Transcranial Alternating Current Stimulation Improves Memory Function in Alzheimer's Mice by Ameliorating Abnormal Gamma Oscillation

Linyan Wu, Wenlong Zhang, Sinan Li, Youjun Li, Ye Yuan, Liang Huang, Tiantian Cao, Liming Fan, Jiawen Chen, Jingyun Wang, Tian Liu, and Jue Wang

***Abstract*— Transcranial alternating current stimulation (tACS) is considered to have a positive effect on the rehabilitation of Alzheimer's disease (AD) as an intervention method that matches stimulation frequency to neurogenesis frequency. However, when tACS intervention is delivered to a single target, the current received by brain regions outside the target may be insufficient to trigger neural activity, compromising the effectiveness of stimulation. Therefore, it is worth studying how single-target tACS restores gamma-band activity in the whole hippocampal–prefrontal circuit during rehabilitation. We used Sim4Life software to conduct finite element methods (FEM) on the stimulation parameters to ensure that tACS intervened only in the right hippocampus (rHPC) and did not activate the left hippocampus (lHPC) or prefrontal cortex (PFC). We stimulated the rHPC by tACS for 21 days to improve the memory function of AD mice. We simultaneously recorded local field potentials (LFPs) in the rHP, lHPC and PFC and evaluated the neural rehabilitative effect of tACS stimulation with power spectral density (PSD), cross-frequency coupling (CFC) and Granger causality. Compared to the untreated group, the tACS group exhibited an increase in the Granger causality connection and CFC between the rHPC and PFC, a decrease in those between the lHPC and PFC, and enhanced performance on the Y-maze test. These results suggest that tACS may serve as a noninvasive method for Alzheimer's disease rehabilitation by ameliorating abnormal gamma oscillation in the hippocampal–prefrontal circuit.**

***Index Terms*— Alzheimer's disease, tACS, cross-frequency coupling, granger causality, Sim4Life.**

Manuscript received 18 September 2022; revised 13 January 2023 and 1 March 2023; accepted 4 April 2023. Date of publication 20 April 2023; date of current version 26 April 2023. This work was supported by the National Natural Science Foundation of China under Grant 31972907, Grant 61431012, and Grant U1913216. *(Corresponding authors: Tian Liu; Jue Wang.)*

This work involved animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Xi'an Jiaotong University's Animal Protection and Use Committee.

Linyan Wu, Wenlong Zhang, Sinan Li, Youjun Li, Ye Yuan, Liang Huang, Tiantian Cao, Liming Fan, Jiawen Chen, Jingyun Wang, and Tian Liu are with the Key Laboratory of Biomedical Information Engineering of Ministry of Education, the School of Life Science and Technology, and the Institute of Health and Rehabilitation Science, Xi'an Jiaotong University, Xi'an 710049, China, and also with the National Engineering Research Center for Healthcare Devices, Guangzhou 510500, China (e-mail: lonathe@foxmail.com; 2621628833@qq.com; lisinan@stu.xjtu.edu.cn; liyoujun1@mail.xjtu.edu.cn; yuanye.xjtu2020@gmail.com; hl2605033935@stu.xjtu.edu.cn; 1194157894@qq.com; flmxjtu@163.com; 1045730832@qq.com; tianliu@xjtu.edu.cn.

Jue Wang is with the Key Laboratory of Biomedical Information Engineering of Ministry of Education, the School of Life Science and Technology, and the Institute of Health and Rehabilitation Science, Xi'an Jiaotong University, Xi'an 710049, China, also with the National Engineering Research Center for Healthcare Devices, Guangzhou 510500, China, and also with the Key Laboratory of Neuro Informatics and Rehabilitation Engineering of Ministry of Civil Affairs, Xi'an 710049, China (e-mail: juewang_xjtu@126.com).

This article has supplementary downloadable material available at https://doi.org/10.1109/TNSRE.2023.3265378, provided by the authors.

Digital Object Identifier 10.1109/TNSRE.2023.3265378

I. Introduction

ALZHEIMER'S disease (AD) is a neurological disease that causes patients' cognitive and memory functions to deteriorate [1]. Local field potentials (LFPs) recordings have become more popular in recent years for studying the pathophysiology of AD and other neurodegenerative illnesses [2], [3]. Recording resting neuronal oscillations in the cortex and hippocampus is thought to be a promising method for identifying many neurological illnesses [4], [5], including Alzheimer's disease [6]. Researchers have discovered that patients with AD have aberrant electroencephalogram (EEG) rhythms that differ dramatically from those of healthy people [7]. However, since EEGs in clinical research are generally taken on the surface of the skin and cannot record from specific brain nuclei, animal experiments are commonly utilized to investigate the mechanism of AD. Studies have shown that the abnormal neural oscillation reported in the brains of Alzheimer's patients is consistent with experimental results from AD animal models [8].

The mouse brain, similar to that of humans, exhibits differences between the left and right hemispheres [9]. Both the left and right hemispheres of the brain contain the hippocampus, and both the hippocampus and the prefrontal cortex have been linked to cognitive performance [10], [11]. Relevant electrophysiological studies have shown that the left hippocampus (lHPC) and right hippocampus (rHPC) of mice play different roles in the short-term memory process. Compared with the left hippocampus, the right hippocampus significantly

promotes memory formation. Therefore, we consider tACS intervention on the right hippocampus to promote memory [12]. Given that AD impacts short-term memory, exploring whether there is a unique interaction between the hippocampus of different hemispheres and the prefrontal cortex (PFC) will be beneficial to understanding the disease's pathogenesis. For the memory functional circuit composed of the hippocampus and prefrontal cortex, there has been evidence that memory defects in AD are related to the interruption of the brain network [13]. In AD research, graph analysis based on EEG data shows that the functional connectivity between brain regions is reduced, especially the vulnerability of networks with small world attributes in the gamma band, and brain connectivity will change [14]. Using functional magnetic resonance imaging (fMRI), some researchers have recently discovered that there are differences in the faulty connections between the hippocampus and the prefrontal cortex in different hemispheres of patients with AD. The connection between the left hippocampus and the prefrontal cortex in AD is amplified, whereas the connections between the right hippocampus and the prefrontal cortex become weaker [15]. These findings suggest that anomalies between these regions may be a characteristic of AD research. This abnormal connection also motivates us to investigate whether LFPs in AD animal models have a similar link from an electrical standpoint.

