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STUDY PROTOCOL

Acupuncture Modulates Spatiotemporal Neuronal Dynamics in Mild Cognitive Impairment: A Protocol for Simultaneous EEG-fMRI Study

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Background: Mild cognitive impairment (MCI) is characterized by abnormal changes in spatiotemporal neuronal specificity responses. Simultaneous electroencephalogram (EEG)-functional magnetic resonance imaging (fMRI) offers a novel approach to measure these changes. Emerging evidence suggests that acupuncture may enhance cognitive function by modulating spatial or temporal central activity in individuals with MCI. However, no studies have investigated the detailed mechanisms underlying this effect.

Methods: This randomized controlled neuroimaging trial will enroll 60 patients with MCI, who will be randomly assigned to one of two groups: a real acupuncture (RA) group or a sham acupuncture (SA) group. The trial period will last 12 weeks, during which participants will receive 24 sessions of acupuncture twice weekly. The primary outcome measure will be the improvement in the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) score from baseline to post-treatment. Secondary outcomes will include improvements in specific cognitive domains such as memory, executive function, language, and attention. Simultaneous EEG-fMRI combined with correlation analysis, regression analysis, and joint independent component analysis (jICA) will elucidate the spatiotemporal central modulatory mechanisms of acupuncture in MCI patients.

Discussion: This study may reveal that real acupuncture can treat cognitive impairment by modulating the brain's spatiotemporal neuronal specificity activity. Our findings will provide scientific evidence for the efficacy of acupuncture in the treatment of MCI and further add to the understanding of the neural mechanisms.

Clinical Trial Registration: ClinicalTrials.gov, identifier [ChiCTR2400084666].

Keywords: acupuncture, mild cognitive impairment, simultaneous electroencephalography - functional magnetic resonance imaging

Introduction

Mild cognitive impairment (MCI) is characterized by memory decline—particularly in episodic memory—along with executive dysfunction and mild language or visuospatial challenges, though without impairing daily activities. As an intermediate stage between normal aging and Alzheimer's disease (AD), MCI affects 10–20% of adults over age 50 globally, presenting a pressing public health issue amid aging populations.[1–3] With US dementia care costs anticipated to reach $360 billion by 2024, early intervention in MCI is essential to mitigate societal and economic burdens.[4]

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Despite extensive research, no pharmacological treatments have proven effective in reversing the progression of MCI or dementia.[5–7] In recent years, traditional Chinese medicine (TCM), including herbal remedies,[8,9] acupuncture,[10] and moxibustion,[11] has attracted considerable attention for its potential therapeutic benefits in AD and MCI. Acupuncture has been used to treat cognitive dysfunction for millennia. Recent randomized controlled trials and meta-analyses suggest that acupuncture can enhance global cognitive function, memory, and attention in MCI patients, either as a standalone or adjunctive therapy.[12–15] While these findings are promising, the complex pathology of MCI indicates that the mechanisms underlying acupuncture's efficacy remain poorly understood.[16,17]

Currently, functional magnetic resonance imaging (fMRI) technology enables the non-invasive detection of underlying neurological changes in diseases such as MCI and AD. Studies have shown significant structural and functional abnormalities in key brain regions responsible for episodic memory processing, including the hippocampus, medial temporal lobe, and frontal cortex, in MCI patients.[18–20] Considering previous research, the brain's intrinsic activity is subject to temporal fluctuations and is responsive to contextual factors and activities. Complementary to fMRI, electroencephalography (EEG) provides direct information about neural electrical activity, particularly capturing rapid changes on the millisecond timescale.[21] The integration of simultaneous EEG-fMRI offers high spatiotemporal resolution, identifying significant spatiotemporal abnormalities in MCI/AD patients' brain networks, providing new insights into pathological mechanisms.[22,23]

Growing evidence indicates acupuncture at specific acupoints can modulate cerebral function by enhancing neural plasticity and facilitating functional reorganization.[24–26] For instance, electroacupuncture at Baihui (GV20) and Shenting (GV24) can improve the neurogenesis function of immature granule cells in the dentate gyrus of the hippocampus.[27] Acupuncture at Taixi (KI3) significantly regulates the functional connectivity within and between the sensory/somatomotor, cingulate-frontal, and dorsal attention networks.[28] However, the mechanistic exploration of acupuncture is hindered by the limitations of single neuroimaging techniques. Multimodal approaches, such as simultaneous EEG-fMRI, present a promising avenue to better understand how acupuncture influences brain function. Based on spatiotemporal abnormalities observed in patients with MCI and AD, it is hypothesized that acupuncture may exert therapeutic effects by modulating disrupted spatiotemporal brain dynamics.

Acupuncture has demonstrated effectiveness as a treatment for mild cognitive impairment (MCI). Our previous systematic review revealed that acupuncture can modulate the activity of critical brain regions and networks, in MCI patients.[29] However, the dynamic spatiotemporal mechanisms of brain regulation through acupuncture are still not well understood. Building on this, we propose using simultaneous EEG-fMRI technology to investigate the hypothesis that acupuncture at classical acupoints exhibits high specificity across time scales in altering the functional activity in key brain regions and networks for cognitive function in MCI individuals. Consequently, we developed a randomized, parallel, sham acupoint-controlled clinical trial to investigate the spatiotemporal regulatory mechanisms of acupuncture intervention in MCI using simultaneous EEG-fMRI.

Methods

Study Design

This study is a parallel, randomized, sham-controlled trial designed to evaluate the spatial and temporal specificity of brain activity related to acupuncture in individuals with MCI using simultaneous EEG-fMRI. The trial will be conducted across five hospitals in China, including Chengdu University of Traditional Chinese Medicine Hospital, Fourth People's Hospital of Chengdu, Sichuan Province People's Hospital, West China Hospital, and Chengdu Pidu District Hospital of Traditional Chinese Medicine. Sixty patients with MCI will be randomly assigned in a 1:1 ratio to either the real acupuncture (RA) group or the sham acupuncture (SA) group. Participants will undergo baseline evaluations (weeks −2 to 0) and receive clinical treatment for 12 weeks. All participants will undergo clinical assessments along with simultaneous EEG-fMRI scans at baseline. Following the treatment period, we will evaluate the therapeutic effects of acupuncture on MCI. To assess changes in brain activity within acupuncture-related areas, EEG-fMRI scans will be repeated at a 12-week interval. This protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement (Supplementary Material 1). The study's flowchart is presented in Figure 1.

