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RESEARCH ARTICLE

TRanscranial AlterNating current Stimulation FOR patients with Mild Alzheimer's Disease (TRANSFORM-AD study): Protocol for a randomized controlled clinical trial

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Abstract

Introduction: Recently, transcranial alternating current stimulation (tACS), which can interact with ongoing neuronal activity, has emerged as a potentially effective and promising treatment for Alzheimer's disease (AD), and the 40 Hz gamma frequency was suggested as a suitable stimulation frequency for AD.

Methods: The TRANSFORM-AD study is a double-blind, randomized-controlled trial that will include 40 individuals with mild AD. Eligible patients need to have amyloid β (Aβ) loads examined by Pittsburgh compound B (PiB) positron emission tomography (PET) or decreased Aβ level in cerebrospinal fluid. Participants will be randomized into either a 40 Hz tACS group or a sham stimulation group. Both groups will undergo 30 one-hour sessions across 3 weeks (21 days). The outcome measures will be assessed at baseline, at the end of the intervention, and 3 months after the first session. The primary outcome is global cognitive function, assessed by the 11-item cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog), and the secondary outcomes include changes in other neuropsychological assessments and in PiB-PET, structural magnetic resonance imaging (MRI), resting electroencephalography (EEG), and simultaneous EEG–functional MRI (EEG-fMRI) results.

Results: The trial is currently ongoing, and it is anticipated that recruitment will be completed in June 2021.

Discussion: This trial will evaluate the efficacy and safety of 40 Hz tACS in patients with AD, and further explore the potential mechanisms by analyzing amyloid deposits using

PiB-PET, brain volume and white matter integrity by structural MRI, and neural activity by EEG and EEG-fMRI.

KEYWORDS

Alzheimer's disease, gamma frequency, randomized controlled clinical trial, transcranial alternating current stimulation

1 | INTRODUCTION

Alzheimer's disease (AD) is incurable and increasing in prevalence, and has become one of the largest global health challenges. It is well known that the amyloid β (Aβ) protein deposition is the major pathological feature of AD. However, as a complex disease, multiple pathophysiological mechanisms participate and interact with each other in the development of AD.[1] The Aβ deposition induces and in the meantime could be aggravated by synaptic depression and neural activity and network abnormalities. The default network with aberrant neural activity is susceptible to Aβ accumulation.[2] The network activity impairments were found to be an early pathogenic change in AD, including hyperexcitability and spontaneous epileptiform activity, and the previous study suggested that network hypersynchrony occurred during reduced gamma rhythm activity.[3]

Previous studies have shown reduced activity of gamma oscillations in hippocampus and entorhinal cortex in AD transgenic models,[4,5] which are key regions involved in the formation of declarative memory. Theta and gamma oscillations are the predominant activities in the entorhinal-hippocampal circuit in healthy brains. The gamma synchronization and theta-gamma cross-frequency coupling are closely related to memory encoding and retrieval. The abnormality of gamma oscillation in AD is one of the causes for cognitive impairments. Recently, the studies of gamma frequency stimulation in AD provided further evidence. It was shown that using non-invasive 40 Hz gamma stimulation, but not other frequencies, significantly improved cognitive functions and reduced Aβ levels in the AD model.[6,7] Thus the modulation of the gamma activity of neurons with 40 Hz stimulation represents a novel non-pharmacological treatment for patients with AD.[8] These previous studies used light flicker or auditory tone stimulation to entrain gamma oscillations in visual or auditory cortices. In this study, we will use transcranial alternating current stimulation (tACS), characterized by modulating neuronal activity at a particular frequency, to evoke gamma oscillations.

Transcranial current stimulation is a non-invasive method that stimulates the brain with weak electric fields, which can alter brain network dynamics and behavior. tACS oscillates a sinusoidal current at a particular frequency, usually chosen to be close to the frequency of the circadian oscillation of neuronal activity. tACS synchronizes or manipulates the intrinsic oscillations of the brain and interacts with ongoing neuronal activity during cognitive processes, thereby causing cognitive function changes.[9,10] Studies reported that gamma-tACS over the frontal and temporal lobes enhanced episodic memory recognition, working memory, and problem-solving ability.[11–13] In addition, tACS is a safe intervention with possible mild and transient side effects and no serious adverse events.[14] As a potential effective and safe therapy for AD, there have been no studies published on tACS intervention for AD. We hypothesized that 40 Hz tACS targeting frontal and temporal cortices could improve cognitive functions of patients with AD.

