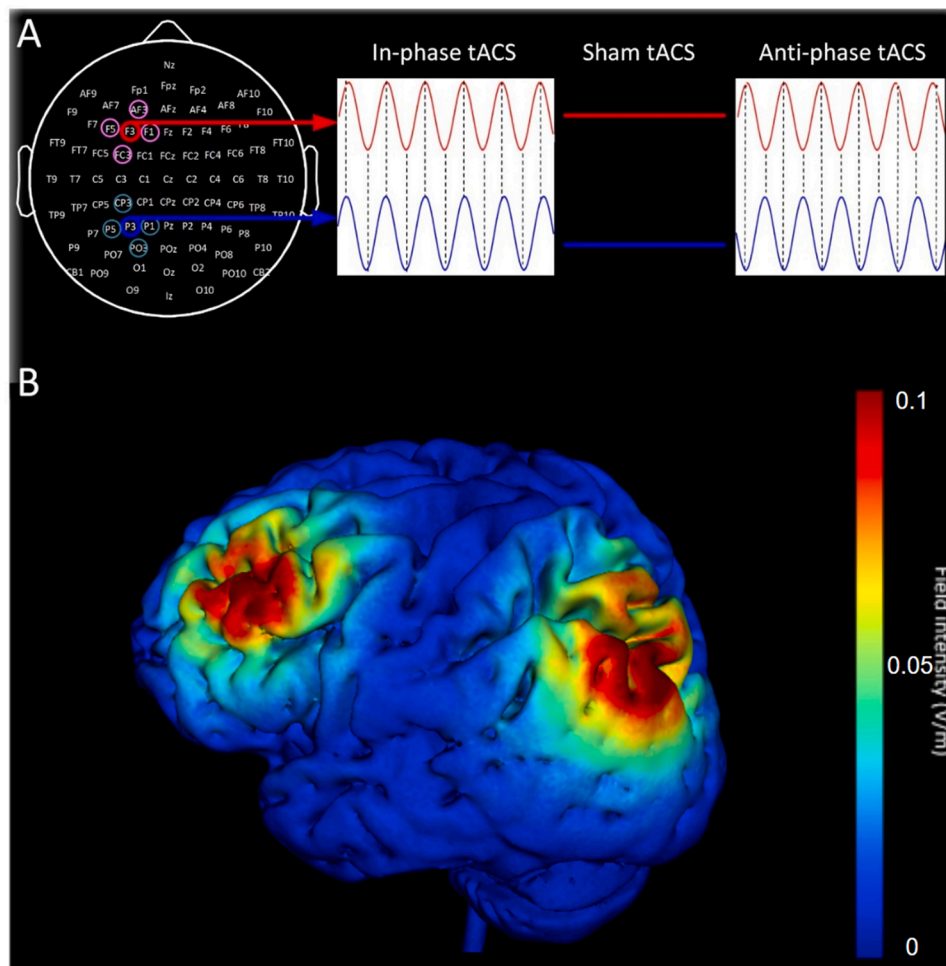

Fig. 2. tACS arrays, stimulation schemes and simulated electric field distribution. (A) Position of the two 4 × 1 HD-tACS arrays and the three stimulation protocols: in-phase (0° phase difference), anti-phase (180° phase difference), and sham. Within each array, the center electrode and the four surrounding electrodes have opposite polarity, forming a closed circuit. The center-surround, source-sink arrangement of the five electrodes enables better focality of electrical stimulation (high definition or HD). (B) Current flow under in-phase stimulation shown on 3D reconstruction of the cortical surface demonstrates maximal electrical field intensity over the left frontal and parietal cortex.

active stimulation sessions (Reinhart et al., 2017). For the pre-stimulation and post-stimulation sessions, the stimulating electrodes were replaced by EEG recording electrodes.

Data acquisition and preprocessing

Data Acquisition. Experiments were performed in an electromagnetically shielded room. Throughout the entire experiment, the subject's pupil diameter was measured at a sampling rate of 1 kHz with an EyeLink 1000 infrared eye-tracker (SR Research, Mississaugu, ON, Canada). The subject's EEG was recorded using a 128-channel Biosemi ActiveTwo system (Biosemi, Amsterdam, The Netherlands) at a sampling rate of 1 KHz.