Transcranial alternating current stimulation (tACS) is a new type of noninvasive neural intervention that uses stimulation frequency coupling to stimulate neurogenesis at the target site [16]. tACS is available at a variety of frequencies. The gamma frequency band is a critical frequency band that impacts memory function, according to recent studies, while irregular frequency oscillations in the gamma band of the EEG are a prominent symptom of AD [17]. As a result, gamma-tACS has been widely studied as a feasible tool for intervention in AD. Some researchers have found that gamma-tACS has a positive effect on AD; however, the mechanism is still unknown [18]. At present, small experiments have shown that tACS can improve the memory function of healthy adults by directly interacting with cortical activities [19]. For example, a crossover trial of 24 healthy adults showed that tACS significantly improved the retrieval accuracy of subjects [20]. However, scalp EEG is used in these clinical trials, and scalp EEG is only the projection of neuronal discharge on the scalp, and it cannot accurately describe neuronal discharge; therefore, it cannot effectively explain the intervention mechanism of tACS. On the other hand, since tACS is a noninvasive type of stimulation delivered close to the scalp or skull, there is a relatively clear effect on the skin, and usually it will only have the strongest stimulation effect at the position of the stimulation electrode. Therefore, selecting the tACS target is very important in AD intervention. Meanwhile, the study of how single target tACS affects the neural oscillation of the whole hippocampal–prefrontal circuit will also help us to understand the intervention mechanism of tACS. Due to the different roles of the left and right hippocampus in short-term memory function, we speculated that gamma tACS of the right hippocampus can have a positive result on all brain regions in the hippocampal–prefrontal circuit and studied the stimulation effect of tACS in the rHPC. As a result, this research has three goals: 1) to detect oscillatory activity between different hippocampi and the prefrontal cortex in Alzheimer's model mice; 2) to detect connectivity between different hippocampi and the prefrontal cortex in Alzheimer's model mice; and 3) to investigate the effects of tACS intervention on oscillatory activity and connectivity of these structures. We examined the connection of these structures by simultaneously recording LFPs in the left and right hippocampus and prefrontal cortex. Our results suggest that gamma tACS in the right hippocampus can significantly ameliorate the abnormal gamma oscillatory activity induced in the hippocampal–prefrontal circuit by AD, offering a potential treatment for this disease.

II. Materials and Methods

A. Animals

This study employed 15 male APP/PS1 transgenic mice with proven memory impairment and 5 common C57/BL6-Tg mice (all purchased from the Chinese Academy of Medical Sciences' Institute of Medical Laboratory Animals). The animals were kept on a 12/12-hour dark-light cycle (lights on at 7:00 a.m.) with ad libitum access to water and food to maintain body weight. Fifteen transgenic mice were divided into 3 groups of 5 mice each: the no-stimulation group, sham-stimulation group, and 21-day tACS group. 5 C57 mice were included in the C57 group. The Animal Ethics Committee of Xi'an Jiaotong University approved and supervised all procedures. The transgenic mice showed memory dysfunction at different times, while the rehabilitation effect of transgenic mice that were too old was poor. Therefore, we bought three APP/PS1 mice one month ahead of the experiment. When these three mice showed memory impairment in the Y maze, we began our experiment. An interval of one month was chosen because it is longer than our stimulation time.

B. Experimental Procedure

After acclimating to the environment, mice from the sham-stimulation group and 21-day tACS group were implanted with stimulating electrodes and then allowed to rest for 2 days. The mice in these two groups were then subjected to stimulation for 21 days. Fifteen APP/PS1 mice were implanted with acquisition electrodes immediately after stimulation. Subsequently, 5 C57 mice were also implanted with field potential acquisition electrodes made of Ni-Cr alloy. The mice were familiarized with the maze for 10 minutes per day for 4 days following implantation.

Four days later, the mice were subjected to the Y-maze behavioral tests. The electrophysiological signals of mice entering and exiting the central waiting area of the Y maze were recorded, and each mouse was tested 80 times. On the first day, the LFPs of the sham-stimulation group (5 mice) and 21-day tACS group (5 mice) were collected according to the sequence of stimulation in the previous 21 days. On the second day, the LFPs of the C57 group (5 mice) and the no-stimulation group (5 mice) were recorded. Figure 1 depicts the experimental procedure.

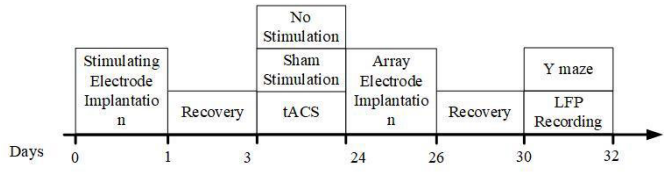

Fig. 1. The detailed timeline of this experiment.

