

Differential Effects of 10 and 20 Hz Brain Stimulation in Chronic Stroke: A tACS-fMRI Study

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Abstract—Transcranial alternating current stimulation (tACS) is an emerging non-invasive neuromodulation technique, which could be potentially applied to enhance stroke rehabilitation. In this study, we aimed to characterize the frequency-specific effects of tACS in chronic stroke. Therefore, concurrent tACS and functional magnetic resonance imaging (fMRI) were conducted in 13 chronic stroke individuals. Resting-state and task-based fMRI were collected for each subject under different frequencies (10 Hz, 20 Hz, Sham). Task-based fMRI showed that increased activation was found in the ipsilesional precentral area during paretic hand movements after 10 Hz tACS, while increased activation was found in the contralesional precentral area during non-paretic hand movements after both 10 and 20 Hz tACS. Resting-state seed-based functional connectivity (FC) analysis showed that 10 Hz tACS mainly modulated FC within motor-related regions, while 20 Hz tACS also modulated regions beyond the motor-related areas. Graph theory analysis further demonstrated the functional interaction modulated by tACS in the whole-brain level. Taken together, our results showed that tACS might exhibit frequency-specific modulation in chronic stroke. 20 Hz tACS facilitates the functional interaction between the sensorimotor regions and brain regions involved in executive control, while 10 Hz and sham tACS has limited effect on motor-related brain activity. Our results reveal the neural response process under external current stimulation, providing new insight into the neuromodulation mechanism of tACS in a lesioned brain.

Index Terms—Transcranial alternating current stimulation, stroke, functional magnetic resonance imaging, functional connectivity, graph theory.

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I. INTRODUCTION

STROKE is a leading cause of disability, and the demand for stroke rehabilitation is increasing [1]. The existence of neuroplasticity makes it possible for various rehabilitation methods [2], [3]. Therefore, more and more clinical studies have been focused on novel interventions aiming at enhancing the neuroplasticity in stroke subjects, including non-invasive brain stimulation [4], [5].

Transcranial alternating current stimulation (tACS) opens a new era of non-invasive electrical stimulation by directly interfering with cortical rhythms [6]. Although the neurophysiological mechanisms of tACS are still not thoroughly understood. It is believed that the frequency-specific entrainment effects play an important role in modulating endogenous brain oscillations [7]. Through modulation of neural activity, tACS is a powerful tool to investigate the neural correlates of perception [8], memory [9], as well as motor [10] and cognitive function [11]. TACS is usually applied at conventional electroencephalography (EEG) frequencies (0.1–80 Hz), which may be able to interact with the ongoing rhythms in the cortex [6]. The effects of tACS applied in the EEG range also depend on the stimulation frequency. Alpha-band (8–12 Hz) and beta-band (13–30 Hz) oscillations are known to be highly associated with motor preparation [12], motor imagery [13], and motor execution [14]. The modulation of sensorimotor cortical excitability is also related to the manipulation of alpha- and beta-band oscillations [15], [16]. Alpha- and beta-components of the sensorimotor rhythms (SMR) are supposed to represent different functional loops within the sensorimotor system. Oscillatory beta-activity has been observed at various levels of the motor system, including motor cortex, basal ganglia and muscles [17], [18]. By contrast, the alpha rhythm has been linked to the transformation of visual and auditory information into movements [19]. Therefore, in this study, we focused on two representative frequencies of 10 Hz and 20 Hz in alpha-band and beta-band, respectively. The effects of 10 and 20 Hz tACS might be driven by the same neurophysiological mechanism, from the modulation of dynamic shunting inhibition exerted by GABAergic interneurons over a local neuronal ensemble [20]. A number of studies have investigated the stimulation effects on the primary motor cortex (M1) in healthy subjects [21], [22]. Over the past decade, studies have shown that tACS can increase the corticospinal

excitability of the M1 [23], [24]. However, few studies have explored the effects of tACS in stroke subjects [25], [26], and the underlying mechanisms are still unclear. Understanding the mechanisms of such external stimulation could help us develop more effective stimulation protocols.

Functional magnetic resonance imaging (fMRI) has emerged as one of the most popular neuroimaging tools to evaluate the cortical effects associated with neuromodulation [27]. It is widely acknowledged that brain regions do not operate in isolation but continuously interact with each other in cortical networks [28]. An increasing number of neuroimaging studies are exploring functional connectivity (FC) between brain regions [29], [30]. Graph theory is also widely adopted to evaluate the stimulation effects using either resting-state EEG [31] or fMRI [32], [33]. Task-based fMRI is able to depict the functional reorganization in the brain after stroke recovery in various domains, such as motor, language, and attention [34].

Despite its promising potential for clinical utility, the underlying modulation mechanism of tACS remains unclear, especially for stroke subjects. It is still not thoroughly understood whether alpha- and beta-band stimulation over the ipsilesional primary motor cortex would increase the motor-related cortical excitability and further induce whole-brain level functional modulation. To fill this gap, the current study aims to thoroughly explore the tACS stimulation effects using rs-fMRI and task-based fMRI. Due to the different functional roles of alpha and beta oscillations within the sensorimotor system, we hypothesized that both 10 Hz and 20 Hz tACS would enhance the functional interaction within the sensorimotor regions, while only 20 Hz tACS would also modulate the interaction between sensorimotor regions and more widespread brain regions. First, we investigated the activated area changes in the motor cortex during a motor task with either the paretic or non-paretic hand. After that, resting-state seed-based FC analysis was performed at each time point (*Pre*, *During*, and *Post*) and stimulation frequency to investigate the whole-brain changes in the FC with the stimulation site. Furthermore, graph theory analysis at a whole-brain level was also conducted to further explore the modulation of tACS on the interactions within local communities as well as between communities. The findings in this study might shed light on the underlying mechanism of the neural modulation process using tACS, which could further provide insights into stimulation protocol design for stroke.