The aim of this double-blind, randomized controlled trial is to examine the efficacy and safety of 40 Hz tACS in patients with mild AD dementia. The tACS instrument includes three electrodes; one is placed on the forehead and the other two are placed on each mastoid area. The simulation results showed that the current flows through bilateral frontal and temporal lobes (our unpublished data). In this study, the subjects are individuals with mild AD with Aβ plaques in the brain verified by Pittsburgh compound B (PiB) positron emission tomography (PET) imaging or cerebrospinal fluid (CSF) Aβ levels. In addition to neuropsychological assessments, we will also explore the potential mechanism of tACS on AD using PiB-PET, structural magnetic resonance imaging (MRI), resting electroencephalography (EEG), and simultaneous EEG–functional MRI (EEG-fMRI) techniques.

2 | METHODS

2.1 | Study design

This single-center, double-blind trial has been registered on clinicaltrials.gov (NCT 03920826). All participants will be recruited from Xuanwu Hospital, Capital Medical University, Beijing. This study will be reported in accordance with both the CONsolidated Standards of Reporting Trials (CONSORT) statement and the CONSORT statement for nonpharmacological interventions.

The participants will be randomized into either a tACS group or a sham stimulation group. Both groups will undergo 30, 1-hour sessions of tACS or sham stimulation across 3 weeks (21 days). The outcomes will be assessed at baseline, at the end of the intervention, and at 3-month follow-up. The outcomes will include multiple neuropsychological assessments to examine the effect of tACS on cognitive functions, structural MRI to measure brain volume and white matter integrity, resting-state EEG and EEG-fMRI to measure brain connectivity and neural activity, and PiB-PET to analyze Aβ deposits in the brain.

2.2 | Participants

Forty patients with mild AD will be recruited according to the following inclusion and exclusion criteria.

2.2.1 | Inclusion criteria

1. Subjects with informed consent;
2. Literate Han Chinese, 45 to 75 years of age;
3. At least 6 years of education;
4. Diagnosed with AD according to the National Institute on Aging and the Alzheimer's Association (NIA-AA) guidelines[15];
5. The global score of Clinical Dementia Rating Scale (CDR) is 1.0[16];
6. Positive findings in amyloid PET imaging or decreased CSF levels of $A\beta_{1\text{-}42}$; and
7. On a stable dose of cholinesterase inhibitors (eg, donepezil or rivastigmine) for at least 6 consecutive weeks and without any intention to modify the dosage during the intervention or observation periods.

2.2.2 | Exclusion criteria

1. Current or past history of any neurological disorder other than AD, such as epilepsy, stroke, progressive neurological disease (eg, multiple sclerosis), poorly controlled migraines or intracranial brain lesions, and history of previous neurosurgery or head trauma that resulted in residual neurological impairment;
2. Contraindication for undergoing MRI or receiving tACS;
3. Eczema or sensitive skin;
4. Familial AD;
5. Depression or other psychiatric disorders;
6. Abnormal brain structural MRI scan, including hydrocephalus, stroke, structural lesions, which could potentially confound the outcome;
7. Clinically significant gastrointestinal, renal, hepatic, respiratory, infectious, endocrine, or cardiovascular diseases, cancer, alcoholism or drug addiction; and
8. Other conditions that, in the investigator's opinion, might not be suitable for the study.

2.3 | Randomization and masking

Participants will be randomly allocated to either the tACS group or the sham stimulation group at a ratio of 1:1. After informed consent, randomization will be performed by an independent statistician who is blinded to the patients' interventions, using simple randomization with the random number table method in SAS software (SAS Institute, Inc., Cary, NC). Both the tACS stimulators and sham stimulators used