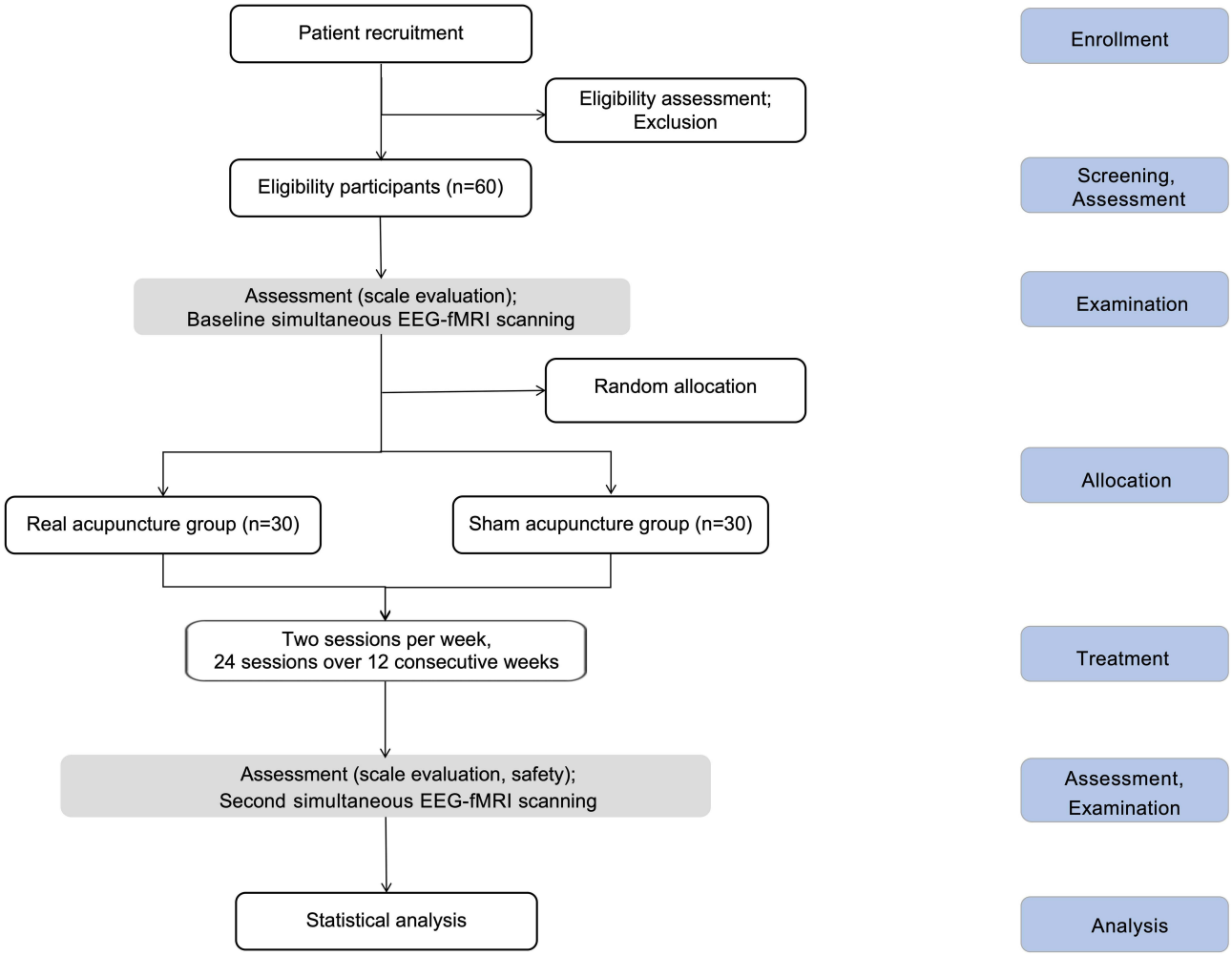

Figure 1 A flow chart of the trial.

Participants

Enrollment will be advertised through a combination of online and offline methods, and participants who meet the inclusion criteria will be invited to participate in the study. Following confirmation of the details of the study process, they will be asked to sign an informed consent form and receive baseline assessments.

Inclusion Criteria

Inclusion criteria comprise conformity to the MCI diagnostic criteria (the Jak/Bondi 2014 diagnostic criteria),[30] age between 50 and 80 years, right-handedness, Mandarin-speaking ability, disease duration exceeding 3 months, a Clinical Dementia Rating score of 0.5, a Hachinski Ischemic Score less than 4, a minimum of 8 years of education with the ability to understand and complete assessments, voluntary participation and signed informed consent, and no contraindications for MRI, such as metal implants.

Exclusion Criteria

Exclusion criteria include participants currently undergoing treatments that interfered with cognitive functions, those with a history of neurological diseases affecting cognitive function (except for early suspected Alzheimer's disease cases), brain MRI indicative of infection, focal lesions, multiple infarctions, or severe white matter changes (Fazekas score ≥ 3), a history of tumors, psychiatric disorders, or severe anxiety (Hamilton Anxiety Scale (HAMA)$\geq$ 29) and depression (Hamilton Depression Scale (HAMD)$\geq$ 24), hemorrhagic diseases, bleeding tendencies, or severe skin infections, severe

drug dependency, smoking, drug abuse, or alcoholism, pregnant or breastfeeding women, or those suspected to be pregnant, and those who had undergone acupuncture or participated in other clinical studies within the last six months.

Withdrawal Criteria

Withdrawal criteria include disease progression or other serious adverse events deemed unsuitable for continued study participation by the investigator. Participants demonstrating poor compliance or voluntarily withdrawing during treatment were also excluded. Those combining treatments prohibited by the protocol or failing to adhere to medical advice were likewise ineligible. Additionally, participants experiencing severe illness or significant family upheavals that make continued treatment unsuitable will be withdrawn. Researchers will record the details of all study withdrawals. The final time of needling and associated assessments for these patients will be documented in a case report form (CRF) to ensure reliability and transparency.

Randomization and Blinding

All patients will be randomly assigned (1:1 ratio) to either the RA or SA group after providing informed consent. An independent researcher will generate the randomization sequence using R software version 4.1.2, without stratification. Randomization will be concealed using opaque, sealed envelopes. Acupuncturists will be informed of the allocation plan via telephone. Participants will be informed that they will receive one of two effective interventions (acupuncture following Traditional Chinese Medicine principles or another type) assigned randomly post-enrollment.