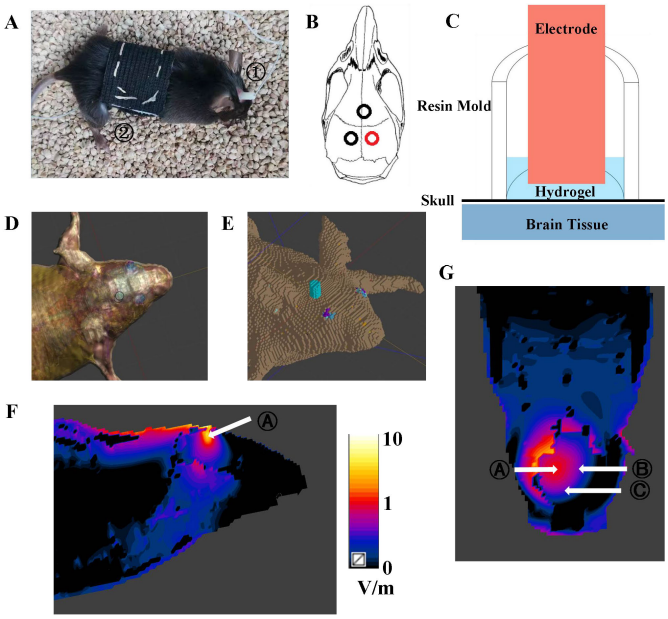

Fig. 2. tACS and the estimated current distribution. (A) Schematic diagram of mouse stimulation. 1 and 2 are two electrodes. (B) The red circle is the rHPC; the black circles are the lHPC and PFC. (C) tACS is delivered through the hydrogel in the resin tube. (D, E) Three-dimensional brain model, based on a mouse model constructed from MRI and Nissl histology. An electrode (shown as a cylinder) was placed in the right hippocampus of the model mouse. (F, G) Simulation diagram of stimulation effect; A is rHPC, B is lHPC, C is PFC.

C. Modeling Transcranial Alternating Current Stimulation to Target the Hippocampal–Prefrontal Circuit

To assess the effective parameter range for activation of the rHPC without activation of the lHPC or the PFC using tACS, we used FEM to simulate the distribution of electric fields in a three-dimensional mouse brain model. Our 3D mouse model is a detailed set of high-resolution anatomical animal models created from magnetic resonance and cryosection image data, consisting of 71 distinct tissues (Fig. 2 D, E). We focused on the head of the mouse model, assigned conductivity and relative permittivity (at 40 Hz [20]) to the head-related tissues and rendered the 3D model so that it contained a total of 189 × 178 × 496 voxels with a voxel resolution of ∼400 × 400 × 400 μm^3 (Table I). We performed quasi-electrostatic FEM simulations using the Sim4Life platform (ZurichMedTechAG) [21] to calculate current distributions in the brain model. The stimulation effect of tACS in the rHPC at 135 μA and 40 Hz and its effect on the lHPC and PFC were simulated. By simulation, we simulated right hippocampal tACS voltage field strength to determine the effectiveness of our stimulation parameters.

TABLE I
VARIOUS ORGANIZATIONAL PARAMETERS

Tissue	Permittivity	Conductivity
Blood vessel	280.0936	1.0968
Skull	53.7737	0.0828
Cerebellum	464.6029	0.3775
Cerebral hemisphere	247.6784	0.2251
Cerebrospinal fluid	108.5928	2.0022
Fat	29.5811	0.0526
Midbrain	247.6784	0.2251
Muscle	159.9523	0.6445
Nerve	155.0744	0.2229
Olfactory bulb	247.6784	0.2251
Skin	361.6703	0.1973

D. Transcranial Alternating Current Stimulation in APP/PS1 Mice

The animals were scalped with 2% (v/v) isoflurane oxygen anesthesia prior to tACS, and the right hippocampus location (AP = −2 mm, ML = 1.35 mm) was calculated using a stereotaxic device. The animals were fixed after anesthesia with 0.8% (v/v) isoflurane oxygen. The scalp was shaved and sterilized, and a hollow 3D printed mold was placed in the center of the skull immediately above the right hippocampus and covered with a dental base acrylic. One stimulating electrode was a printed mold filled with hydrogel, while another stimulation electrode was placed on the mouse's abdomen when it was in use (Figure 2 A, B, C). The specific stimulation process can be found in our previous reports. The following were the experimental parameters: frequency 40 Hz, amplitude 135 μA (signal generated and monitored by a RIGOLDG4202 waveform generator), stimulation for 20 minutes per day for 21 days, and the mice in the stimulation group were numbered to ensure that the stimulation sequence on each day was consistent. During the stimulation process, the oscilloscope monitors the stimulation current in real time to ensure stable stimulation. The APP/PS1 sham group and tACS group received the same anesthesia and procedures. In the sham group, 0.5 s stimulation with the identical settings of the tACS group was administered at the beginning and end of stimulation to simulate switching of stimulation. During the scheduled tACS treatment period, body weight was monitored once a day to ensure the safety of each tACS group. The duration of tACS was selected as 21 days because our previous research showed that 21 days of stimulation had a better effect on the recovery of memory function in AD mice than a shorter duration of tACS.

E. Surgery

Mice were immobilized in a stereotaxic device after being sedated with 2% isoflurane. To prevent infection, the animals were given penicillin before surgery. In the left hippocampus (AP = −2 mm, ML = −1.35 mm), right hippocampus (AP = −2 mm, ML = 1.35 mm), and prefrontal cortex (AP = 2 mm, ML = −0.2 mm), craniotomy was performed according to the stereotaxic map written by George Paxinos [22]. Three groups of 9 electrode wires (diameter: 50 μm, grouped in a 3 × 3 × 3 form; spacing between each microwire in one region: 250 μm; the electrode wire at the prefrontal