Data Preprocessing. For continuous EEG data, the preprocessing was performed using EEGLAB (http://sccn.ucsd.edu/eeglab/index.html) and custom Matlab scripts (The Mathworks, Natick, MA, USA). The continuous EEG data were band-passed between 0.1 and 30 Hz using a FIR filter (EEGLAB function pop_eegfiltnew()), down-sampled to 256 Hz, and re-referenced against the average reference. For continuous pupil diameter data, blinks were detected using software provided by the manufacturer SR Research, and linear interpolation was carried out in Matlab. EEG and pupil data were epoched identically, from -1–7 s with 0 s denoting the onset of the memory set (also referred to as cue). Trials with either excessive noise in the pupillary data or EEG were manually identified and removed. Trials with incorrect responses were also excluded from further analysis. For the remaining EEG trials, independent components analysis (ICA) (Delorme and Makeig, 2004) was applied to remove artifacts due to eye movements and blinks. ICA was performed in EEGLAB and the bad components were manually marked and removed. In general, about 5 % of the components were removed. For each memory load, the data from the middle 1 s of the retention period (3000–4000 ms) was selected as the time period of interest. Here, the first 1 s of the retention period, 2000–3000 ms, was excluded to avoid the negative impact of cue-offset-evoked activities on the spectral analysis of ongoing neural oscillations, and the last 1 s of the retention period, 4000–5000 ms, was excluded to avoid the negative impact of the anticipation of probe processing on neural activity (Wang and Ding, 2011). To minimize the negative effects of volume conduction and common reference on connectivity analysis, the artifact-corrected scalp voltage data were converted to reference-free current source density (CSD) by calculating 2D surface Laplacian algorithm (Kayser and Tenke, 2006). All subsequent analyses were performed on the CSD data.

Data analysis

Working Memory Capacity. The participants' individual WMC was assessed via the operation span task (OSPAN) (Unsworth et al., 2005) during Week 1. In each trial of this task, the subject was shown a series of letters to remember, and the number of letters to be remembered varied from 3 to 7 depending on the trial. A simple mathematical problem was inserted between letters, and at the end of the trial, the subject was asked to recall the letters from memory. There was a total of 15 trials. The OSPAN score, taken as a measure of WMC, was the sum of all correctly recalled letters across the 15 trials. The maximum OSPAN score is 75.

Theta Power Estimation. Fast Fourier transforms (FFT) were applied to the data in the time period of interest to estimate the power spectra. Normalization by power in the precue baseline period was done on a subject-by-subject basis (1–30 Hz) (Jensen et al., 2002). This normalization procedure removed the influence of amplitude variability from subject to subject and allowed more straightforward averaging across participants. Theta power were the averaged power from 4 to 8 Hz from the normalized power spectrum.

Phase Synchrony Estimation. We used phase locking value (PLV) (Lachaux et al., 1999) in theta frequency between two signals as a measure of neural synchrony. Specifically, the PLV at time t is defined as:

$$PLV(t) = \frac{1}{N}\left| \sum_{n=0}^{N} \exp(j(\varnothing_1(n,t) - \varnothing_2(n,t))) \right|$$

where n is the trial index, N = total number of trials, and the instantaneous phase value $\varnothing_1(n,t)$ and $\varnothing_2(n,t)$ in the theta band are extracted from the two signals using Hilbert transform. The PLV value measurers the inter-trial variability of the phase difference. PLV is close to 1 when the two signals are strongly coupled and close to zero if they are uncoupled. This procedure was repeated for all the pairwise channel combinations between the 5 frontal (AF3, F5, F3, F1, FC3) and 5 parietal (CP3, P5, P3, P1, PO5) recording channels. The averaged PLV from 25 such pairs were used as the frontoparietal PLV.