Participant blinding to treatment allocation will be maintained through the use of sham acupuncture, which will elicit a comparable sensory stimulus to real acupuncture. Given the specificity of the acupuncture procedure, acupuncturists will not be blinded, whereas the outcome assessor and statistician will be blinded to the procedure, the results of the randomization, and the intervention in the study. The researcher, acupuncturist, outcome assessor, and statistician will operate independently. Additionally, each patient will be evaluated by the same evaluator.

Intervention

Interventions will be reported following the Standards for Reporting Interventions in Controlled Trials (STRICTA) (Tables 1 and 2).[31] Patients in the RA and SA groups will receive 24 sessions of acupuncture treatment over 12 weeks (twice a week), with each session lasting 30 min. All treatments will be performed by an acupuncturist who is certified and has more than 5 years of experience in the treatment process. Before the official start of the study, we will provide uniform training to the acupuncturists and assess the relevant acupuncture operations.

Real Acupuncture Group

Our selection of acupoints for the RA group is based on expert consensus and literature research.[32,33] The following five acupoints will be used: Shenting (GV 24), Baihui (GV 20), Taixi (KI 3), Dazhong (KI 4), and Sanyinjiao (SP 6) (Figure 2). The positioning of acupoints will be determined based on the World Health Organization guidelines in 2010 (Table 3).[34] After routine skin disinfection, disposable sterile acupuncture needles (25 mm in length and 0.25 mm in diameter, Hwato, China) will be inserted into the acupoints. Both techniques will be applied uniformly to facilitate the sensation of *deqi*, adjusting according to the patient's tolerance. Needle stimulation will be conducted every 10 minutes to maintain the sensation of *deqi*, with a needle retention time of 30 minutes.

Sham Acupuncture Group

The SA group will receive superficial penetration at non-acupuncture points. To minimize physiological effects, non-acupoints were selected based on previous studies, which do not correspond to any known meridians and are not conventional acupoints.[35–37] The locations of the four sham acupoints are bilateral, as shown in Figure 3 (Table 3). After skin disinfection, acupuncture needles will be inserted into four bilateral non-acupoints. Instead of attempting to induce a sensation of *deqi*, the non-acupoints will be punctured with 0.25 mm × 13 mm acupuncture needles (Hwato, China) for 2.5–7.5 mm.

Table 1 Real Acupuncture Treatment Details Based on the STRICTA 2010 Checklist

Item	Item Number	Detail
1.Acupuncture rationale	1a) Style of acupuncture	Traditional Chinese Medicine
	1b) Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate	The treatment is carried out according to traditional acupuncture theory, previous studies, and the acupuncturists' consensus.
	1c) Extent to which treatment was varied	Standardized acupuncture treatment
2. Details of needling	2a) Number of needle insertions per subject per session	8
	2b) Names of points used	GV 24 (Shenting, unilateral), GV 20 (Baihui, unilateral), KI 3 (Taixi, bilateral), KI 4 (Dazhong, bilateral), and SP 6 (Sanyinjiao, bilateral)
	2c) Depth of insertion, based on a specified unit of measurement, or on a particular tissue level	From 15 to 25 mm.
	2d) Response sought	Deqi (soreness, numbness, heaviness, distention, etc.)
	2e) Needle stimulation	Manual acupuncture
	2f) Needle retention time	30 minutes
	2g) Needle type	Sterile, disposable acupuncture needles (length, 25 mm; diameter, 0.25 mm; Hwatuo, China)
3. Treatment regimen	3a) Number of treatment sessions	24
	3b) Frequency and duration of treatment sessions	Twice per week (once per 2–3 days interval), for 12 successive weeks.
4. Other components of treatment	4a) Details of other interventions administered to the acupuncture group	None
	4b) Setting and context of treatment, including instructions to practitioners, and information and explanations to patients	The trial will be implemented at departments the Chengdu University of Traditional Chinese Medicine Hospital, the Fourth People's Hospital of Chengdu, the Sichuan Province People's Hospital, the West China Hospital, the Chengdu Pidu District Hospital of Traditional Chinese Medicine. All information and explanations will be offered to participants.
5. Practitioner background	5) Description of participating acupuncturists	Trained, licensed acupuncturists with at least 6 years in acupuncture clinical practice.
6. Control or comparator interventions	6a) Rationale for the control or comparator in the context of the research question, with sources that justify this choice	The treatment is carried out according to previous studies, and the acupuncturists' consensus.
	6b) Precise description of the control or comparator. If sham acupuncture or any other type of acupuncture-like control is used, provide details as for Items 1 to 3 above.	Sham acupuncture will be performed at 8 non-acupoints.
	6b.1) Style of acupuncture	The sham acupuncture is invasive (penetrating the skin).
	6b.1) Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate	The sham acupuncture treatment based on previous studies.
	6b.1) Extent to which treatment was varied	Standardised sham acupuncture treatment.

EEG-fMRI Scanning Procedure

To obtain high-quality simultaneous EEG-fMRI data, acquisitions will be uniformly performed at the Chengdu No. 4 People's Hospital (Chengdu, China) using a Siemens 3.0 T Magnetom Skyra MRI system (Siemens Medical, Erlangen, Germany) with a 32-channel head coil. Participants will be instructed during the scan that they must remain awake and relaxed, and will also be advised to keep their eyes closed and place earplugs in their ears to minimize noise stimulation from the equipment. In addition, foamed pads will be used to immobilize the subject's head to avoid movement. After obtaining standard tri-plane localizer images, T_1 structural imaging scans will be conducted using the T_1-weighted fast spoiled gradient-recalled echo sequence (FSPGR). Images will be acquired with the following parameters: TR/TE/TI = 2300/2.32/900ms, field of view