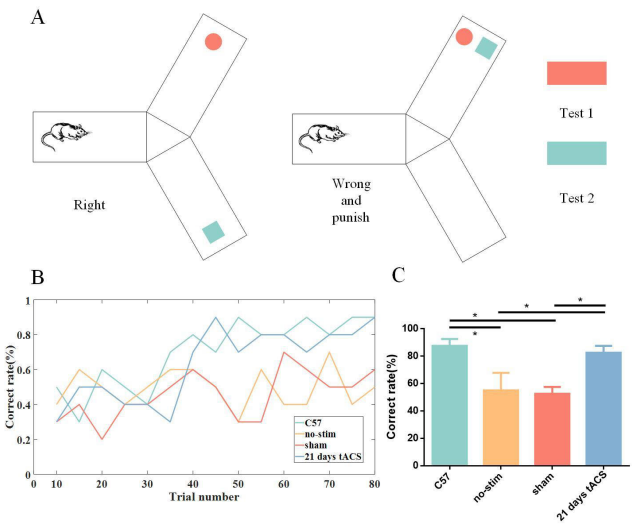

Fig. 3. Experimental test. (A) The schematic graph of Y-maze training. (B) The correct rate in the learning and memory stage. (C) The mean correct rate in the last 20 trials.

cortex was 200 μm longer than that at the hippocampus) were slowly and simultaneously placed on the lHPC, rHPC and PFC. The electrode was then lowered to a predefined depth (DV = 1.3 mm for the hippocampus, DV = 1.5 mm for the prefrontal cortex). A screw in contact with the dura mater was implanted as a ground in the cerebellum at the same time, and the screw and array electrodes were secured to the skull with dental cement. After implantation, the entire electrode was enclosed by copper mesh, and screws were linked to the copper mesh to decrease external electromagnetic interference.

F. Behavioral Tests

We used the Y-maze to study short-term memory in mice. The Y-maze is made of a clear acrylic sheet. It consists of 3 arms, each 50 cm long, 10 cm wide and 25 cm high. We specifically prepared the learning process in the maze to improve the learning process and generate electrophysiological signals that are more indicative of the short-term memory process. First, the mouse was placed in any arm of the Y maze during each trial. There were two tests in each trial. For two successive tests, the mouse had to go to different arms. If the mouse started in arm A and moved to arm B the first time, the second time it had to move to arm C and not arm B, as shown in Figure 3(A). If the mouse made an incorrect choice, it was punished with 15 V pulsed electrical stimulation. After each trial, the equipment was carefully cleaned with a towel containing 70% ethanol. This was done to prevent the exploratory behavior of other mice from being influenced by olfactory stimuli produced by previous mice. The setting of the behavior experiment is based on Komorowski's experimental setting [23]. Meanwhile, the number of experiments is selected as 90 according to the research of torts and our experience in the experiment [24]. The continuous 90 experiments are regarded as the memory process of mice.

G. Electrophysiological Recording

The mouse LFPs were recorded using a 64-channel data acquisition system (Cerebus, Blackrock Microsystems, Salt Lake City, USA). LFPs were captured at a 10 kHz sampling rate, amplified ($\times$300), and bandpass filtered (0.3–500 Hz).

H. Data Analysis

The LFP data collected in the selected state of the maze were analyzed using NeuroExplorer software, and data with severe interference during movement were removed. Power spectral density, cross-frequency coupling and Granger causality of LFPs were calculated from 5 s epochs without notable artifacts when mice made decisions in the Y-maze. The data with the least interference from the three electrodes in each brain region were chosen as the field potential data of the brain region for each mouse in each behavioral condition.

The power spectra density was computed using the MATLAB toolbox Chronux (http://chronux.org) using multitaper spectral estimation. Multiplying the time series with multiple tapers achieves an optimal balance between spectral resolution and spectral variation and effectively prevents the spectral leakage phenomenon of common spectral analysis and wavelet analysis. Therefore, the results are more detailed and realistic [25], [26]. The calculated parameter values are as follows: params.fpass = [0 48] (the frequency range of interest is 0-48 Hz; because 50 Hz power frequency interference will inevitably be encountered during the acquisition process, the data were not processed at 50 Hz) and params.tapers = [3 5] (the best spectral smoothness at the time bandwidth product of 3 and 5 tapers).

Memory function is evaluated using CFC, which is a measurement of the interaction between LFPs in multiple frequency bands [27]. The intervention had a gamma frequency band and was placed at the right hippocampus. As a result, we examined the link between the hippocampus's gamma-band phase and the prefrontal cortex's theta-band amplitude. A 30-45 Hz filter filters the gamma band, while an 8-12 Hz filter filters the theta band. According to Belluscio, the average amplitude of each phase bin is calculated using a composite time series consisting of instantaneous phase and amplitude values [28]. The modulation index (MI) was then used to determine the intensity of the phase–amplitude coupling between the theta amplitude and gamma phase.

We computed Granger causality in the frequency domain to further investigate the causal linkages between lHPC, rHPC and PFC and the direction of information flow between these structures. We want to look at the information flow between three regions, but typical Granger causal analysis can only look at flow between two regions. As a result, we apply multivariate Granger causality (MVGC) to analyze our data [29], [30]. The first stage is to estimate the model order using the Akaike information criterion (AIC) to select the optimum model order. Next, the Levinson Wiggins Robinson (LWR) algorithm is used to estimate the vector autoregression (VAR) model using the selected model order, and the autocovariance sequence is generated from the VAR model. Finally, the autocovariance sequence is used to compute partial Granger causality in the frequency domain.

I. Statistics

Learning curves depict the behavioral consequences of mice. The learning curve is a line graph created by computing the average accuracy throughout 10 tests. Each window's accuracy is determined using a sliding window with a total length of 10 and a stride of 5. A bar chart represents the accuracy rate of the last 20 experiments to examine the learning results of the mice in the maze.

The mean PSD of the collected LFPs at 30-45 Hz, the mean value of Granger causal analysis at 30-45 Hz, and the MI produced by CFC were all subjected to homogeneity of variance analysis. After determining homogeneity of variance, one-way ANOVA and the least significant difference (LSD) post hoc test were used. Normal mice, Alzheimer's mice, sham-stimulated mice, and 21-day tACS mice were studied for differences in gamma-band power, gamma–theta phase–amplitude coupling, and Granger causality. The significance level was set at 0.05.