II. MATERIALS AND METHODS

A. Subjects

Thirteen chronic stroke individuals (8 males, age = 61 ± 10) with a unilateral hemisphere (7 left hemisphere) impairment were recruited in this study. Fugl-Meyer Assessment for upper-extremity and Action Research Arm Test were used to evaluate the motor function of all participants. The demographics of the participants are in the Supplementary Materials. All subjects suffered from a single cerebrovascular accident. The inclusion criteria were as following:

(1) sufficient cognition to understand the experimental instructions, with Hong Kong Montreal Cognitive Assessment score exceeds 22 [35], (2) with a single unilateral brain lesion, (3) stroke onset more than 6 months. Subjects with (1) serious cognitive deficits, (2) history of alcohol, drug abuse, or epilepsy, and (3) any contraindications to MRI or brain stimulation were excluded.

This study was approved by the Joint Chinese University of Hong Kong New Territories East Cluster Clinical Research Ethics Committee. All subjects had written consent before the intervention. This study was registered at <https://clinicaltrials.gov> (NCT04638192)

B. Experimental Design

1) TACS Administration: Two tACS electrodes ($5 \times 5 \text{ cm}^2$) were attached to each subject's scalp and fixed with straps. The electrodes were put over the ipsilesional M1 (C3/C4) and the contralesional supraorbital ridge (FP1/FP2), respectively [21], [36]. Fig.1(B) illustrates the electrodes placement. For 10 Hz and 20 Hz tACS, 1 mA (peak-to-peak) current was delivered for 20 mins by an MRI-compatible DC-stimulator (NeuroConn GmbH, Ilmenau, Germany) with 30 seconds' ramp-up and 30 seconds' ramp-down. For the sham stimulation, only 30 seconds' ramp-up and 30 seconds' ramp-down was applied [21]. The sham stimulation was delivered using 10 Hz or 20 Hz tACS, and the frequency was randomized across subjects. A single-blinded design was adopted, and the investigators were informed of which stimulation protocol was given, but the subjects were blinded about the protocols. Fig.1(C) illustrates the current density change in each cycle of the tACS period. The simulation was performed on the standard MNI brain template provided by the software using SIMNIBS 3.2 [37]. The total impedance was kept below $20 \text{ k}\Omega$ during the whole stimulation period. For the MRI-compatible cables, each of the two cables contained a $5 \text{ k}\Omega$ resistor to reduce induction voltage due to high radiofrequency (RF) impulses.

2) Image Acquisition: MRI data were acquired using a 3T Philips MR scanner with an 8-channel head coil (Achieva TX, Philips Medical System, Best, Netherlands). T1-weighted anatomical images and blood-oxygen-level dependent (BOLD) fMRI images were collected for each subject. The detailed MRI sequence parameters are listed in the Supplementary Materials. MRI data were acquired for all 13 subjects. During rs-fMRI acquisition, the participants were instructed to focus on a white fixation cross in the black background. Rs-fMRI data were collected before, during as well as after tACS, with one 6-minute run before stimulation, three 6-minute runs during stimulation and one 6-minute run after stimulation. Each 6-minute fMRI run consisted of 180 volumes. During task-based fMRI acquisition, subjects were asked to grasp their hand with the maximum force using either their paretic hand or non-paretic hand when a letter of "L" or "R" (indicating the left hand or right hand) was displayed on the screen, and they were also instructed to maintain for 6 s until the letter disappeared from the screen. Task-based fMRI data were