Table 2 Sham Acupuncture Intervention Details Based on the ACURATE Checklist

Category	Item	Description	Detail
1. Type of sham acupuncture	1a	Report the type of sham acupuncture.	Sham acupuncture will be performed at four non-acupoints (Shallow acupuncture, 2.5–7.5mm)
	1b	Report whether the sham acupuncture is penetrating or non-penetrating.	Penetrating
	1c	Rationale for using the chosen sham acupuncture.	Based on our previous clinical trial.
2. Details of sham acupuncture manipulation	2a	Report the number of sham acupuncture applied per subject per session.	8
	2b	Report the depth of sham acupuncture insertion (if there was no penetration, state this within the paper).	2.5–7.5mm.
	2c	Report whether any response was observed during sham acupuncture manipulation (eg de qi or muscle twitch response).	Without attempting to yield the deqi sensation.
	2d	Report if there was any stimulation using sham acupuncture.	Without stimulation or manipulation.
	2e	Report if there was sham acupuncture retention.	30 minutes.
	2f	Report details of other interventions administered in addition to sham acupuncture during one session.	Patients will be allowed to perform basic treatments such as blood pressure control, blood sugar control, and other supportive treatments.
3. Location of sham acupuncture	3a	Report the location of sham acupuncture (eg acupoint/ non-acupoint or the exact location of the sites).	4 non-acupoints (Table 4 and Figure 3).
	3b	Explicitly state in the paper if the points are unilateral or bilateral.	4 non-acupoints are bilateral.
	3c	Describe the reason for the chosen location of sham acupuncture.	Based on our previous clinical trial.
4. Treatment regimen	4a	Report the number of treatment sessions.	30 minutes sessions over 12 weeks.
	4b	Report whether the number of sessions were identical between real and sham acupuncture treatments.	The acupuncture in both groups will be administered with the same, number of sessions, frequency and treatment duration.
	4c	Report the frequency and duration of treatment sessions.	30 min sessions over 12 weeks.
	4d	Report the total trial period.	12 weeks.
5. Practitioner	5a	Report whether the same practitioner is administering both real and control treatments (interventions).	All interventions will be performed by doctors of Traditional Chinese Medicine with more than 6 years of experience. They will administer both real and sham acupuncture.
	5b	Report whether there were conversations between practitioner and patient directly linked to the trial design, other than scripted instructions and preset information, prior to and during the treatment.	Throughout the course of treatment, acupuncturists will be asked to avoid discussing treatment options with patients.
6. Protocol and settings	6a	Report the information regarding sham acupuncture provided to participants.	Before randomization all participants will be informed that they will be allocated to the acupuncture group or sham acupuncture group.
	6b	Report whether the information given to patients include the term to openly state that the control is inert (eg "fake", "sham", "dummy","placebo").	The participants will be informed that they will receive one of the two acupuncture treatments.
	6c	Describe how sham device was blinded from patients, and if done, how the blinding was assessed.	Patients will be treated separately to ensure blind evaluation.
	6d	If done, report any modification in the sham acupuncture treatment procedure, and reason for the modification.	Not Applicable.
	6e	Report any difference in the treatment settings between real and sham acupuncture.	The choice of acupoints and the depth of insertion varied.

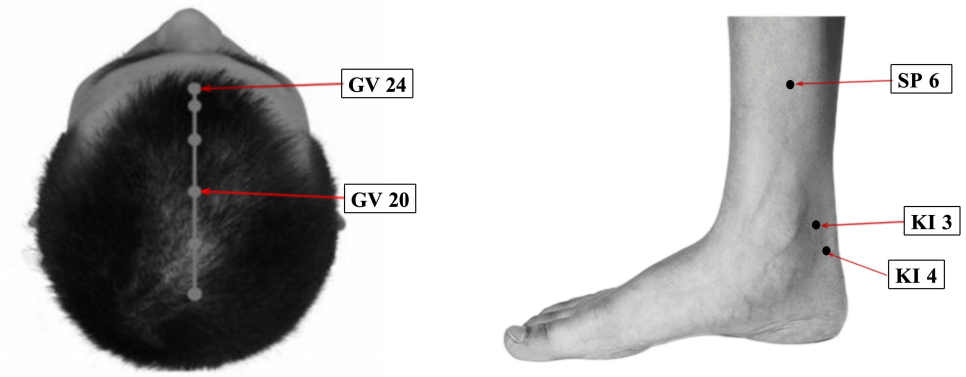

Figure 2 Locations of acupoints: GV 24 (Shenting), 0.5 cun directly above the midpoint of the anterior hairline. GV 20 (Baihui), 5 cun directly above the midpoint of the anterior hairline, at the midpoint of the line connecting the apexes of the two auricles. SP 6 (Sanyinjiao), on the medial side of the shank, 3 cun above the medial malleolus, by the posterior of the tibia. KI 3 (Taixi), posterior to the medial malleolus, in the depression between tip of the medial malleolus and tendo calcaneus. KI 4 (Dazhong), located medially to the foot, posterior and inferior to the medial malleolus, resides in a recess anterior to the attachment of the Achilles tendon.

(FOV) = 240mm × 240mm, voxel size = 0.9mm × 0.9mm × 0.9mm, matrix size = 256 × 256, flip angle = 8°, and slice thickness = 0.9mm, resulting in a total scan duration of 321 seconds. Functional MRI (fMRI) will be performed using a gradient echo-planar imaging sequence (GRE-EPI) with parameters set to TR/TE = 2000/30ms, FOV = 240mm × 240mm, voxel size = 3.8mm × 3.8mm × 4.4mm, matrix size = 64 × 64, flip angle=90°, and slice thickness = 4.4mm. The fMRI sequence will incorporate inter-slice gaps and cover the entire brain, including the cerebellum and brain stem, over a scanning

Table 3 Locations and Manipulations of the Acupoints and Sham Acupoints Selected in This Study

Acupoints	Location	Manipulation
GV 24 (Shenting)	0.5 cun directly above the midpoint of the anterior hairline.	Subcutaneous insertion to a depth of 6–10 mm with manipulation for the deqi
GV 20 (Baihui)	5 cun directly above the midpoint of the anterior hairline, at the midpoint of the line connecting the apexes of the two auricles.	Subcutaneous insertion to a depth of 10–15 mm with manipulation for the deqi
KI 3 (Taixi)	Posterior to the medial malleolus, in the depression between tip of the medial malleolus and tendo calcaneus.	Subcutaneous insertion to a depth of 15–20 mm with manipulation for the deqi
KI 4 (Dazhong)	Located medially to the foot, posterior and inferior to the medial malleolus, resides in a recess anterior to the attachment of the Achilles tendon.	Subcutaneous insertion to a depth of 15–20 mm with manipulation for the deqi
SP 6 (Sanyinjiao)	On the medial side of the shank, 3 cun above the medial malleolus, by the posterior of the tibia.	Subcutaneous insertion to a depth of 15–20 mm with manipulation for the deqi
Non-acupoint 1 (NP-1)	At the medial arm on the anterior border of the insertion of the deltoid muscle at the junction of deltoid and biceps muscles	Subcutaneous insertion to a depth of 2.5–7.5mm without manipulation
Non-acupoint 2 (NP-2)	Half way between the tip of the elbow and axillae	Subcutaneous insertion to a depth of 2.5–7.5mm without manipulation
Non-acupoint 3 (NP-3)	Ulnar side, half way between the epicodylus medialis of the humerus and ulnar side of the wrist	Subcutaneous insertion to a depth of 2.5–7.5mm without manipulation
Non-acupoint 4 (NP-4)	Edge of the tibia 1–2 cm lateral to the Zusanli (ST36) horizontally	Subcutaneous insertion to a depth of 2.5–7.5mm without manipulation