Highlights

- Transcranial alternating current stimulation (tACS) interacts with neural activity at a particular frequency.
- Gamma oscillations are impaired in Alzheimer's disease (AD), and 40 Hz entrainment is a potential treatment.
- The TRanscranial AlterNating current Stimulation FOR patients with Mild Alzheimer's Disease (TRANSFORM-AD) study is a randomized-controlled trial to evaluate the effects of 40 Hz tACS in mild AD.
- The intervention is 30 one-hour sessions of tACS or sham stimulation across 3 weeks.
- Cognitive assessments, Pittsburgh compound B (PiB) positron emission tomography (PiB-PET), magnetic resonance imaging (MRI), and simultaneous electroencephalography–functional MRI (EEG-fMRI) will be examined.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using the traditional sources. Although there are no studies published on transcranial alternating current stimulation (tACS) intervention for Alzheimer's disease (AD), some reports showed that tACS could enhance cognitive abilities. Furthermore, studies suggested that 40 Hz gamma entrainment improved cognitive function and reduced amyloid β (Aβ) levels in the AD model. These relevant articles are appropriately cited.
2. Interpretation: The TRanscranial AlterNating current Stimulation FOR patients with Mild Alzheimer's Disease (TRANSFORM-AD) study is a double-blind, randomized-controlled trial to examine the effects of 40 Hz tACS in patients with mild AD dementia. The intervention is 30 one-hour sessions of tACS or sham stimulation across 3 weeks. Neuropsychological assessments, Pittsburgh compound B (PiB) positron emission tomography (PiB-PET), structural magnetic resonance imaging (MRI), resting electroencephalography (EEG), and simultaneous EEG-fMRI (functional MRI) will be used to evaluate the effects of tACS.
3. Future directions: The purpose of this pilot study is to verify the possibility of tACS as a treatment for AD. Results of this study are valuable for future large sample studies on tACS and other non-pharmacological intervention for AD.

in the treatment group and sham-controlled group, respectively, are provided by the NEXALIN company (Nexalin Technology, Houston, TX). The sham instrument's operating procedures, parameter displays, and prompts are the same as those of a real instrument. During the trial, the distribution of the stimulators will be based on the random allocation table. Neither participants nor operators will be able to discern whether the stimulator is real based on its appearance or patients' feelings. Study participants, their caregivers, and all assessors will remain masked to treatment assignment throughout the study. Randomization data are confidential until the time of unblinding and will not be accessible except by the independent data monitoring committee or for medical emergencies. Once the blinding is broken, the participant will be managed as off-trial.

2.4 | Intervention

The NEXALIN ADI transcranial alternating current stimulator is the tACS instrument we will use in this study.[17] The alternating current is administered through medical grade conductive pads that are produced specifically for the NEXALIN technology. The pads are placed on the forehead and behind each ear and are connected to the NEXALIN device with thin cables. As mentioned before, 40 Hz gamma frequency stimulation has the most evidence supporting its effects in AD.[8] Hence, 40 Hz was chosen as the stimulation frequency.

In the treatment group, participants will receive tACS with gamma frequency (40 Hz) and a peak-to-peak amplitude of 15 mA in 30 one-hour sessions across 3 weeks (21 days).

In the sham stimulation group, electrodes will also be placed on the patient's forehead and behind each ear. The sham stimulator has the same appearance as the real stimulator. However, when the device is started, no current flows through the electrodes. Participants in this control group will also receive sham stimulations with 30, 1-hour sessions across 3 weeks (21 days).

2.5 | Outcome measures

2.5.1 | Neuropsychological assessments

The primary outcome of the intervention will be assessed by the 11-item cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog), in which scores range from 0 to 70, with a higher score representing a worse outcome.[18]

The Mini-Mental State Examination (MMSE),[19] Montreal Cognitive Assessment (MoCA) ,[20] and Clinical Dementia Rating Scale Sum of Boxes (CDR-SB, scores range from 0 to 18, with higher scores indicating worse dementia)[21] will be used to evaluate general cognitive function.

The World Health Organization-University of California-Los Angeles auditory verbal learning test (WHO-UCLA AVLT) will be used to assess memory function. Scores range from 0 to 45, with a higher value representing a better outcome.[22] Digit span forward will be used to assess attention. The trail making test (TMT) B minus A score and digit span backward will be used to assess executive processing. Semantic tasks will be examined by the Boston Naming Test (BNT). The assessment of daily function will use the activities of daily living (ADL) scale. In addition to cognitive assessments, the Neuropsychiatric Inventory (NPI)[23] and the Geriatric Depression Scale (GDS)[24] will be used to measure neuropsychiatric symptoms.

2.5.2 | MRI data collection

Participants will undergo brain MRI at baseline, at the end of the intervention, and 3 months after the first session.

T1-weighted images of the whole brain will be obtained using a sagittal three-dimensional (3D) magnetization prepared rapid gradient echo (MPRAGE) sequence: repetition time (TR) = 1690 ms; echo time (TE) = 2.56 ms; slice thickness, 1 mm; flip angle, 12°; field of view (FOV) = 256 × 256 mm^2; matrix = 256 ×256; slice number = 176.