Granger Causality (GC). Neural synchrony measured by PLV was further decomposed into directional components using nonparametric GC (Dhamala et al., 2008; Ding et al., 2006). The nonparametric approach for estimating pairwise GC consists of the following steps: (i) using the multitaper method (Mitra and Pesaran, 1999) to construct spectral density matrix $S(f)$ from Fourier transforms of two signals, (ii) factorizing spectral density matrix: $S = \Psi\Psi^*$ via Wilson's algorithm (Wilson, 1972, 1978) where Ψ is the minimum-phase spectral factor, (iii) deriving noise covariance matrix $\sum$ and transfer function H from Ψ according to equations $\sum = A_0 A_0^T$ and $H = \Psi A_0^{-1}$, (iv) using S, H, and $\sum$ in Geweke's formula (Geweke, 1982) to compute the causality from y to x at each frequency f according to:

$$I_{y \to x}(f) = \ln \frac{S_{xx}(f)}{S_{xx}(f) - (\sum_{yy} - \sum_{xy}^2 / \sum_{xx})|H_{xy}(f)|^2}$$

Reversing y and x in the above formula we can compute the causality from x to y at each frequency f. For each direction (e.g., frontal → parietal), GC were computing for all the pairwise channel combinations between the 5 frontal and 5 parietal recording channels (25 pairs) and averaged. The averaged value was taken as the GC for that direction.

Results

Working Memory Capacity: The average OSPAN across the subjects was 51.35 ± 14.75. Based on a median split, the subjects were divided into a low WMC group (WMC=40.80 ± 11.42) and a high WMC group (WMC=61.90 ± 9.02), with the low WMC group consisting of 6 women and 4 men (22 ± 3 years of age) and the high WMC group consisting of 5 women and 5 men (25 ± 3 years of age).

TACS Effects on Task Performance. Over the entire sample, there were no significant differences in accuracy and response times for any of the three memory load conditions following in-phase, anti-phase, and sham stimulation (Fig. 3A and C, all $p > 0.1$). After splitting subjects into low and high WMC groups, as shown in Fig. 3D, we found that under the high memory load (load 6) condition, the response time post in-phase stimulation was significantly faster than that post sham stimulation ($t_9 = -2.47$, $p = 0.035$) in subjects with high WMC; in-phase tACS yielded no behavioral benefits for the low WMC group ($p > 0.1$). No significant effects were observed following the anti-phase tACS (Fig. 3B and D, $p > 0.1$). A 2-way ANOVA in the high WMC group (load 2/4/6 vs. sham/in-phase/anti-phase stimulation) found that there is no interaction between load and stimulation scheme ($p = 0.92$).