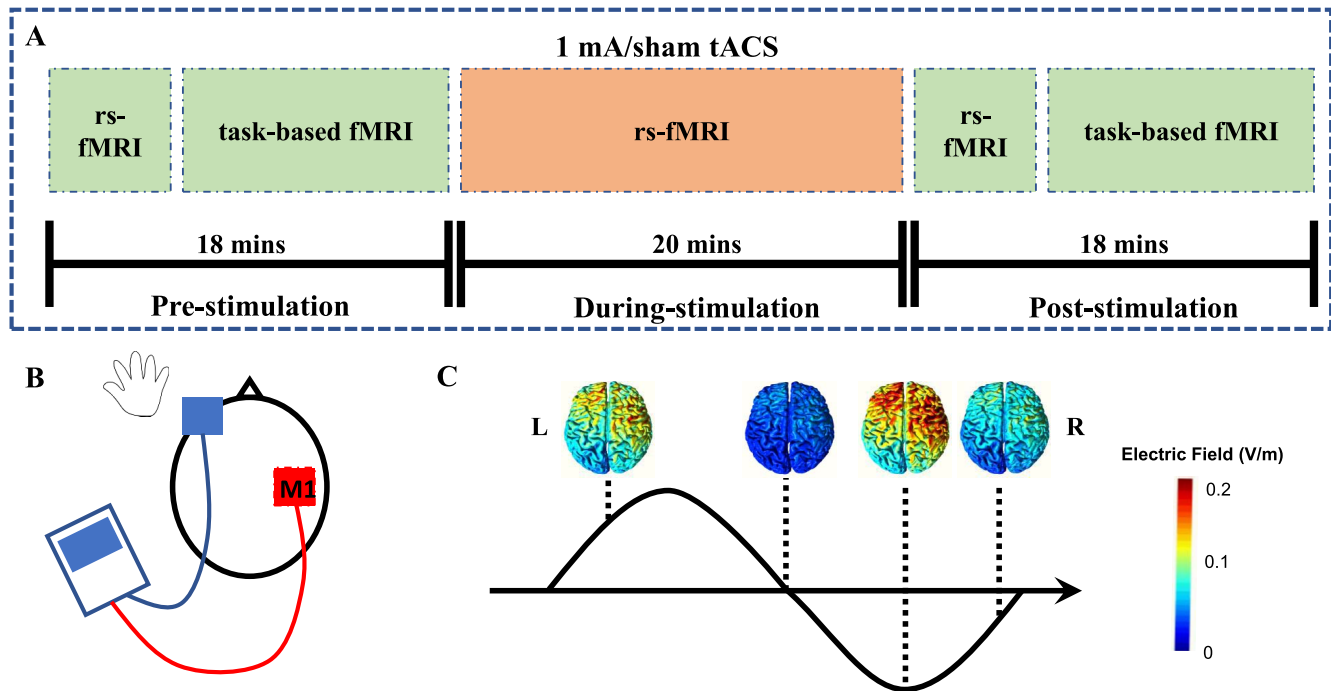


Fig. 1. (A) The sequence of MRI acquisition protocol. (B) The electrode placement of tACS. One electrode was put on the ipsilesional primary motor cortex (M1), and the other one was on the contralesional supraorbital ridge. (C) The electric field changes periodically in tACS.

collected before and after the stimulation, with two 6-minute task-based fMRI runs at each stage. In each task-based fMRI run, 16 hand contraction tasks were performed. All the trials were used in the task-based fMRI analysis. One subject was excluded in the task-based fMRI analysis due to large motion artifacts (see Supplementary Materials). The sequence was designed in an event-related manner, and the inter stimuli interval was randomized from 14 to 20 seconds. Fig.1(A) illustrates the MRI acquisition protocol.

C. Image Preprocessing

DPARSF toolbox [38] was adopted to preprocess the rs-fMRI and task-based fMRI data. In order to perform group-level analysis later, the images for subjects with the lesion in the left hemisphere were flipped along the midsagittal plane. Thus, all subjects have a right hemispheric lesion. More information about the preprocessing steps is listed in the Supplementary Materials.

D. Task-Based fMRI Analysis

First-level analysis for each subject was carried out using a general linear model (GLM). Three different kinds of events (paretic hand movement, non-paretic hand movement, fixation only) were convolved with a canonical hemodynamic response function and formed up three regressors in the GLM. Besides, six typical motion parameters were also fitted into the GLM to further deal with the motion-related fluctuations. The effect of fixation was subtracted in the contrasts for paretic hand movement and non-paretic hand movement. Therefore, each subject had two *t*-maps at each session, with one for the

paretic hand movement and another for the non-paretic hand movement. One-sample *t*-tests for paretic hand movement and non-paretic hand movement in the *Pre* sessions were performed to demonstrate the different activation patterns in the brain using each side of the upper-limb. Paired *t*-tests were carried out for *t*-maps from *Pre* and *Post* sessions under different stimulation protocols. Voxels were thresholded at $p < 0.005$, and cluster-level corrected.

E. Seed-Based Functional Connectivity Analysis

To evaluate the effects of tACS on the whole-brain FC, seed-based FC analysis was performed. The stimulation site, the ipsilesional lateral sensorimotor area, was chosen as the seed area. Therefore, the right lateral sensorimotor area in the Harvard-Oxford atlas was used as the seed. The time courses of the voxels within the seed area were averaged in order to calculate the FC with voxels in the whole brain, and FC maps were produced for different stages (*Pre*, *During*, and *Post*), separately. Fisher *r*-to-*z* transform was used on the FC maps. Repeated-measure ANOVAs with time (*Pre*, *During*, and *Post*) as the within-subject effect were performed to explore the stimulation effects for each stimulation protocol (10 Hz, 20 Hz, and Sham). Voxels were thresholded at $p < 0.005$, and cluster-level corrected.

F. Graph Theory Analysis

The whole cortical areas were parcellated into 30 regions in 7 networks (sensorimotor (SMN), default-mode (DMN), visual (VN), salience (SN), dorsal attention (DAN), frontoparietal (FPN), and language network (LN)) according to the

Harvard-Oxford atlas, together with 2 cerebellar (CB) regions according to the AAL atlas. Therefore, there were 32 regions of interest (ROI) for each subject. The detailed information regarding the regions could be found in the Supplementary Materials. Functional connectivity between each pair of regions was calculated using CONN toolbox [39], producing 32×32 matrices for each stage (*Pre*, *During*, and *Post*) under each stimulation protocol (10 Hz, 20 Hz, and Sham). Nodes N (brain regions) and edges k (FC between regions pairs) are the key elements in a graph. We adopted Pearson's correlation to calculate the functional connectivity between each pair of ROIs. Fisher's r -to- z transform was performed on the correlation coefficients. The matrices were thresholded by 0.2 using absolute value, producing binary matrices [40]. All the analyses were based on the thresholded binary networks.