Resting-state fMRI will be conducted using a multiband echo-planar imaging (EPI) sequence: TR = 2000 ms; TE = 30 ms; 40 slices; slice thickness, 3 mm; gap 1 mm; flip angle, 83°; FOV = 234×234 mm^2; pixel size = 3.0 mm×3.0 mm; matrix = 64×64; duration: 6 minutes.

The diffusion tensor imaging (DTI) will be acquired by using a diffusion-weighted double spin-echo EPI sequence: TR, 8500 ms; TE, 63.0 ms; 64 diffusion-weighted directions with a b value of 1000 s/mm^2 and 10 images with a b value of 0 s/mm^2; flip angle, 90°; FOV, 224 × 224 mm^2; matrix = 128 ×128; in-plane resolution, 2.0×2.0×2.0 mm^3 voxels; 60 contiguous 2-mm thick axial slices.

The diffusion spectrum imaging (DSI) method was developed to image the complex distribution of fiber orientation. This method may overcome the limitations of DTI, such as estimating crossing or kissing fibers, to add more detailed information about the white matter microstructure. DSI generalizes DTI by acquiring more directions in q-space.[25] In this trial, DSI data will be acquired with equal spacing in q-space on a cartesian grid in 259 directions with a maximum b value of 7000 s/mm^2. The scan parameters are TR = 5548 ms; TE = 84.1 ms; FOV = 224× 224 mm^2; voxel size = 2.0× 2.0 × 2.0 mm^3 voxels; flip angle: 90°.

2.5.3 | Resting EEG and EEG-fMRI

EEG signals will be recorded using the Neuroscan Synamps 2 system, together with an MRI-compatible 64-channel (Ag/AgCl) electrode cap, positioned according to the international 10-20 system. The ground electrode is placed on the forehead, and the reference electrode is predefined in the cap and is positioned between Cz and Pz. Electrooculography electrodes are measured above and below the left eye, and there is an additional electrode to record an electrocardiography to control for heartbeat artifacts. The electrode impedance values will be monitored and should be <50 kΩ. The participants will be instructed to remain in an alert state. Resting-state EEG will be recorded for 10 minutes with the eyes closed and open (5 minutes each). The sample rate

is 1000 Hz, and the bandpass filter is between 0.5 and 100 Hz. After resting-state EEG is recorded, participants will enter the MRI scanner room with the electrode cap, and the amplifier will be placed outside the scanner room. The EEG will be recorded continuously during fMRI using the above parameters and synchronized with the scanner's internal clock to remove the gradient artifacts in subsequent analyses. During the EEG-fMRI recording, all participants will be instructed to close their eyes and relax without falling asleep.

2.5.4 | PiB-PET imaging

The production of PiB and radio labeling with ^{11}C will be synthesized using the method described previously.[26] In our preliminary experiment, the mean administered activity was 555 MBq (range 318 to 684 MBq). The PET is 30-minute dynamic acquisition, 40-minute post-injection (50- to 70-minute summation). PET sinograms are iteratively reconstructed into a 256 × 256 mm^2 FOV with a slice thickness of 1.4 mm. We will use the PMOD image quantitative software to process the PET data.[27] A global cortical PiB-PET retention ratio is formed by calculating median uptake over voxels in the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus regions of interest normalized to the cerebellar gray median.[28] The result is considered positive if this ratio is higher than 1.25.[29]

2.5.5 | Adverse events

Side effects will be assessed after every five sessions. Every participant will be asked to complete a self-report questionnaire. According to previous literature and recommendations, the questionnaire includes the following symptoms: headache, neck pain, scalp pain, tingling, itching, ringing/buzzing noise, burning sensations, skin redness, sleepiness, trouble concentrating, acute mood change, dizziness, flickering lights, and other symptoms.[30–32] Participants will rate each symptom on a scale of 0 to 3 according to the presence and severity of these symptoms (0, absent; 1, mild; 2, moderate; or 3, severe) and will be asked to what extent they believe the reported symptoms are related to tACS (0, none; 1, possible; 2, probable; or 3, definite).

2.6 | Primary outcome

The primary outcome measure is the score change in ADAS-Cog from baseline to the end of the intervention.