TACS Effects on Frontoparietal Theta Synchrony and Granger Causality. The inter-areal phase synchrony between frontal and parietal regions in the post-stimulation sessions was assessed via phase-locking value (PLV). Over the entire sample, there were no significant differences in theta PLV between left frontal and left parietal ROIs for any of the three memory load conditions in any of the three post stimulation sessions (all $p > 0.1$). However, after splitting subjects into low and high WMC groups, we found that in subjects with high WMC (Fig. 4A Top), in-phase stimulation enhanced left frontoparietal theta synchronization relative to both sham and antiphase stimulation during working memory
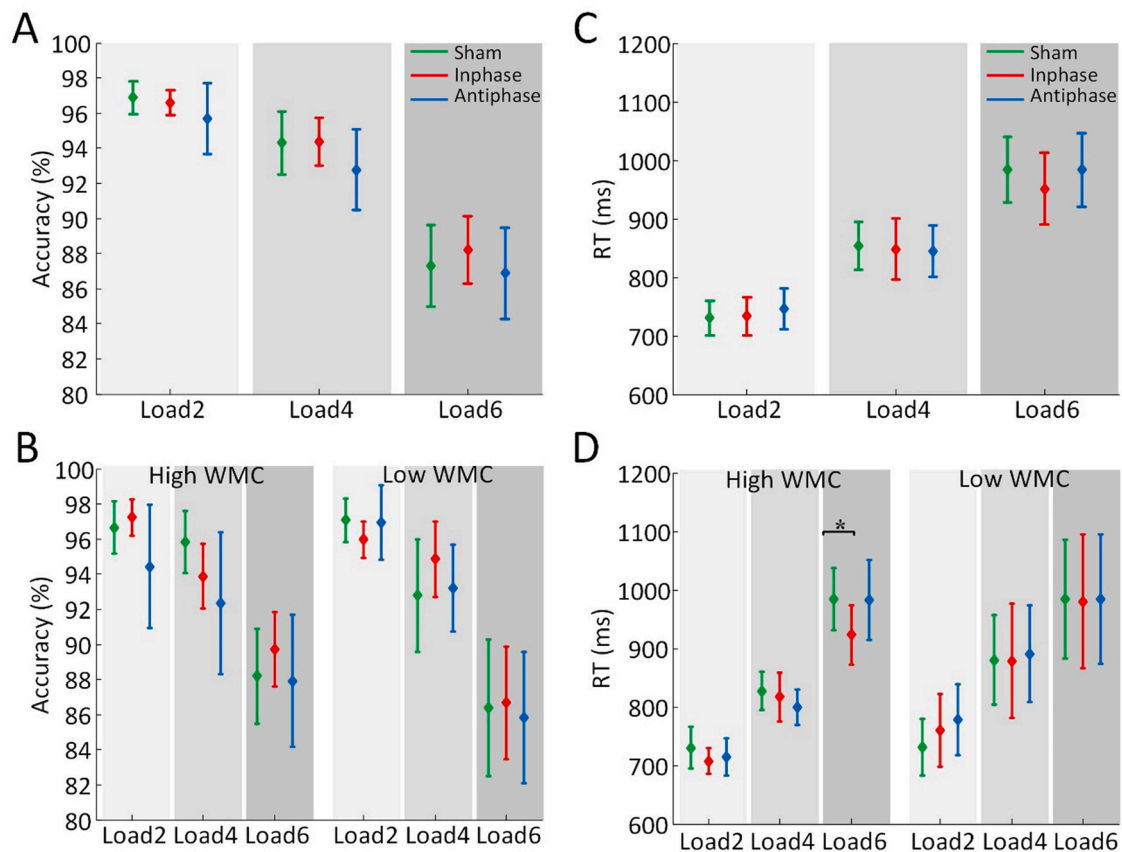
Fig. 3. TACS aftereffects on task performance. (A) Mean accuracy and (C) RT under different WM load conditions following the three stimulation protocols in all subjects (n = 20). (B) Mean accuracy and (D) RT under different WM load conditions following the three stimulation protocols in high (n = 10) and low (n = 10) WMC subjects. *p < 0.05. Green: post sham, red: post in-phase, blue: post anti-phase. *p < 0.05.

retention under memory load 4 and 6 (load 4: in-phase > sham, $t_9 = 3.12$, $p = 0.012$; in-phase > antiphase, $t_9 = 2.60$, $p = 0.029$. load 6: in-phase > sham, $t_9 = 2.44$, $p = 0.037$; in-phase > antiphase, $t_9 = 2.71$, $p = 0.024$). There was no evidence of enhanced theta synchronization (a) in the left frontoparietal network for low WMC individuals (Fig. 4A Bottom, $p > 0.1$) and (b) in the right frontoparietal network for either of the two WMC groups (Fig. 4B, all $p > 0.1$).

Applying Granger causality (GC), the frontoparietal theta synchronization in the left hemisphere was decomposed into its directional components, frontal→parietal and parietal→frontal. As shown in Fig. 4C Top, in-phase stimulation enhanced left frontal→parietal theta GC as compared to sham stimulation during WM retention under memory loads 4 and 6 in subjects with high WMC (load 4: in-phase > sham, $t_9 = 2.33$, $p = 0.045$; load 6: in-phase > sham, $t_9 = 4.01$, $p = 0.0031$). In contrast, there was no evidence of increased frontal→parietal theta GC in low WMC individuals (Fig. 4C Bottom, $p > 0.1$), and there was no difference in parietal→frontal theta GC in either groups (Fig. 4D, all $p > 0.1$). Thus, the increased left frontoparietal synchrony in the theta band following in-phase stimulation under higher memory load conditions in high WMC individuals is mainly driven by increased left frontal→parietal theta drive, whereas left parietal→frontal GC is not modulated by theta tACS.

TACS Effects on Pupil Diameter. Pupil diameter was examined to assess whether different stimulation schemes differentially affected arousal levels in the post stimulation sessions. Both at the entire sample level and at the level of the high and low WMC groups, we found that there were no significant differences in pre-cue pupil diameter from the three post-stimulation sessions (all $p > 0.1$), indicating that the arousal level was not different whether the session was preceded by sham, in-phase, and anti-phase stimulations. These results suggested that the

observed effects on task performance, left frontoparietal theta synchronization, and left frontal → parietal theta GC in the high WMC groups were not due to differences in arousal levels.