Community structure is a sensitive marker of brain network organization [41], which consists of nodes with dense connections within its own community and sparse connections with other communities. The measurement of modularity depicts the goodness of such network partitioning. To calculate the community structure, the modularity Q could be used as the objective function, in order to maximize within-module connections as well as minimize between-module connections. The modularity Q could be formulated as [42], [43]:

$$Q = \sum_{u \in M} \left[e_{uu} - \left(\sum_{v \in M} e_{uv} \right)^2 \right] \quad (1)$$

where the network is parcellated into several modules M that are not overlapped with each other, and e_{uv} means the connections between nodes in module u and nodes in module v . The community structure calculation was performed in the GREYNA toolbox [44]. The resulting group-level community structure for 20 Hz stimulation was showed in Fig.4(A) as an example.

Once the community structure is determined, the within-module degree z -score could be adopted to describe the how well-connected the nodes are in their own communities, and is formulated as:

$$z_i = \frac{k_i(c_i) - \bar{k}(c_i)}{\sigma_{k(c_i)}} \quad (2)$$

where c_i means the module that contains node i , $k_i(c_i)$ means the degree of node i within module c_i , $\bar{k}(c_i)$ and $\sigma_{k(c_i)}$ stand for the mean and standard deviation of the nodal degree distribution within module c_i , respectively. From a different perspective, the participation coefficient is regarded as a metric of the goodness of inter-modular connections [45]. Specifically, it describes whether the edges of a node is well-distributed to different communities, and is formulated as:

$$P_i = 1 - \sum_{j=1}^{n_c} \left(\frac{k_{ij}}{k_i} \right)^2 \quad (3)$$

where k_{ij} means the number of connections that links node i and the nodes in community j , k_i means total degree of node i , and n_c means the total number of communities.

The $P-z$ plot demonstrates how a node is located in its own community, and its relationship with other nodes [33]. Nodes that share similar roles should also have close topological properties, which could be represented by the locations in the $P-z$ plot.

III. RESULTS

A. Effects of tACS on Hand Movement Task

From Fig.2(A), the activated area during the paretic hand movements was sparsely distributed in both hemispheres of the brain, and not only the ipsilesional hemisphere but the contralesional hemisphere was also highly involved. However, the activated brain area during the non-paretic hand movements was mainly distributed in the contralesional side, and the ipsilesional hemisphere was less activated. Following 10 Hz tACS, paired t -tests revealed that significantly increased activation was found in the ipsilesional precentral area with an MNI coordinate of (57, -5, 44) and contralesional insula (-38, -13, 19) during paretic hand movements (Fig.2(B)), and significantly increased activation was found in the contralesional precentral area (-59, -2, 22) during non-paretic hand movements (Fig.2(C)). After 20 Hz tACS, paired t -tests showed that significantly increased activation was only found in the contralesional precentral area with an MNI coordinate of (-48, -10, 28) during non-paretic hand movements (Fig.2(C)); no significant change was found during paretic hand movements. For sham stimulation, paired t -tests exhibited that no significant result was found neither during paretic hand movements nor non-paretic hand movements.

B. Results of Seed-Based Analysis

For 10 Hz tACS, the repeated-measure ANOVA with time (*Pre*, *During*, and *Post*) showed that significantly modulated FC was found in contralesional central area (-8, -24, 50) (Fig.3(A)), with significant modulation between *Pre* and *During* ($t(12) = -3.189$, $p = 0.024$), as well as between *During* and *Post* ($t(12) = 3.347$, $p = 0.018$). For 20 Hz tACS, the repeated-measure ANOVA with time showed that significantly modulated FC was found in contralesional central operculum cortex (-60, -18, 14) and anterior cingulate gyrus (-8, 42, 14) (Fig.3(B).) Increased FC from *Pre* to *Post* ($t(12) = 5.714$, $p < 0.001$), as well as from *During* to *Post* ($t(12) = 4.214$, $p = 0.003$) was observed in contralesional central operculum cortex, and increased FC from *Pre* to *Post* ($t(12) = 2.821$, $p = 0.045$), as well as from *During* to *Post* ($t(12) = 4.937$, $p < 0.001$) was observed in anterior cingulate gyrus. For sham tACS, the repeated-measure ANOVA with time showed that significantly modulated FC was found in the ipsilesional central area (36, 2, 48) and another cluster in posterior cingulate gyrus and precuneus (6, -36, 38) (Fig.3(C)). Decreased FC from *Pre* to *During* ($t(12) = -3.806$, $p = 0.009$), from *Pre* to *Post* ($t(12) = -6.972$, $p < 0.001$), as well as from *During* to *Post* ($t(12) = -4.479$, $p = 0.003$) was observed in the ipsilesional central area, while increased FC from *Pre* to *During* ($t(12) = 4.259$, $p = 0.003$), as well as from *Pre* to *Post* ($t(12) = 3.770$,