2.7 | Secondary outcome

The secondary outcomes for neuropsychological assessments include the score change in ADAS-Cog from baseline to 3-month follow-up and the score changes from baseline to the end of the intervention and 3-month follow-up in other scales, including the MMSE, MoCA, CDR-SB, WHO UCLA AVLT, digit span, TMT B−A, ADL, NPI, and GDS. PiB-PET will be reassessed at the end of the intervention if patients undergo PiB-PET at baseline, and the change between these two assessments is one of the secondary outcomes. The MRI and EEG results will be assessed at baseline, at the end of the intervention, and at 3-month follow-up. The secondary outcomes contain structural changes, including gray matter volume, measured by voxel-based morphometrics; white matter microstructure, measured by DTI and DSI; and neuronal activity and connectivity changes assessed by EEG and EEG-fMRI. Side effects will be assessed by the above-mentioned questionnaire.

2.8 | Data collection

Inclusion and exclusion criteria will be assessed at screening. After written informed consent is acquired, the following data will be collected: demographic data (gender, age, education, and occupation); medical history; concomitant medications; and findings from a complete physical examination and neurological examination. Then, neuropsychological assessments, MRI, and EEG will be carried out. The follow-up assessments will be scheduled at the end of the intervention and 3 months after the first session. The study schedule of the trial is shown in Table 1. The study flowchart is presented in Figure 1.

2.9 | Data monitoring

The study will comply with the Declaration of Helsinki and the good clinical practice (ICH-GCP) guidelines of the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use. The neuropsychological functions of all participants will be evaluated by the same trained neuropsychologists. MRI and PET data will be acquired according to the same parameters and using the same scanner.

2.10 | Sample size

There are no published randomized controlled trials on tACS in AD. In our preliminary experiments, 30 sessions of tACS intervention resulted in improvement in ADAS-Cog scores (mean ± SD: 3.12 ± 2.35 vs 0.67 ± 2.13). According to these data, to obtain a statistical power of 80% with a significance level of 5%, the sample size needs to be 15 for each group. To allow for a maximum dropout rate of 20%, the sample size is set to 40 participants, 20 in each group.

2.11 | Statistical analysis

All data will be analyzed according to intent-to-treat principles. To explore the demographic characteristics of our participants, independent sample *t* tests for continuous data and chi-square tests for dichotomous variables (Fisher's exact tests, if needed) will be used.

TABLE 1 Schedule of the trial

	Study period				
Time point	Screening	Baseline	tACS	End of the intervention	3-Month follow-up
Eligibility screen	X				
Informed consent	X				
Randomization		X			
Demographic data		X			
Physical examination		X		X	X
Assessments					
CDR	X			X	X
ADAS-Cog		X		X	X
MMSE		X		X	X
MoCA		X	↔	X	X
AVLT		X		X	X
Digit span		X		X	X
TMT		X		X	X
BNT		X		X	X
ADL		X		X	X
NPI		X		X	X
GDS		X		X	X
MRI		X		X	X
EEG		X		X	X
PiB-PET or CSF $A\beta_{1\text{-}42}$	X			X (only PiB-PET)	

Abbreviations: ADAS-Cog, cognitive subscale of the Alzheimer's Disease Assessment Scale; ADL, activities of daily living; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDR, Clinical Dementia Rating Scale; GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NPI, Neuropsychiatric Inventory; TMT, trail making test.

The effects of tACS on neuropsychological scores, PiB-PET, EEG, and MRI data will be examined using linear mixed-effects models nested within individuals. Time will be assigned as the repeated variable. Group, time, and group-by-time will be included as fixed effects. We will analyze the changes in the outcomes from baseline to the end of the intervention and from baseline to 3-month follow-up. For adverse events, chi-square tests or Fisher's exact tests will be used to compare the frequencies between groups. Correlation analyses between significant neuroplasticity changes and neuropsychological scores will be conducted to explore neural mechanisms for cognitive functional changes. For all analyses, significance levels will be set to 0.05.