Summary. We summarized the findings reported so far in Table 1. It is clear that the effects of in-phase stimulation in the post-stimulation session were only observed under more demanding cognitive conditions in subjects with stronger executive functions (i.e., higher WMC).

Discussion

We applied in-phase, anti-phase, and sham theta tACS to the left frontal (F3) and parietal (P3) sites via two 4 × 1 high-definition stimulation arrays during verbal working memory. Focusing on behavioral and neurophysiological aftereffects, we presented preliminary results showing that during the post stimulation session, in subjects with higher WMC and under more cognitively demanding conditions, in-phase theta tACS (1) improved WM task performance and (2) enhanced left frontoparietal theta synchrony and frontal→parietal theta Granger causality. There were no behavioral and neurophysiological aftereffects in subjects with low WMC and in the entire sample. The pupil diameter, an established marker of arousal, was found to be not different between stimulation schemes in the entire sample as well as in each of the two WMC groups.

Theoretically, this work was guided by two considerations. According to the neuronal communication via neuronal coherence (NCNC) hypothesis (Fries, 2005), in-phase oscillation between distant sites of an oscillatory network facilitates neuronal communication, whereas anti-phase oscillation hinders it. By promoting synchronous firing, in-phase tACS is conducive to the induction of synaptic changes via spike-timing dependent plasticity (Bi and Poo, 2001; Caporale and Dan,
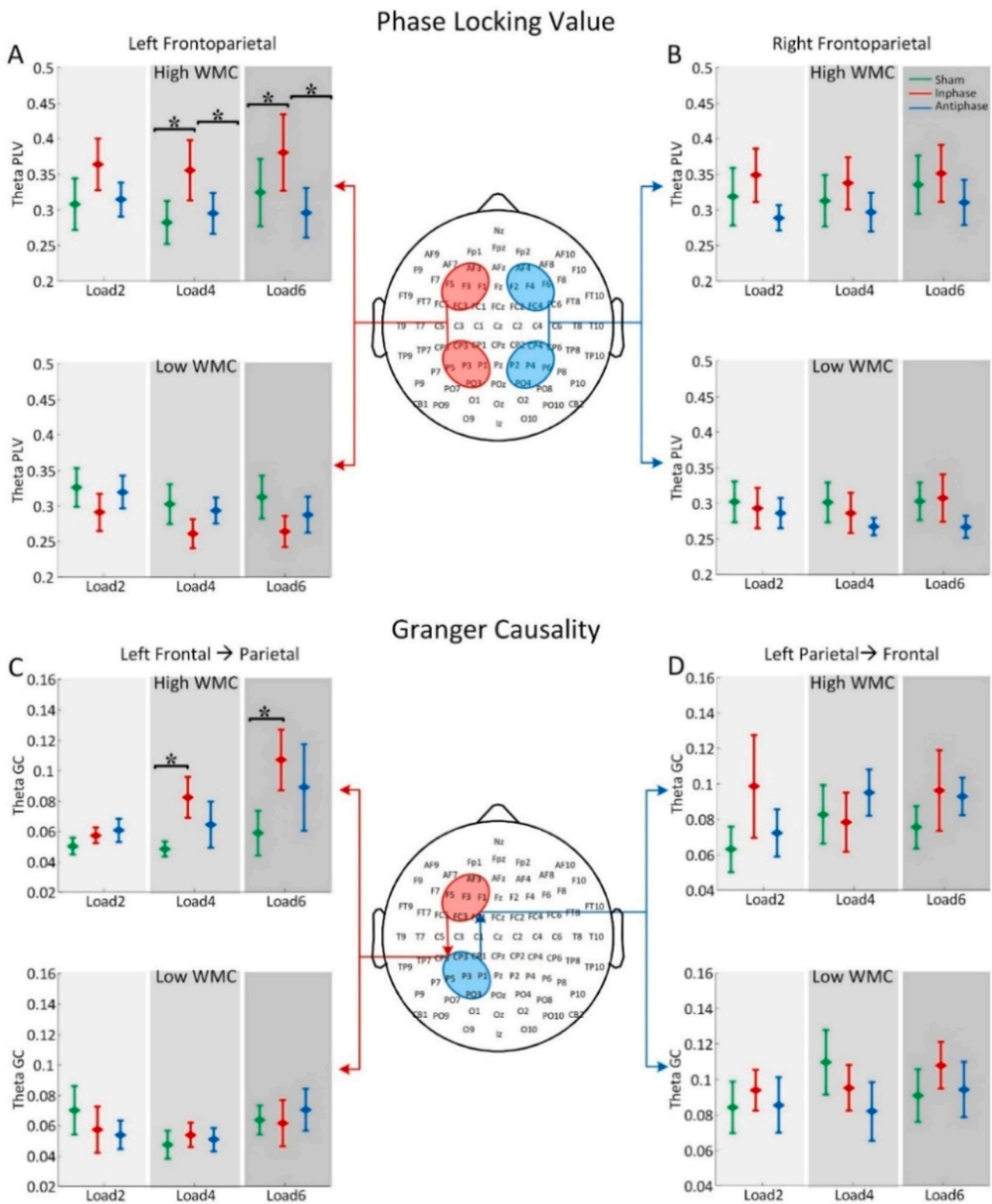
Phase Locking Value