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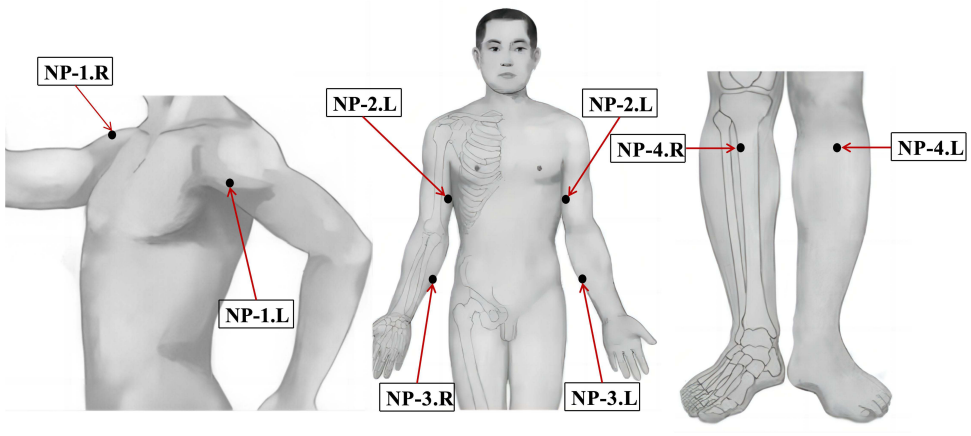

Figure 3 Locations of acupoints: Non-acupoint 1 (NP-1), At the medial arm on the anterior border of the insertion of the deltoid muscle at the junction of deltoid and biceps muscles. Non-acupoint 2 (NP-2), Half way between the tip of the elbow and axillae. Non-acupoint 3 (NP-3), Ulnar side, half way between the epicodylus medialis of the humerus and ulnar side of the wrist. Non-acupoint 4 (NP-4), Edge of the tibia 1–2 cm lateral to the Zusanli (ST36) horizontally.

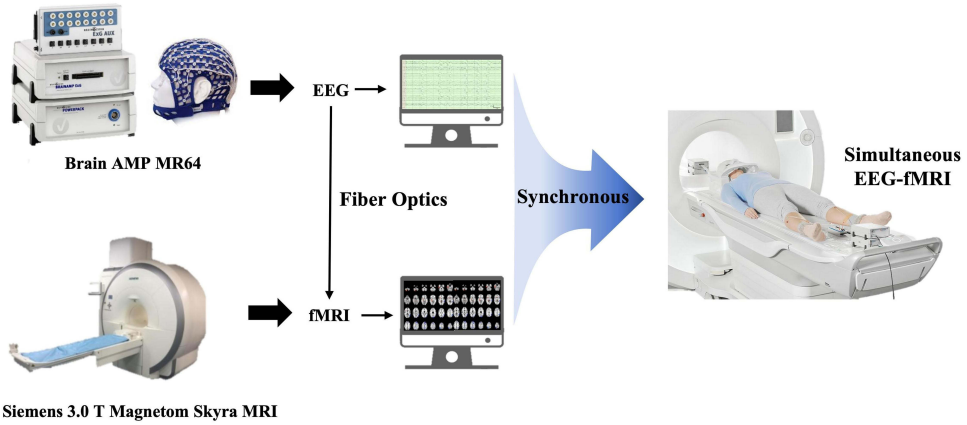

Figure 4 Scanning setup of the simultaneous EEG-fMRI.

period of 516 seconds. Simultaneously, EEG recordings will be synchronized with the resting-state fMRI scans using the Brain AMP MR64 captured using a 64-channel MR-compatible cap, which includes an additional electrode for ECG, arranged according to the International 10/20 system. During EEG data acquisition, FCz will serve as the reference electrode, and AFz as the ground electrode, with a sampling rate set at 5000 hz and scalp impedance maintained below 10 kΩ. EEG signals will be transmitted through fiber optics to an amplifier located outside the MRI suite and stored on a dedicated recording computer. Time-invariant sampling of image acquisition artifacts will be ensured by synchronizing the EEG sampling with the gradient switching clock of the MR scanner (SyncBox, Brain Products, Gilching, Germany). Figure 4 shows the scanning setup of the simultaneous EEG-fMRI.

Outcome Measures

The study will employ a suite of psychometric instruments to assess cognitive and functional capabilities holistically. Neuropsychological assessments will be conducted by researchers in a controlled, quiet environment. To ensure consistency, each participant's evaluation will be performed by the same researcher throughout the study. Additionally, data on medication usage, adherence to treatment, blinding efficacy, treatment satisfaction, and cases of dropout and exclusion will be meticulously documented. Table 4 provides detailed information about the time points at which outcome assessments were conducted.

Table 4 Enrolment, Intervention, and Measurement Schedule

Timepoint	Baseline	Treatment Phase			
	−2-0 week	0 week	→		12 week
ENROLLMENT:					
Eligibility screen	X				
Informed consent	X				
Examination	X				
Randomization	X				
INTERVENTIONS:					
Real acupuncture group		X	→		X
Sham acupuncture group		X	→		X
EEG-fMRI:					
Real acupuncture group	X				X
Sham acupuncture group	X				X
ASSESSMENTS:					
ADAS-Cog	X				X
AVLT-H	X				X
STT-A&B	X				X
BNT	X				X
AFT	X				X
SDMT	X				X
DST	X				X
FAQ	X				X
HAMA	X				X
HAMD	X				X
PSQI	X				X
Adverse events		X	→		X
Blinding assessment					X
Overall satisfaction		X	→		X

Abbreviations: ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; AFT, Animal Verbal Fluency Test; AVLT-H, Auditory Verbal Learning Test - Huashan; BNT, Boston Naming Test; DST, Digit Span Test; EEG-fMRI, Electroencephalography-functional magnetic resonance imaging; FAQ, Functional Activities Questionnaire; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; PSQI, Pittsburgh Sleep Quality Index; SDMT, Symbol Digit Modality Test; STT, Shape Trail Test.