III. Results

A. Modeled tACS in the Hippocampus and Prefrontal Cortex

We first confirmed through simulation that tACS can fully activate the right hippocampus of mice (Figure 2 F). Simulations show a strong electric field (>10 V/m) in the rHPC when electrodes are placed on the skull surface above the rHPC. Since the current comes from the electrode, the strongest electric field occurs in the cortical region closest to the electrode. The electric field strength gradually decreases deep into the brain. However, due to the presence of cerebrospinal fluid, skin and other tissues, the current will flow to the tissues with low impedance, and there is also a relatively large field strength in these tissues. Since the right hippocampus is close to the electrodes, it receives a relatively strong electric field. As the distance from the electrode increases, the electric field received by other brain regions will attenuate rapidly (Figure 2 G). The left hippocampus and prefrontal cortex can only receive an electric field below 1 V/m under a tACS of 135 μA. According to the literature [31], the threshold of field strength activated in the brain region is 1 mv/mm. Therefore, we can see that under current stimulation of 135 μA, only the right hippocampus is activated, while the left hippocampus and prefrontal cortex are not activated. In this case, we can explore how inactive regions of the hippocampal–prefrontal circuit (lHPC, PFC) are affected by active regions (rHPC) when using tACS to rehabilitate AD mice.

B. Behavioral Improvement After Gamma Transcranial Alternating Current Stimulation

Figure 3 (B, C) displays the behavior of various mouse groups, and each group had 5 mice. We established a goal of achieving 80% selection accuracy in the maze 20 consecutive times as the mice learned the task. After 80 experimental trials, the normal mouse group (correct rate = 87.5%) and the 21-day tACS group (correct rate = 82.5%) were able to complete the learning task, whereas the no-stimulation group (correct rate = 55%) and the sham-stimulation group (correct rate = 52.5%) were still unable to complete the task.

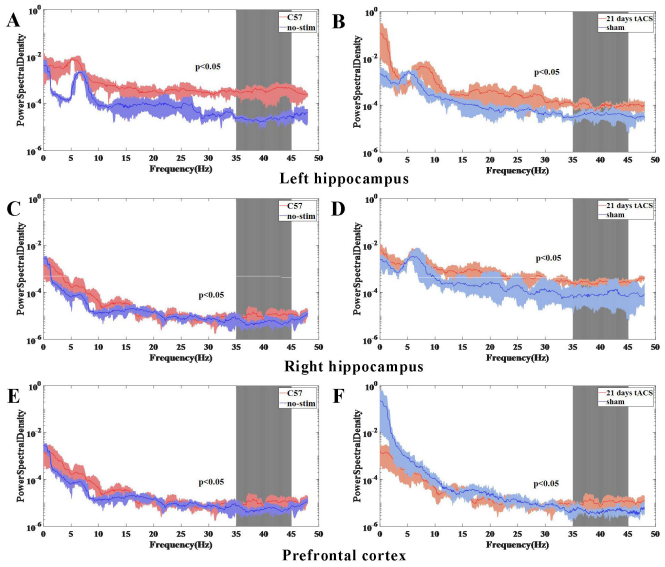

Fig. 4. Power spectral density of LFPs recorded in the left hippocampus, right hippocampus, and prefrontal cortex. (A)(B) is the power spectrum of the left hippocampus of 4 groups of mice, 5 mice in each group. (C)(D) is the power spectrum of the right hippocampus of the 4 groups of mice, while (E)(F) is the power spectrum of the prefrontal cortex of the 4 groups of mice. (A, C, E) AD mice compared with C57 mice; power in the gamma band (35-45 Hz) was significantly decreased in these three regions. (B, D, F) AD mice after tACS compared with sham stimulation mice; power was significantly increased in these three regions. Data are presented as the mean ± standard error of the mean (SEM). The shaded area depicts the frequency range of significant group differences.

C. Local Field Potential Power Changes After Gamma Gamma Transcranial Alternating Current Stimulation

We calculated the PSD of each brain area by recording LFPs from the left hippocampus, right hippocampus, and prefrontal cortex during selection in the Y-maze. There were 5 mice in each group, and we used one-way ANOVA for different groups. The gamma band LFP power in all three areas of the Alzheimer's mouse group was considerably lower than that of normal mice during the short-term memory process (Fig. 4) ($p = 0.007$). In addition, LFPs in the lHPC and rHPC had a peak in the theta band power spectrum. Compared with AD mice, the frequency band of the left hippocampus-evoked peak was higher in normal mice, while the frequency band of the right hippocampus-evoked peak was lower. After tACS, the gamma band LFP power of AD mice were restored, as was the peak of theta band LFPs in the lHPC and rHPC. At the same time, while the prefrontal cortex's PSD improved in the gamma frequency band, the PSD in the low frequency band dropped.