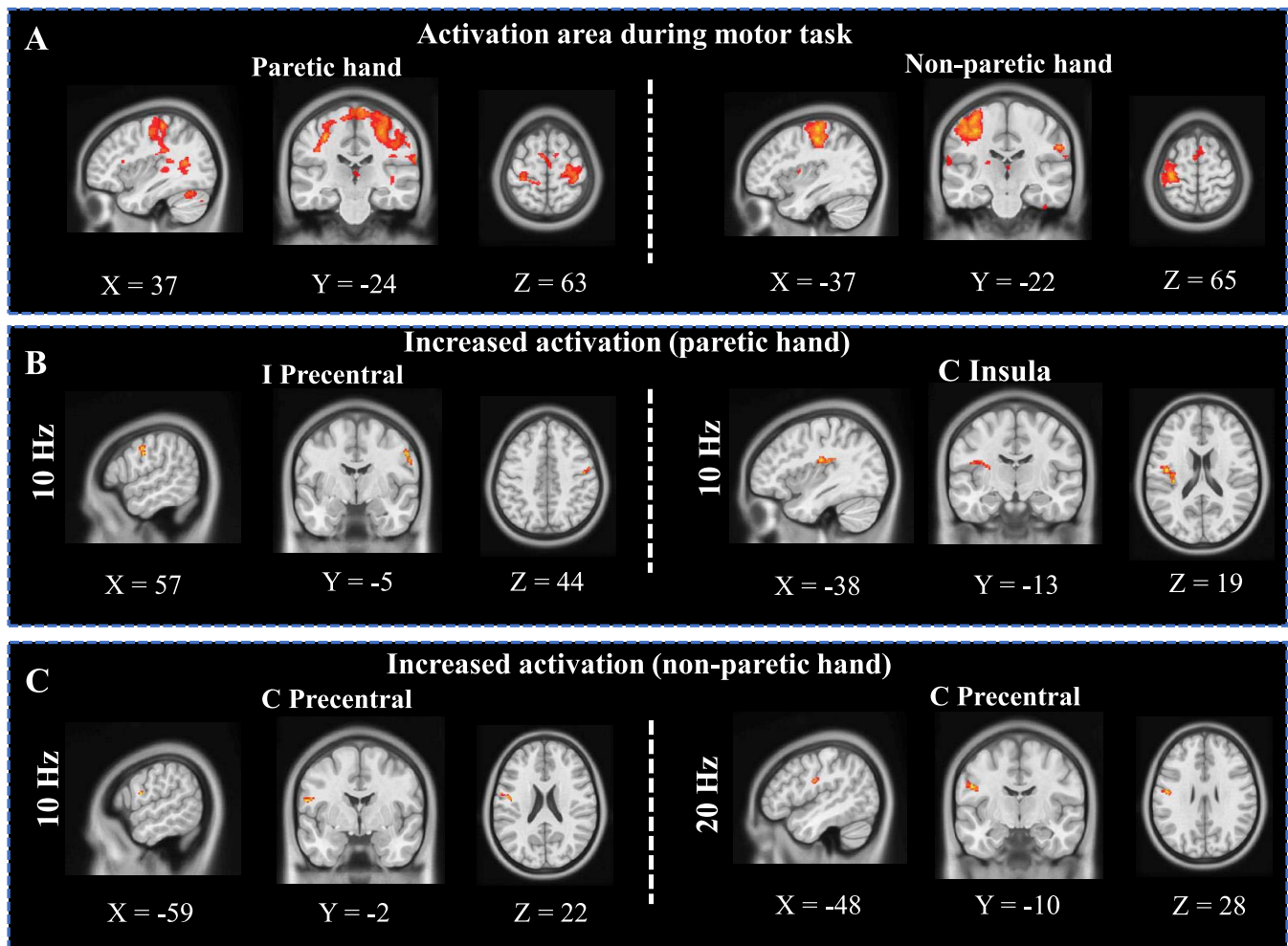


Fig. 2. (A) The left panel demonstrated the the activated area during paretic hand movements, and the right panel demonstrated the activated area during non-paretic hand movements. The number below indicated the coordinate in the MNI space. (B) For 10 Hz stimulation, significant increased activation was found in the ipsilesional precentral area with a MNI coordinate of (57, -5, 44) and contralesional insula (-38, -13, 19) during paretic hand movements. (C) In the left panel, significant increased activation was found in the contralesional precentral area (-59, -2, 22) during non-paretic hand movements after 10 Hz tACS; in the right panel, significant increased activation was found in the contralesional precentral area with a MNI coordinate of (-48, -10, 28) during non-paretic hand movements after 20 Hz tACS.

$p = 0.009$) was observed in posterior cingulate gyrus and precuneus. Bonferroni correction was used to correct the multiple comparisons.

C. Results of Graph Theory Analysis

The resulting group-level community structure for 20 Hz stimulation could be visualized in Fig.4(A). Regions in the same community were divided into the same red square, producing four different communities for each stage. The community structure at 10 Hz and sham stimulation showed similar outcomes, which could be found in the Supplementary Materials. $P - z$ plots in Fig.4(B) demonstrate the clear difference in P distribution between *Pre* and *Post* sessions for 20 Hz stimulation.

IV. DISCUSSION

The current study thoroughly explored the frequency-specific tACS effects in chronic stroke subjects. The results

showed that tACS might exhibit frequency-specific modulation in chronic stroke. 20 Hz tACS facilitated the functional interaction between the sensorimotor regions and brain regions involved in executive control, while 10 Hz and sham tACS had limited effect on motor-related brain activity. The findings in this study might shed light on the mechanism of the neural modulation process using tACS, which could further provide insights into stimulation protocol design for stroke.