Primary Outcome

The anticipated primary outcome is an enhancement in cognitive functioning, as measured by the change in scores from baseline to week 12 on the Alzheimer's Disease Assessment Scale - Cognitive (ADAS-Cog).[38] This scale is a widely recognized tool in the assessment of Alzheimer's Disease and related cognitive impairments. For this research, the ADAS-Cog will be employed to evaluate participants with MCI. The scale comprises 12 tasks, including word recall,

naming, commands, constructional and ideational praxis, orientation, word recognition, comprehension of spoken language, word-finding difficulty, recall of test instructions, and attention. The ADAS-Cog's scoring system ranges from 0 to 75, with higher scores indicating more severe cognitive impairment.

Secondary Outcomes

Secondary outcomes, assessed by blinded investigators, will evaluate changes in participant performance at week 12 relative to baseline (weeks −2 to 0):

1) Episodic memory function: The Auditory Verbal Learning Test - Huashan (AVLT - H) is a well-established neuropsychological test of learning and memory function in China.[39] In the AVLT-H, there are five parts: immediate recall (N1-3), short-term delayed recall (N4), long-term delayed recall (N5), cued recall (N6), and recognition (N7). Higher scores in each section indicate better memory functioning. We will measure the changes in N5 and N7 scores in this study.

2) Executive function: The executive function will be gauged through the Shape Trail Test (STT).[40] This instrument, a Chinese adaptation of the Shape Trace Test, comprises two subtests: STT-A, which measures processing speed, and STT-B, which assesses executive function. Completion time and errors provide data on executive function. In this study, the completion time of STT-A and STT-B will be used to assess executive function in patients with MCI.

3) Language ability: Language ability will be evaluated using two distinct neuropsychological tests. The 30-item Chinese version of the Boston Naming Test (BNT) will be administered to assess confrontational word retrieval abilities, which involves naming pictures of common objects (total score range 0–30).[41] The Animal Verbal Fluency Test (AFT) will measure a subject's semantic memory and categorical verbal fluency by requiring them to generate as many animal names as possible within 1 minute.[42] Together, these tests offer a comprehensive profile of a person's linguistic capacity, with higher scores indicating better performance.

4) Attention function: The Symbol Digit Modality Test (SDMT) will be used to measure attention, concentration, and processing speed, providing a multifaceted view of cognitive processing abilities.[43] In the SDMT, individuals refer to a key to match symbols with their corresponding numbers, earning one point for each accurate pairing within a 90-second timeframe. The SDMT score is the number of correct answers. Additionally, the Digit Span Test (DST) will be employed to evaluate the participant's attention span and working memory.[44] It consists of two subtests, digit span forward (DSF) and digit span backward (DSB). Scores are calculated by repeating the maximum digit span in both directions. Higher scores on both tests indicate better attentional functioning.

5) Daily activity. The Functional Activities Questionnaire (FAQ) will be used to evaluate the impact of cognitive function on daily living activities.[45] As a widely utilized tool for assessing daily living skills, the FAQ encompasses tasks such as paying bills, engaging in hobbies, cooking, keeping up with current events, remembering appointments, and traveling. The scoring for the FAQ ranges from 0 to 30, with lower scores indicating less impairment in functional abilities.

6) Emotional disorders. Emotional states, which can be both a contributor to and a consequence of cognitive impairment, will be assessed using the HAMD and HAMA, two of the most commonly applied scales to measure depression and anxiety levels, respectively. The HAMD comprises 17 items, each rated on a scale from 0 to 4, to evaluate various aspects of depression, including despair, somatization, sleep disturbances, and anxiety, thereby assessing the overall severity of depressive symptoms.[46] The HAMA, with its 14 items also rated from 0 to 4, measures both psychological and physiological manifestations of anxiety, quantifying the severity of an anxiety disorder.[47]

7) Sleep Quality. The Pittsburgh Sleep Quality Index (PSQI) will be used to quantify sleep quality.[48] The PSQI is a self-reported questionnaire that assesses sleep quality and disturbances across seven domains over the past month. An aggregate score, derived from nineteen items, yields a global score, with higher scores indicating poorer sleep quality.

Blinding Assessment

At the 12th week of treatment (session 24), participants will be asked: "Do you think you received a traditional acupuncture treatment? (Yes or no)" to assess the success of blinding.[49]

Overall Satisfaction

A custom-designed scale will be employed to assess patient satisfaction in both groups post-treatment.

Safety Assessments

During the study, all adverse events will be meticulously recorded and reported, with participants receiving immediate and appropriate care. Researchers will be responsible for explaining to participants the importance of accurately reporting any post-treatment health changes. Particular attention will be paid to adverse events such as hematoma, severe pain, nausea, fainting, and local infection. These incidents will be documented in detail, including their onset, symptoms, severity, duration, treatment, and outcomes. The cause of each adverse event will be analyzed. Severe cases will be immediately reported to the Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, and participants affected by adverse events may choose to withdraw from the study at their discretion.

Quality Control, Data Management, and Monitoring

We will produce a comprehensive clinical study manual prior to the trial's commencement to ensure that all researchers involved in the trial are thoroughly trained. To maintain data integrity, all patient data will be initially documented in case report forms and then securely stored in a restricted area to ensure confidentiality. Subsequently, this data will be transferred to a specially designed electronic database, protected by a password. This process will be overseen by an investigator who is blinded to the participant group allocations, thereby maintaining objectivity. Access to this database will be restricted to select members of the research team only. The Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine will have the authority to review these study records and monitor the conduct of the trial to ensure adherence to ethical guidelines and the study protocol.

Sample Size

For neuroimaging, sample size estimation differs from classical randomized controlled trials. Previous studies have recommended that each group should include 12–15 participants to ensure stable statistical effects in brain fMRI data analysis.[50,51] Previous research indicates that achieving 80% power at the single-voxel level requires approximately 12 subjects with a 0.05 threshold, but 24 subjects are needed for a stricter threshold used in multiple comparisons correction.[50] Considering a potential 20% loss rate in this study, including subject dropout and unavailability of data due to head movements, the sample size in our study will be 60 participants (30 patients per group).