D. Gamma–Theta Phase–Amplitude Coupling Changed After Gamma Transcranial Alternating Current Stimulation

Because the gamma-band LFP and theta-band LFP of mice in different groups are different, we then analyzed the results of theta-gamma CFC of mice in different groups. There were 5 mice in each group, and we used one-way ANOVA for different groups. In Alzheimer's mice, CFC research revealed that the left hippocampal gamma-band and prefrontal theta-band

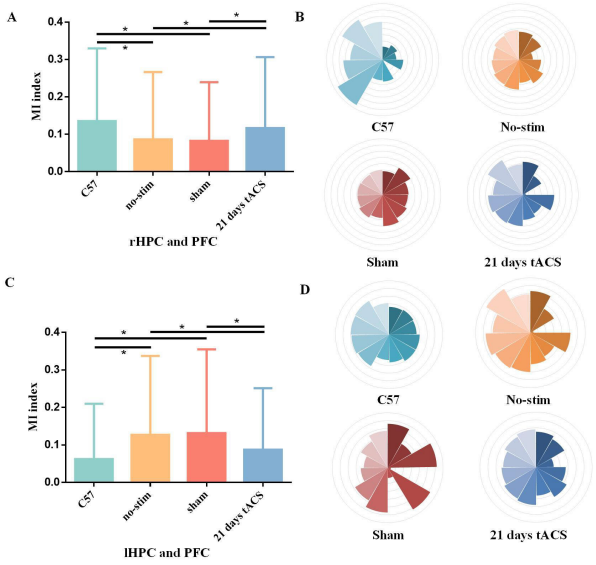

Fig. 5. Cross-frequency coupling between the right hippocampus (rHPC) and prefrontal cortex (PFC) (A, B) and between the left hippocampus (lHPC) and prefrontal cortex (C, D) in each group. (A) The MI index of rHPC-PFC was decreased in the frequency ranges of 4-8 Hz in the rHPC and 30-45 Hz in the PFC. (B) Phase amplitude nightingale rose chart between the right hippocampus and prefrontal cortex. The 360° phase at the prefrontal cortex is represented as a circle in polar coordinates, and the radius of the concentric circle is the amplitude at the hippocampus. The more regular the circle is, the smaller the CFC is; the more irregular the circle is, the larger the CFC is. (C) The MI index of lHPC-PFC was increased at this frequency. (D) Phase amplitude nightingale rose chart between the left hippocampus and prefrontal cortex. Data are depicted as the mean ± SEM. The shaded area depicts the frequency range of significant group differences.

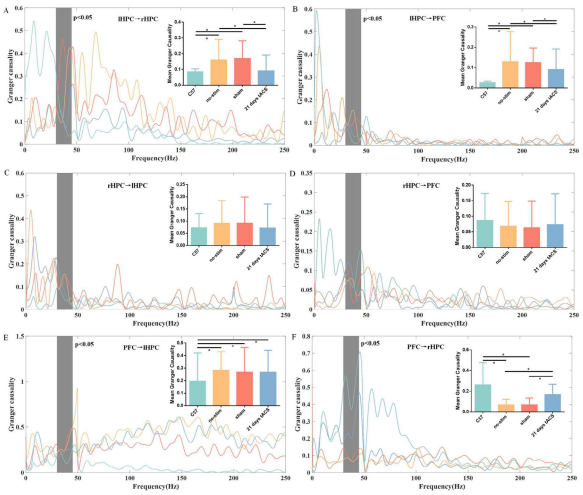

Fig. 6. Granger causality in the frequency domain and gamma causality between pairs of structures. (A, B) Gamma Granger causality was significantly higher in lHPC → rHPC and lHPC → PFC in AD mice than in C57 mice, and tACS decreased this higher causality. (C, D) No significant group differences were observed in rHPC → lHPC and rHPC → PFC. (E) Gamma Granger causality was significantly higher in PFC → lHPC in AD mice than in C57 mice, but tACS had no effect. (F) Gamma causality was significantly lower in PFC → rHPC in AD mice than in C57 mice, and tACS increased this lower causality. Linear graphs (left in each graph) show averaged Granger causality from 1 to 250 Hz for the four groups. Data for Granger causality in the gamma band (right in each graph) are depicted as histograms. The shaded area depicts the frequency range of significant group differences.

coupling strength rose (from 0.062±0.147 to 0.127±0.21, $p = 0.008$), whereas the right hippocampal gamma-band and prefrontal theta-band coupling strength dropped (from 0.135±0.194 to 0.086±0.18, $p = 0.017$). The aberrant coupling between the left hippocampal gamma band and the prefrontal theta band was improved after tACS (from 0.127±0.21 to 0.087±0.163, $p = 0.029$), while the coupling strength between the right hippocampal gamma band and the prefrontal theta band increased (from 0.086±0.18 to 0.117±0.189, $p = 0.025$) (Fig. 5). The alterations remained significant ($p < 0.05$) even if they did not recover to the level of normal mice.

E. Granger Causal Analysis of Gamma Band Oscillations Changed After Gamma Transcranial Alternating Current Stimulation

After analyzing the PSD and CFC, we discovered that tACS improved gamma-band LFPs in three brain areas of different groups of mice. There were 5 mice in each group, and we used one-way ANOVA for different groups. To further investigate the functional connectivity of the three brain areas across the gamma band (30–45 Hz, based on CFC results) before and after tACS, we employed MVGC analysis. There were changes in these three regions in AD mice, similar to the CFC results (Fig. 6). The flow from the left hippocampus to the prefrontal cortex was increased in AD mice (from 0.026±0.117 to 0.129±0.149, $p = 0.011$), whereas the flow from the prefrontal cortex to the right hippocampus was decreased (from 0.26±0.171 to 0.068±0.054) ($p = 0.004$). These two connections were still significantly different from AD mice after tACS, but they were also improved compared to the no-stimulation group (lHPC→PFC: from 0.129±0.149 to 0.091±0.101, $p = 0.031$, PFC→rHPC: from 0.068±0.054 to 0.165±0.101, $p = 0.023$) ($p < 0.05$). Furthermore, in AD mice, the flow from the left to the right hippocampus was larger than that in normal mice (from 0.087±0.098 to 0.16±0.131, $p = 0.017$), and tACS improved this aberrant flow (from 0.16±0.131 to 0.09 ±0.101, $p = 0.025$). Furthermore, in AD mice, the flow direction from the prefrontal lobe to the left hippocampus was increased (from 0.195±0.164 to 0.283±0.147, $p = 0.009$), but tACS had no effect on this anomalous flow direction.