Evidence has accumulated that the essential role of brain oscillations in various brain functions by affecting the coordination of neuronal spiking at the microscale, which would synchronize the involved distributed cortical regions at the macroscale [46], [47]. Some studies have proposed that the interaction between task-relevant regions is routed by functionally blocking off the pathways between task-irrelevant regions, which is reflected by oscillatory activities in the alpha band [20]. Beta oscillations depend on a balance

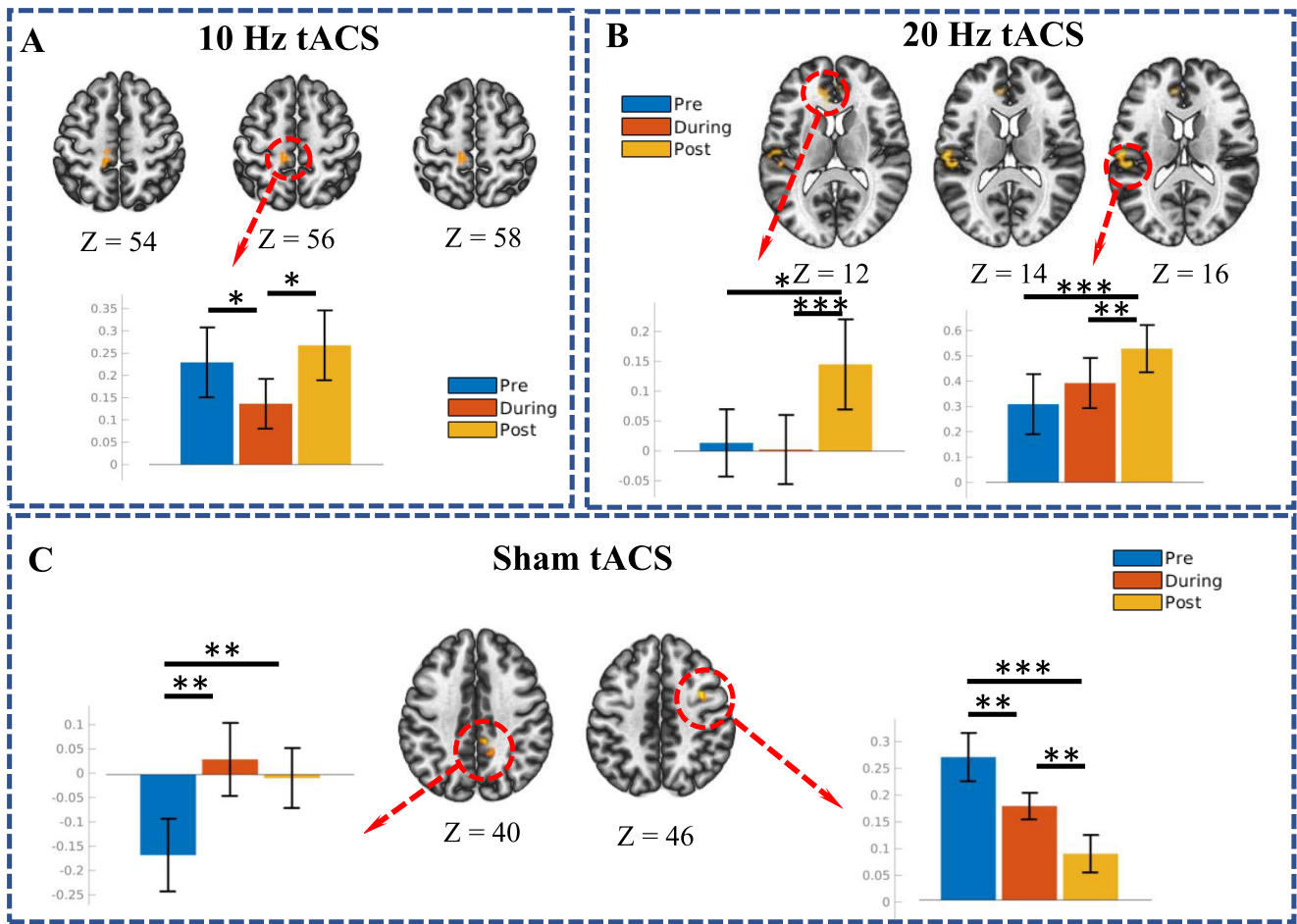


Fig. 3. (A) For 10 Hz tACS, the repeated-measure ANOVA with time (*Pre*, *During*, and *Post*) showed that significant modulated FC was found in contralesional central area ($-8, -24, 50$). The Bar plot demonstrated the FC changes in the three sessions. Error bars stand for standard deviation. Pair-wise comparisons between each pair of time points were carried out using paired *t*-tests. *** means $p < 0.001$, ** means $p < 0.01$, and * means $p < 0.05$ (B) For 20 Hz tACS, the repeated-measure ANOVA showed that significant modulated FC was found in contralesional central operculum cortex ($-60, -18, 14$) and anterior cingulate gyrus ($-8, 42, 14$). (C) For sham tACS, the repeated-measure ANOVA showed that significant modulated FC was found in ipsilesional central area ($36, 2, 48$) and another cluster in posterior cingulate gyrus and precuneus ($6, -36, 38$).

between inhibitory (GABAergic) and excitatory (glutamatergic) input [48]. One study exploring the causal contributions of alpha and beta oscillations in movement selection indicated that these two oscillations contribute independently to how oscillations orchestrate motor behavior. Furthermore, their findings highlighted the 10 Hz stimulation could potentially be adopted as a neurophysiologically grounded intervention to promote human performance, which might be utilized to facilitate rehabilitation for subjects suffering neural injuries [49]. Few studies have investigated the potential clinical utilities of brain stimulation techniques to boost the rehabilitation effects after neural injuries, especially for tACS [26]. Our study might give some insights into the mechanisms of how tACS manipulates the neural correlates of stroke brains.