Statistical Analysis

Clinical Data Analysis

Statistical analysis of the clinical data will be performed by a blinded statistician using IBM SPSS V26.0 (IBM Corp, New York). All analyses will be conducted following the intention-to-treat (ITT) principle, including all randomized participants who received at least one treatment and completed a comprehensive baseline assessment. Data missing due to participant attrition was imputed utilizing the multivariate imputation by chained equations (MICE) method within the R Project for Statistical Computing (version 4.2.1, www.r-project.org).[52] This approach facilitated the imputation of continuous, binary, and categorical variables. For continuous variables, data will be described using means and standard deviations when the normality assumption is satisfied; for non-parametric data, medians and interquartile ranges will be used. Continuous variables will be analyzed using t-tests or Mann–Whitney *U*-tests. Categorical variables will be described using percentages (%) and analyzed using Chi-square or Fisher's exact tests. A two-tailed test will be applied to the available data, and a *P*-value of less than 0.05 will be considered statistically significant.

Neuroimaging Data Analysis

Data Pre-Processing

fMRI data will undergo preprocessing in MATLAB 2017b (MathWorks, Inc., Natick, MA, USA) utilizing the Data Processing Assistant for Resting-State fMRI (DPARSF) toolbox.[53] The first ten time points of the fMRI data are discarded to mitigate scanner equilibration effects. Slice timing correction addresses temporal offsets between slice

acquisitions, and rigid-body transformations perform head motion correction, with exclusion criteria for movements exceeding 3 mm of translation or 3° of rotation. Spatial normalization is then applied, aligning functional images to the Montreal Neurological Institute (MNI) standard space with a 3 × 3×3 mm isotropic voxel size for uniform spatial resolution. Gaussian smoothing using an 8-mm full-width half maximum (FWHM) kernel reduces noise and enhances the signal-to-noise ratio. Linear trends are removed through detrending, and temporal filtering isolates low-frequency fluctuations (0.01–0.1 hz), providing insights into resting-state neural activity. Rigorous quality control is undertaken through visual inspection of artifacts and motion parameters, ensuring data integrity.

EEG data will be analyzed using Brain Vision Analyzer software 2.0 (Brain Products GmbH, Munich, Germany). Initial preprocessing involves removal of gradient artifacts and ballistocardiographic (BCG) noise, followed by down-sampling the EEG data to 250 hz for computational precision and compatibility with fMRI data. Independent Component Analysis (ICA) removes artifacts from ECG signals and ocular activities, such as eye blinks. Signals are re-referenced to the average of all electrodes to standardize amplitude scale. A band-pass filter (0.5–30 hz) isolates neural activity-relevant frequencies, while low-frequency drifts and high-frequency noise are excluded. Data segments with amplitudes exceeding ±100 μV are discarded, ensuring artifact-free segments for analysis.[54] Synchronization of EEG signals with fMRI acquisition timelines is achieved using scanner trigger pulses.

Data Analysis

Resting-state BOLD-fMRI and EEG data will be analyzed using established software packages, including the FMRIB Software Library (FSL), Analysis of Functional NeuroImages (AFNI), and MELODIC. To explore the spatial and temporal specificity of brain activity influenced by acupuncture, integrated EEG and fMRI activation analyses will be conducted using Brainstorm 3.0 software (https://neuroimage.usc.edu/brainstorm/Introduction). Fusion analyses will incorporate correlation analysis (evaluating the correlation between EEG power and voxel-wise BOLD signal), regression analysis (modeling BOLD signal variance based on EEG features), and joint independent component analysis (jICA) to identify shared spatiotemporal components. To control the family-wise error rate in multiple comparisons, we will employ appropriate methods, such as Gaussian random field (GRF) theory, false discovery rate (FDR) control, and the like. To address motion-related artifacts, six motion parameters (three translation dimensions and three rotation dimensions) will be included as covariates in the General Linear Model (GLM) analysis of fMRI data. For cross-modal data integration, artifacts identified and removed from EEG data (such as ICA) will be synchronously applied to corresponding time windows in the fMRI data, thereby enhancing the reliability of the integrated datasets. Statistical evaluation will involve the use of two-sample t-tests for between-group comparisons and paired-sample t-tests for within-group comparisons, with statistical significance set at $P < 0.05$.

The handling of missing data in EEG-fMRI involves detailed strategies tailored to different data sources, including artifact removal, interpolation methods for specific losses (eg, EEG channel or fMRI timepoints), statistical modeling techniques (eg, multiple imputation or maximum likelihood), and quality assurance through sensitivity analyses and comprehensive reporting to ensure the reliability and validity of results.

Efficacy-Brain Network Correlation Analysis

Structural equation modeling analysis will be applied to investigate the relationship between changes in brain function and cognitive performance across groups. Parameter estimates of brain regions exhibiting significant intergroup differences will be correlated with improvements in neuropsychological scale scores, with a significance threshold of $P < 0.05$. Figure 5 shows the flow diagram of simultaneous EEG-fMRI data analysis.

Discussion

The MCI stage is considered the optimal period for dementia prevention. Given the limited availability of pharmacologic interventions, there is a pressing need to investigate effective non-pharmacologic therapies. A meta-analysis indicates that acupuncture demonstrates cognitive improvement in individuals with MCI and exhibits fewer adverse effects compared to medication.[12] However, the neuroimaging mechanisms underlying the efficacy of acupuncture for MCI