IV. Discussion

In our experiment, we found that c57 mice can achieve 90% accuracy in Y maze. Because the symptoms of AD mice, as a neurodegenerative disease, can only be improved rather than completely recovered to the same level as that of ordinary mice, we chose 80% of the maze accuracy rate as the criterion to judge whether the memory function of AD mice recovered. After the experiment, we found that the correct rate of behavior of AD mice could reach 80% after 21 days tACS intervention, so we think that the behavior of AD mice was restored by 21 days tACS. The memory function of AD mice improved after tACS intervention. During the short-term memory exercise in the Y-maze, we recorded LFPs in the left and right hippocampus as well as the prefrontal

cortex of normal mice, Alzheimer's mice, and tACS-treated Alzheimer's mice. We found that abnormal gamma oscillations are the cause of memory dysfunction in AD mice, and tACS can improve these abnormal gamma oscillations to improve the memory function of AD mice. Three primary discoveries emerged from the analysis of PSD, CFC, and Granger causality of LFPs: 1) After tACS intervention, gamma-band oscillations were elevated in all three regions of the maze in mice; 2) Compared with C57 mice, the CFC between the right hippocampus and the prefrontal cortex of AD mice decreased, while the CFC between the left hippocampus and the prefrontal cortex increased, and tACS could improve this abnormal coupling phenomenon; 3) In Alzheimer's mice, the abnormal information flow direction of the oscillations is lHPC→PFC, lHPC→rHPC, PFC→lHPC, and PFC→rHPC, and tACS could improve this abnormal coupling phenomenon in lHPC→PFC, lHPC→rHPC, and PFC→rHPC.

The specific frequency ranges of the aberrant gamma band oscillations found in AD are currently unknown across diverse animal trials and AD patients. Some studies looked at the relationship between the low band (30-60 Hz) and AD [32], [33], whereas others looked at the upper gamma band (60-120 Hz) [18], [34]. Researchers focus on the range from low gamma stimulation to high gamma stimulation, but there is no consensus on the tACS frequency band that leads to successful intervention. Studies have demonstrated that not only 40 Hz electrical stimulation but also 40 Hz acoustic stimulation and light stimulation can help with AD [35], [36], [37], and 40 Hz may be a critical frequency. Thus, we chose 40 Hz tACS to treat AD mice. Researchers have focused more on the functional distinctions between the left and right hippocampus in recent years, and some studies have found that the functions of the left and right hippocampus in memory are different [38], [39]. Compared with the left hippocampus, the right hippocampus more clearly promotes memory formation [40]. As a result, we chose the Y-maze to assess short-term memory function in AD mice, and we discussed whether activating the right hippocampus could aid in the creation of short-term memory. Furthermore, the prefrontal cortex is involved in memory function. A number of fMRI studies have indicated that in Alzheimer's patients, the connection between the prefrontal lobe and the hippocampus is aberrant [41], [42]. fMRI is an indirect way to analyze neurogenesis. Clinically, it is difficult to use invasive methods to collect nerve signals in the brain. However, because animal studies are not limited in this way, we looked at the aberrant phase–amplitude coupling and functional link between the prefrontal cortex and hippocampus from the standpoint of animal field potential and sought to determine how tACS ameliorated this anomaly.

A. Gamma Transcranial Alternating Current Stimulation Can Entrain Gamma Band Oscillations

The LFPs of AD mice were frequently weakened in the gamma band, which could be one of the explanations for the cognitive impairment of AD mice. This viewpoint has been supported by numerous investigations [43]. The gamma-band PSDs of the lHPC, rHPC and PFC were significantly increased after tACS of the right hippocampus, prompting us to investigate how the intervention affected the connection between the three regions to produce this phenomenon. After tACS, the peak of the theta band in the lHPC and rHPC of AD mice returned to the level of normal mice, indicating that tACS can induce electrical signals in the theta band and gamma band of the hippocampus. In our previous study, we calculated the CFC coefficient between theta and gamma bands of the hippocampus, and the positive results were consistent with this experiment. However, after stimulating the hippocampus, the power of the prefrontal cortex dropped in the theta band. This abnormal phenomenon prompted us to investigate the interaction between the hippocampus and prefrontal cortex from the standpoint of theta-gamma CFC.

B. Gamma Transcranial Alternating Current Stimulation Can Activate Hippocampal–Prefrontal Coupling

The communication between the left and right hippocampus and prefrontal cortex is shown in quantitative analysis employing CFC, which can effectively reflect functional impairment of connectivity [44]. Cognitive functional abnormalities in neurodegenerative disorders are thought to be caused by abnormal couplings between these regions [45]. In fact, we found that in AD mice, the CFC coefficient (MI index) in the lHPC-PFC and rHPC-PFC was abnormal, with increased coupling between the left hippocampus and the prefrontal cortex and decreased coupling between the right hippocampus and the prefrontal cortex. Given that the short-term memory function of AD mice has been demonstrated to be impaired in earlier studies, it is concluded that the neurons in the right hippocampus rather than in the left hippocampus have a greater positive effect on the formation of short-term memory [46]. Since the hippocampus is anatomically a whole, we believe that the coupling between the bilateral hippocampus and the prefrontal cortex should have been in a balanced state. This balance was broken in Alzheimer's mice, resulting in high coupling between the left hippocampus and the prefrontal cortex. This situation makes the coupling between the right hippocampus and prefrontal cortex unable to compete with the high coupling between the left hippocampus and prefrontal cortex, thus showing a state of low coupling. According to the results of CFC, we speculate that the power spectrum of the prefrontal cortex is modulated by the CFC of the hippocampus, and the abnormal coupling between the bilateral hippocampus and prefrontal cortex finally leads to a decrease in the gamma power of the prefrontal cortex. After gamma tACS of the right hippocampus, the coupling between the right hippocampus and prefrontal cortex was activated, resulting in a decrease in the coupling between the left hippocampus and prefrontal cortex. The coupling between the hippocampus and prefrontal cortex then returned to normal, and the whole hippocampal–prefrontal gamma circuit was reconstructed. The enhanced coupling between the right hippocampus and the prefrontal cortex enhances the gamma power spectrum of the prefrontal cortex and weakens the theta power spectrum of the prefrontal cortex, which is consistent with our experimental results.