In this study, significantly increased activation in the ipsilesional precentral area during the paretic hand movement was only found after 10 Hz stimulation, which indicated higher involvement of the ipsilesional motor region during the paretic hand movement. The increased activities in the ipsilesional

motor area were consistent with the findings of transcranial magnetic stimulation (TMS) studies [23], [50]. One study investigated the effects of tACS on cortical excitability for both young and old healthy subjects, and found that applying tACS at the individual alpha peak frequency (8-12 Hz) could increase cortical excitability in both groups [50]. Increased activation in the ipsilesional motor area was not found after 20 Hz tACS during the paretic hand movement in our study. Whereas several studies have exhibited increased motor evoked potentials during beta-tACS [51], and some have not found any effects [52]. A recent meta-analysis on tACS-TMS studies indicated that beta-tACS targeting at the primary motor cortex could boost the cortical excitability if the stimulation intensity is set to be above 1 mA [53], and the montage of the electrodes plays an important role. Our stimulation protocol met with the requirement of the stimulation intensity, the electrode placement and the inter-individual differences might also affect the outcome. We could not claim 20 Hz tACS was not effective in promoting cortical excitability in chronic stroke subjects based on our current findings. We also observed

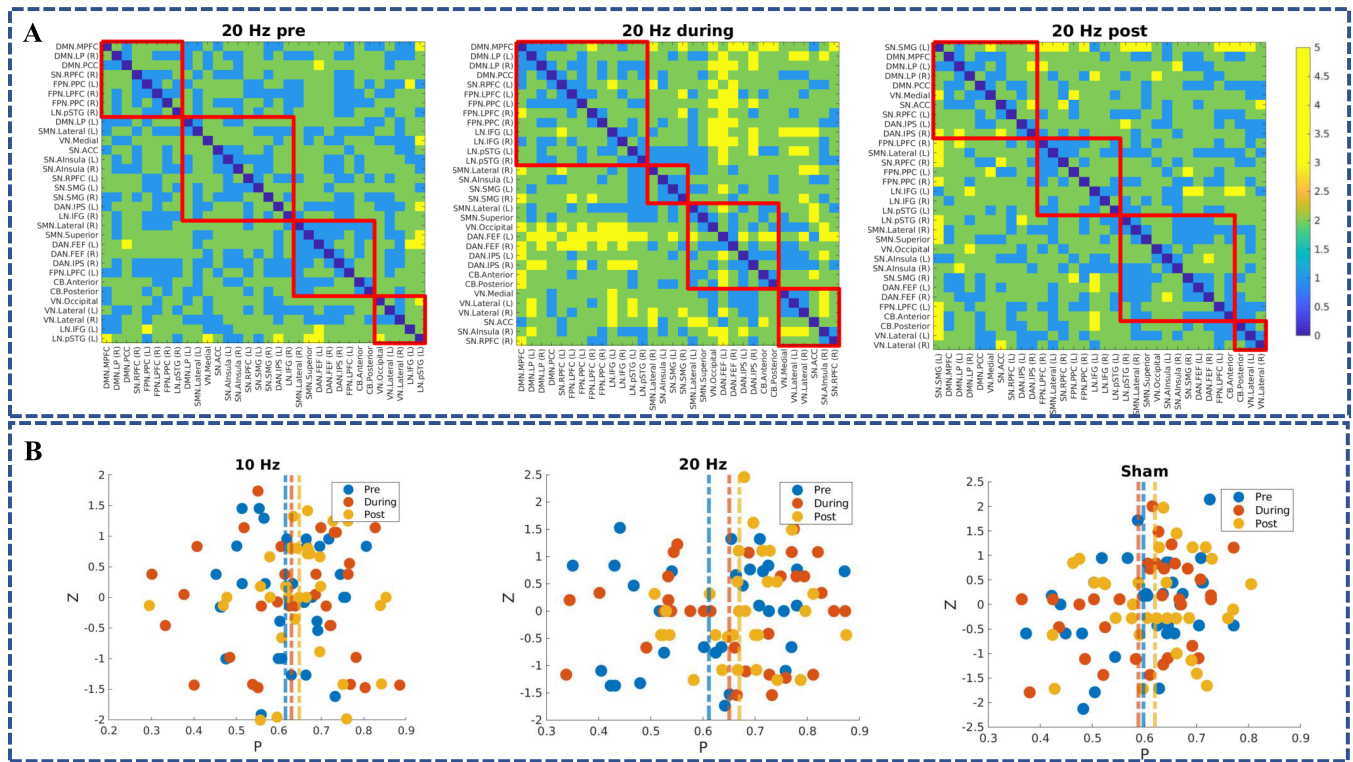


Fig. 4. (A) The group-level community structure for 20 Hz stimulation. The three matrices stand for the distance matrix of all the brain regions at *Pre*, *During*, and *Post* sessions respectively. The regions within each red square were divided into the same community. (B) The P – z plots of all the regions under different stimulation protocols (10 Hz, 20 Hz, Sham). Each point stands for one brain region.

increased activation in the contralesional motor area during the non-paretic hand movement for both 10 Hz and 20 Hz stimulation, which might indicate the interhemispheric effects [54].