Fig. 4. TACS effects on frontoparietal theta synchrony and Granger causality (GC). (A) Left frontoparietal theta phase locking value (PLV) for high (top) and low (bottom) WMC subjects. (B) Right frontoparietal theta PLV for high and low WMC subjects. (C) Left frontal → parietal theta band GC in high (top) and low (bottom) WMC subjects. (D) Left parietal → frontal theta band GC in high and low WMC subjects. Green: post sham, red: post in-phase, blue: post anti-phase. *p < 0.05.

2008; Dan and Poo, 2006). The theta tagging hypothesis (Vertes, 2005) further posits that, long-term potentiation (LTP), which is the main mechanism underlying short-term synaptic plasticity, is more likely to occur in the presence of stronger theta activity. It is known that the magnitude of theta oscillations and synchrony is a function of the

cognitive state, with more cognitively demanding tasks associated with stronger theta oscillations and synchrony, and the magnitude of theta oscillations and synchrony exhibits significant subject-to-subject variability, with individuals with stronger executive functioning (higher WMC) showing stronger theta modulation by cognitive conditions. In

Table 1
Summary of in-phase tACS effects.

	Low WMC			High WMC		
	Load2	Load4	Load6	Load2	Load4	Load6
Accuracy						
RT						✓
Frontoparietal theta synchrony (l)					✓	✓
Frontoparietal theta synchrony (r)						
Frontal → parietal theta GC (l)					✓	✓
Parietal → frontal theta GC (l)						
Pupil diameter						

l: left and r: right.

light of the foregoing, our results, showing that in-phase frontoparietal stimulation enhanced post-stimulation verbal working memory performance only in subjects with stronger executive functioning and under higher cognitively demand conditions, can be seen as in broad agreement with the theoretical considerations.

It is worth noting that, although the NCNC hypothesis, when applied to network level stimulation predicts that: in-phase vs anti-phase stimulation should result in enhanced vs hindered neuronal communication and behavioral performance respectively, empirically, the predicted detrimental effects of anti-phase stimulation are often not observed. Kleinert et al. (2017) applied 5 Hz tACS at fronto-parietal sites during a visuospatial match-to-sample task and reported that there were no significant differences between in-phase and anti-phase stimulation in both behavioral and EEG measurements. Miyaguchi et al., (2019) applied tACS at 70 Hz over the left M1 and the right cerebellar hemisphere in a visuomotor control task and found that the anti-phase stimulation decreased task error compared to the sham condition but did not differ from the in-phase stimulation. Violante et al. (2017) found a decrease in reaction time for in-phase tACS relative to sham and anti-phase tACS but did not observe any difference between anti-phase tACS and sham (Violante et al., 2017). These results could be explained by the fact that under anti-phase stimulation, the current density is more diffuse and less focal (Saturnino et al., 2017), which could render the ani-phase stimulation less effective in modulating the relevant networks. For the present study, which focused on the aftereffects of stimulation, while we observed enhanced theta synchrony and behavioral performance following in-phase stimulation, there is no evidence of declining theta synchrony and task performance following anti-phase stimulation. One possible reason is that anti-phase stimulation, by disrupting rather than promoting neural synchrony, did not result in short-term synaptic changes in the stimulated network, which therefore did not give rise to observable changes in neurophysiology and behavior in the post stimulation session.