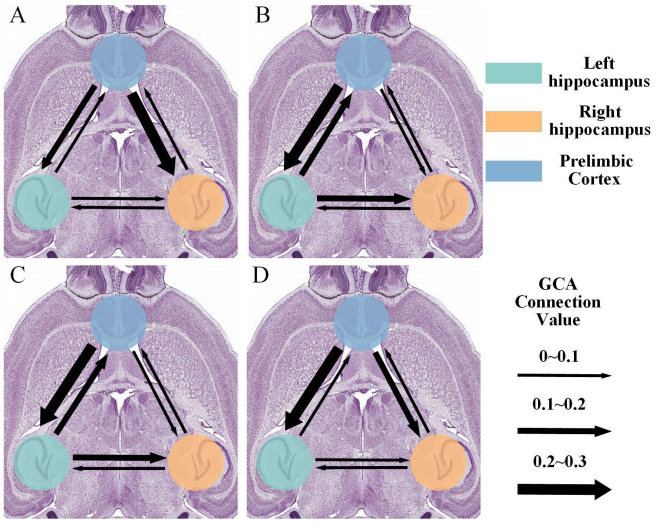

Fig. 7. The network of gamma activity in the left hippocampus, right hippocampus, and prefrontal cortex. Arrows show the directions of information flow for gamma band oscillations among the three structures. (A) C57 group. (B) no-stim group. (C) Sham group. (D) 21-day tACS group.

C. Gamma Transcranial Alternating Current Stimulation Can Reconstruct the Hippocampal-Prefrontal Gamma Circuit

Cross-frequency coupling research in Alzheimer's mice indicated an aberrant connection between the hippocampus and prefrontal cortex in gamma oscillation. Using Granger causality analysis, we determined whether this aberrant effect was one-way or two-way. In AD mice, we discovered that the connections between the left hippocampus and the prefrontal cortex and between the left hippocampus and the right hippocampus were abnormally increased, and the connection from the prefrontal cortex to the right hippocampus was abnormally decreased, while the connections in other directions remained unchanged (Fig. 7). After comparing the results of CFC and Granger causality analysis, we found that the strong coupling between the lHPC and PFC is related to the strong connection between them, while the weakening coupling between the rHPC and PFC is affected by the strong connection from the lHPC to the rHPC and the weak connection from the PFC to the rHPC. We discovered that tACS in the right hippocampus could reconstruct the Granger causal connection in the hippocampal–prefrontal circuit: the weak connection between the PFC and rHPC was strengthened, while the strong connection between the lHPC, rHPC and PFC was weakened. Because the location of our stimulation was in the right hippocampus rather than the left hippocampus, the connection from the HPC to the lHPC remained unchanged following tACS. In conclusion, tACS in the right hippocampus reconstructs the Granger causality in the hippocampal–prefrontal circuit, which is consistent with the CFC changes between the prefrontal cortex and the hippocampus. tACS in the hippocampus not only restored the balance of the left and right hippocampus in the hippocampal–prefrontal circuit but also modulated the power spectrum of the prefrontal cortex through cross frequency coupling and finally realized the reconstruction of the gamma band pathway in the hippocampal–prefrontal circuit. This reconstruction can restore the abnormal memory function of Alzheimer's mice.

Our data imply that tACS can activate gamma oscillation in the right hippocampus and restore the gamma-band activity of the hippocampal–prefrontal circuit. Because the firing rate of parvalbumin-expressing interneurons influences the power of gamma oscillations, gamma power can be considered to represent parvalbumin cell activity [47], [48]. In Alzheimer's mice, we can speculate that gamma tACS could help reestablish transhemispheric gamma synchronization between prefrontal and hippocampal parvalbumin cell interneurons. In addition, other frequency bands of oscillatory activity, including alpha and theta, have also been identified in patients with AD [49], [50]. In this study, although the power spectrum of Alzheimer's mice and ordinary mice was clearly different when looking at the whole frequency band, since our stimulation frequency band was gamma, we only focused on the performance of the hippocampal gamma band. Therefore, the performance of tACS in other bands of electrophysiology needs further investigation. In addition, due to the different weights of the bilateral hippocampus in the formation of memory function, to compare with the right hippocampus tACS, it is worth studying how tACS of the left hippocampus can reconstruct the gamma pathway in the hippocampal–prefrontal circuit.

V. Conclusion

We observed aberrant gamma oscillations in the left and right hippocampus, as well as the prefrontal cortex, during short-term memory in APP/PS1 transgenic Alzheimer's mice and discussed the effect and mechanism of gamma tACS in Alzheimer's mice. Our findings imply that attenuation of field potentials and aberrant connection between the hippocampus and the prefrontal cortex contribute to the deterioration in short-term memory performance in Alzheimer's mice, which can be alleviated by gamma tACS. Gamma tACS in the right hippocampus not only reconstructed the gamma oscillation in the stimulation region but also balanced the gamma oscillation in the left hippocampus, thus modulating the gamma oscillation in the prefrontal cortex and reconstructing the gamma power spectrum of the whole hippocampal–prefrontal circuit, thus restoring the memory function of AD model mice. We found that gamma tACS can significantly improve the abnormal gamma oscillation activity caused by AD and may be a potential treatment for this condition.

Acknowledgment

The authors would like to thank Sim4Life by ZMT (www.zmt.swiss) for assistance with model simulation.

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