3 | DISCUSSION

Because attempts at disease-modifying drug discovery for AD have largely failed, non-pharmacological treatments have been suggested

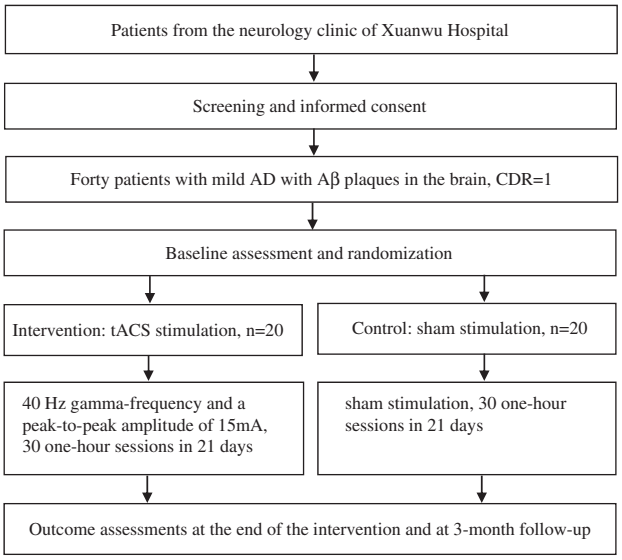

FIGURE 1 The flowchart of this trial

as an alternative and promising treatment for AD, and tACS is a relatively new candidate. Currently, there are no published randomized controlled clinical trials evaluating the effects of tACS in patients with AD. The main aim of this study is to fill this gap by systematically investigating the effects of tACS in AD patients and its potential mechanisms.

This study has multiple strengths. First, tACS is applied to entrain gamma oscillations in this trial. The advantage of tACS is that its current can oscillate at a particular frequency and interact with the brain's intrinsic oscillations. Previous human studies using simultaneous tACS-EEG recordings have suggested that tACS can enhance neuronal activity by synchronizing cortical oscillations to the entrainment frequency and can modify behavioral performance.[33,34] In nonhuman primates, the best available animal model for the human brain, tACS, can influence spike timing, which is critical for neural computation and communication, and the effect of tACS could reach deep brain structures.[35] These data provide strong mechanistic evidence for the application of tACS in improving cognitive functions. Currently, the use of tACS has successfully been shown to enhance working memory,[36] episodic memory recognition,[12] mental rotation performance,[37] and language learning attention,[38] which are commonly impaired in patients with AD.

Second, 40 Hz is the stimulation frequency used in this trial. Before Aβ plaque formation, aberrant neural activity may already occur and could exacerbate the progression of AD pathology.[6,7] Prominently, gamma oscillations, which are closely associated with cognitive functions, have been suggested to be impaired in multiple AD models.[3,39] In AD models, light-emitting diode (LED)–based 40 Hz light flicker or a train of tones flickering at 40 Hz can entrain gamma oscillations in the visual cortex or auditory cortex and cause improvements in cognitive functions and reductions in amyloid and phosphorylated tau levels.[7,40] Chronic gamma entrainment using 40 Hz sensory stimulation could also improve synaptic function, enhance neuroprotective factors, and

reduce neuron damage.[41] This evidence suggests that gamma oscillation entrainment is a promising intervention for AD.

Another strength of this trial is that the effect of tACS will be assessed comprehensively on multiple levels. In 2018, the NIA-AA proposed a research framework that emphasized the diagnosis of AD with biomarkers. The biomarkers are grouped into Aβ deposition, pathologic tau, and neurodegeneration [AT(N)]. The definition of AD shifted from a clinical diagnosis to a biological construct.[42] Thus, in this trial, along with neuropsychological outcomes to assess the direct clinical benefit of tACS, we also use PiB-PET to assess the effects of tACS on Aβ levels. Gray matter degeneration and white matter lesions are common structural changes in AD,[43] and these changes are assessed by MRI in this trial. Furthermore, in addition to conventional EEG, the most commonly used measure for analyzing changes in brain activity in response to brain stimulation, we also use EEG-fMRI technology in this trial. EEG offers a high temporal resolution (milliseconds) but has poor localization; in contrast, fMRI provides a high spatial resolution but inadequate temporal sampling (seconds). Due to a notable degree of complementarity between EEG and fMRI, simultaneous EEG-fMRI could offer new insight into the investigation of brain function and the evaluation of the correlation between electrical brain activity and hemodynamic changes.[44,45] Thus, in this trial, by using EEG-fMRI, the effects of tACS on neural activity can be characterized with both high temporal and high spatial resolution.

4 | CONCLUSIONS

The 40 Hz tACS has the potential ability to enhance neuronal activity, reduce amyloid deposits, and improve cognitive functions in AD. This double-blind, randomized-controlled trial will verify these effects. Through comprehensive measures, this study will identify the effects of tACS on AD at the pathophysiological level and will explore the potential mechanism.

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