Neurophysiologically, we found that in-phase stimulation enhanced frontoparietal theta synchrony during working memory retention, and such enhancement was only observed in the left hemisphere (the stimulated hemisphere) but not in the right hemisphere, suggesting that the stimulation indeed led to short-term plastic changes in the stimulated frontoparietal network, resulting in enhancement in neuronal communication. Similar to behavioral improvement, enhanced neuronal communication was only observed in individuals with stronger executive functioning and under higher cognitive demands, revealing the possible neural underpinnings of the behavioral outcomes. Decomposing neural synchrony into their directional components, Granger causality further revealed that the increased theta synchrony comes from increased frontal→parietal influence, whereas parietal→frontal influence remained unchanged, further demonstrating that improved executive control is likely the mechanism underlying the improved verbal WM performance.

Can any of the changes we see following different tACS stimulation schemes be explained by nonspecific effects such as changes in the brain's overall arousal levels? We used pupillometry data to address this question. Extensive research has shown that pupil size is a good indicator of the level of arousal and cognitive efforts (Aston-Jones and Cohen, 2005; Bradley et al., 2008; Ebitz and Platt, 2015; Eldar et al., 2013; Nassar et al., 2012; Urai et al., 2017). Since visual stimuli and cognitive loads are known to cause event-related pupil changes, to assess non-task related arousal levels, we focused the measurement of pupil diameter during the time period prior to the presentation of memory cues. Our results showed that both at the level of the entire sample and at the level of each of the two WMC groups, the pupil size was the same in the post-stimulation session, regardless of whether the session was preceded by in-phase, anti-phase, and sham stimulations. This can be taken as evidence to support the notion that the observed behavioral and neurophysiological effects following in-phase tACS were mainly due to plastic changes in the frontoparietal theta oscillatory network rather than differences in the arousal levels caused by different stimulation protocols.

Although our study is among the few to have investigated the aftereffects of tACS stimulation at both the behavioral and neurophysiological levels, it has a number of limitations. First, EEG data during tACS stimulation were not analyzed because effectively separating neural activity from stimulation artifacts was difficult. It is thus not known whether different tACS protocols directly modified frontoparietal theta synchrony. This limitation is mitigated to some extent by recent studies showing that external tACS is capable of entraining and modulating endogenous brain oscillations in a frequency-specific manner (Feurra et al., 2011; Pogosyan et al., 2009; Polanía et al., 2012; Reinhart, 2017; Thut et al., 2011; Violante et al. 2017). Second, while the use of HD stimulation arrays improves stimulation focality compared to sponge-style electrodes, scalp-mounted devices still suffer from the lack of very precise spatial targeting ability. Third, the sample size is relatively small. Although the sample size of n = 20 is comparable with other studies employing a cross-over or within-subject design (Biel et al., 2022; Polanía et al., 2012), when the sample was divided into high WMC and low WMC subsamples, the subsample size (n = 10) is small, making the analysis underpowered and not as robust as can be (e.g., multiple comparison correction not implemented). Thus the findings reported here should be viewed as preliminary and interpreted with caution. Having pointed out these limitations, we do hope that the novelty of our findings, including the much needed neurophysiological analyses, will inspire further studies with larger sample sizes to either affirm or refute the findings presented here.

Ethical statement

The experimental protocol was approved by the University of Florida Institutional Review Board (UF IRB). Written informed consent was obtained from all participants. The human experiment was done in accordance to the UF IRB approved protocol. De-identified data were analyzed and presented here.

CRediT authorship contribution statement

Zhenhong Hu: Conceptualization, Data collection, Methodology, Formal analysis, Writing- review & editing. **Immanuel B.H. Samuel:** Data collection, Methodology. **Sreenivasan Meyyappan:** Data collection, Methodology. **Ke Bo:** Data collection, Methodology. **Chandni Rana:** Data collection, Methodology. **Mingzhou Ding:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a

potential conflict of interest.

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