Studies have already demonstrated that transcranial stimulation not only modulates the resting-state functional activities at the stimulation site but also in the whole-brain level [55], [56]. In this study, seed-based functional connectivity analysis exhibited modulated FC in the contralesional central area after 10 Hz stimulation. The stimulation effects in the contralesional central area were consistent with the motor task results, which showed increased activation in the contralesional central area during the non-paretic hand movements. These modulated regions were mainly located in motor-related areas. Seed-based functional connectivity analysis also showed that with modulated FC in contralesional central operculum cortex and anterior cingulate gyrus after 20 Hz stimulation. The anterior cingulate gyrus (ACC) is involved in various higher-level functions, such as attention, decision-making, and emotion [57]. The dorsal ACC integrates diverse task-relevant information and exerts high-level and abstract control over decision and action [58]. As compared with unimanual hand movements, the bimanual hand movements, which need higher demand of executive control, would induce higher activation in the ACC, indicating its modulatory effect on motor areas [59]. In a cued go/no-go task, the response preparation increased connectivity between dorsal ACC and sensorimotor regions to deal with the task execution [60]. Besides, the resting-state FC between dorsal ACC and sensorimotor regions are correlated

with the performance of error monitoring in flanker task, and greater error-related negativity (ERN) difference in amplitude was accompanied by greater functional coupling between these regions [61]. Based on the previous findings, we speculated that the increased FC with the ACC might indicate an improvement in task execution. The stimulation effects in the contralesional central area were also observed for 20 Hz stimulation, which was consistent with the motor task results. The modulated regions after 20 Hz stimulation located in widespread brain areas, not limited to motor-related areas. For the sham tACS, two significant clusters were observed, with one in the posterior cingulate gyrus and precuneus and the other in the ipsilesional central area. The effect of sham-stimulation is also observed by other neuroimaging studies [62], [63]. Further studies are required to thoroughly investigate the effect of sham stimulation. The graph theory analysis further demonstrated the functional interactions modulated by tACS. The within-module degree z score represents the role of a node in its own community, and the participation coefficient P represents each node's connection relative to the community structure of the entire graph [45]. From a functional point of view, the nodes with stronger interactions tend to be formed into the same community, which might act as a module that is responsible for similar topics [41]. Since the brain is a highly dynamic system, the network organization changes over time, especially with external stimulations. As is shown in our study, the community structures of the brain did not change a lot after stimulation. From the P - z plots, we could observe the modulation effects in the whole-brain functional interactions,

as indicated by the shifting of brain regions in the participation coefficient. Beta oscillations have been observed during many perceptual, cognitive, and motor processes in many brain recording studies [64]. Well-established links between oscillatory beta-activity and the sensorimotor system have been recognized, which could be observed at various levels [17], [18]. Cortico-muscular [17] and cortico-thalamic [65] beta-band coherence has been shown to be of functional relevance in the reciprocal communication within the motor system [17], [66] [67], [68]. Several studies have also suggested that beta oscillations might be more suitable with communicating across long conduction delays [69], [70]. The beta-band tACS might induce modulation effects from the motor-related areas, and to a greater extent, widespread brain regions. By contrast, alpha oscillations are known to be relevant for the engagement [19], [71], [72] and disengagement [73], [74], [20] of distant cortical areas that are task-relevant, thus indicating a more general role in cortical physiology [75], [76], [77]. With the general role of alpha oscillations, 10 Hz tACS only had limited effects on motor-related activities and had no effect in more widespread brain regions.

There are several limitations should be mentioned in our study. The stimulation site in this study was the primary motor area, however, the current montage might lead to the diffusion of the electric field. Therefore, high-definition tACS targeting at the primary motor cortex might be adopted in the future. The sample size of the current study was not very large. Studies with larger sample size should be conducted to investigate the brain alterations in subgroups with different residual motor function. Nevertheless, we have further analyzed the data excluding the 4 subjects with high residual motor function (ARAT > 50), which could be found in the Supplementary Materials. The existence of lesions in diverse locations and volume sizes could potentially affect the analysis results and stimulation outcomes. The inaccuracies in spatial normalization due to the lesions might affect voxel-based analysis (i.e., task-based fMRI analysis and seed-based FC analysis), and to a greater extent, the ROI-based analysis (i.e., graph theory analysis). The lesion might also affect the current flow in the brain [78]. To thoroughly investigate the impact of lesions, future studies need to be done to study how the lesion sites and volume sizes would affect the functional correlates of tACS. With the current electrode placement, a return electrode covering the contralateral supraorbital region might yield a flicker sensation, which might affect the quality of the blinding procedure. Behavioral data such as electromyography (EMG) are needed to provide supplementary information about the performance of motor tasks. Due to lack of behavioral data, we could not differentiate that the sparsely distributed activation pattern during the paretic hand movement is solely due to functional reorganization in the brain or due to the involvement of the non-paretic side, although the compensation effects during paretic hand movement are common and not avoidable [79].

V. CONCLUSION

The current study thoroughly explored the frequency-specific tACS effects in chronic stroke subjects using rs-fMRI

and task-based fMRI. The results showed that tACS might exhibit frequency-specific modulation in chronic stroke. 20 Hz tACS facilitated the functional interaction between the sensorimotor regions and brain regions involved in executive control, while 10 Hz and sham tACS had limited effect on motor-related brain activity. The findings in this study might shed light on the mechanism of the neural modulation process using tACS, which could further provide insights on stimulation protocol design for stroke.

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