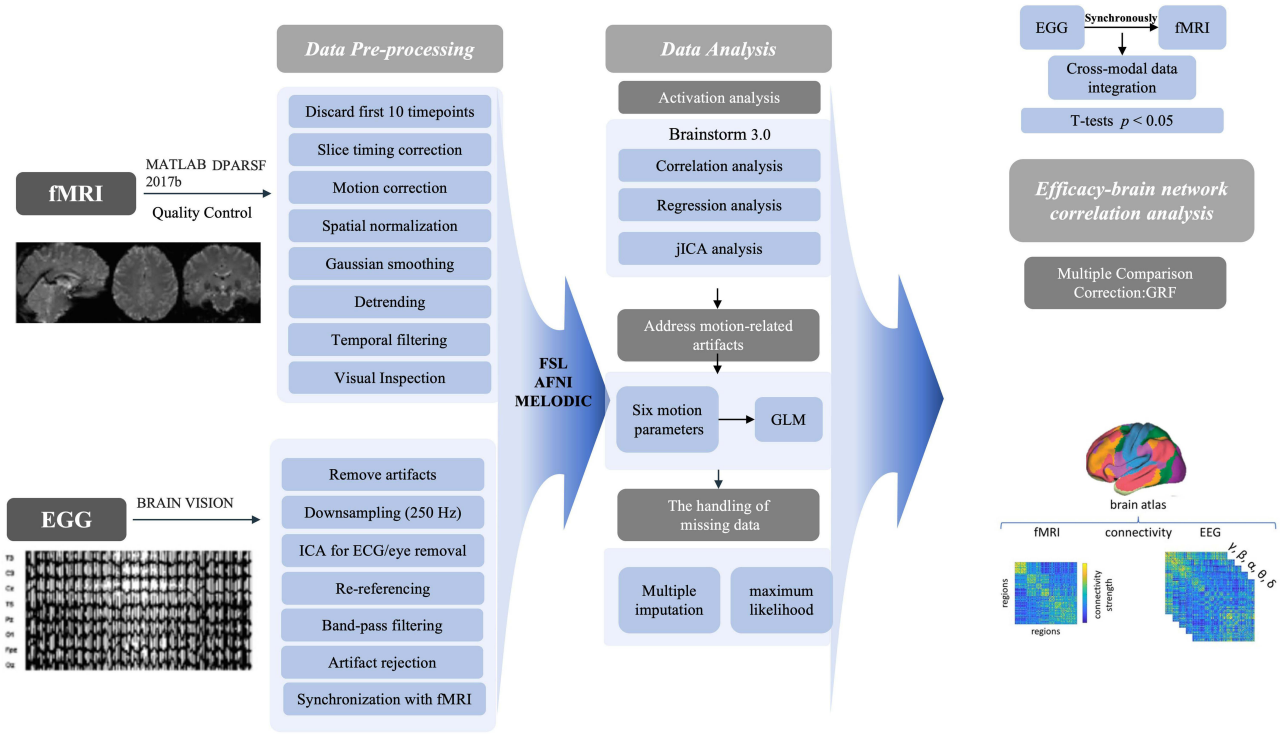

Figure 5 Flow diagram of simultaneous EEG-fMRI data analysis.

are not yet fully understood, hindering its widespread adoption. Therefore, in this study, we propose a randomized controlled trial to investigate its effects on resting-state functional brain activity.

Researchers have observed that the dynamic characteristics of brain activity exhibit greater sensitivity than static characteristics during the early stages of MCI, providing valuable indicators of subtle initial alterations in functional brain connectivity.[55–57] Building on these findings, the combination of fMRI with EEG signals has emerged as a promising approach for investigating cognitive activity with enhanced temporal and spatial precision.[58] Studies have demonstrated a strong positive correlation between fluctuations in alpha-band power within the thalamus and cortex and corresponding fluctuations in the BOLD signal.[59,60] However, in individuals with AD, this association is attenuated in several regions, including the default mode network (DMN), indicating disrupted functional connectivity.[61] Additionally, amyloid accumulation in the brains of MCI patients has been shown to affect EEG-fMRI signals, particularly in the hippocampus and regions associated with cognitive control and visual processing.[62] These findings underscore the potential of simultaneous EEG-fMRI in providing new insights into the neural mechanisms underlying cognitive decline.

The brain's remarkable neuroplasticity and functional compensatory mechanisms suggest opportunities for therapeutic interventions such as acupuncture. Acupuncture has been shown to modulate functional activities both locally within the brain and across interconnected regions, potentially improving cognitive function in MCI patients. For example, a functional near-infrared spectroscopy (fNIRS) study demonstrated that acupuncture increased activation and enhanced the connectivity of the prefrontal cortex in MCI patients.[63] Additionally, a meta-analysis of fMRI studies revealed that acupuncture modulated key brain regions, such as the right insula and the left anterior cingulate/paracingulate gyrus, in MCI patients.[29,64] In our preliminary investigation, we observed that acupuncture not only significantly improved overall cognitive function in MCI patients but also modulated the regional homogeneity (ReHo) of the left dorsolateral superior frontal gyrus.[65]

Despite these promising findings, most existing studies have employed single neuroimaging modalities, thus limiting our understanding of the spatiotemporal mechanisms underlying the cerebral effects of acupuncture. To address this limitation, this study will utilize simultaneous EEG-fMRI to examine the effects of acupuncture on resting-state brain network function in patients with MCI. This approach offers a unique opportunity to investigate the interplay between the

temporal dynamics and spatial patterns of brain connectivity, thereby advancing our understanding of how acupuncture mediates cognitive improvements in MCI. Notably, EEG-fMRI data integration faces challenges like mismatched temporal and spatial resolutions, synchronization errors, and motion artifacts, which can be addressed using techniques such as ICA for artifact removal and spatiotemporal modeling frameworks like JICA. Potential confounding factors, including individual differences (eg, baseline cognitive levels, demographic variables, physiological traits), experimental setup variations (eg, stimulation parameters, environmental noise, synchronization errors), and data acquisition issues (eg, motion artifacts, physiological noise, equipment drift), were controlled using mixed-effects models and further evaluated through sensitivity analyses.

This study also has several limitations that should be acknowledged. First, sham acupuncture was designed as minimally invasive needling at non-meridian points that could elicit physiological responses. However, due to the widespread use of acupuncture in China, non-penetrative sham acupuncture may not ensure patient blinding. Therefore, based on our research group's prior experience, superficial needling at non-acupoints was selected as the placebo control.[35,36] Second, a fixed acupuncture regimen was used without TCM syndrome differentiation, which may not fully capture the potential effects of acupuncture. Furthermore, this study focused on the changes in clinical symptoms and brain activity among patients with MCI before and after acupuncture therapy, without a follow-up period.

This protocol represents the first study to investigate the spatiotemporal neural mechanisms underlying acupuncture in the treatment of mild cognitive impairment (MCI) through simultaneous EEG-fMRI. The findings aim to provide robust clinical evidence supporting acupuncture as a non-invasive, cost-effective, and personalized approach to improving cognitive function and decelerating MCI progression. Furthermore, this research seeks to shed light on acupuncture's effects on cognition-related brain regions, such as the prefrontal cortex and hippocampus, offering valuable insights to guide patient-specific treatment strategies.

Trial Status

Participant recruitment is underway. As of September 1, 2024, we have enrolled 20 participants.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author, Zihan Yin, upon reasonable request.

Ethics Approval and Consent to Participate

The study will strictly follow the Declaration of Helsinki and was approved by the Affiliated Hospital of Chengdu University of TCM (ethical approval number: 2023KL-135-01). All participants will provide written informed consent prior to enrollment, following both verbal and written explanations of the study procedures by the research staff (Supplementary Material 2). The trial was registered on the Chinese Clinical Trial Registry on May 22, 2024 (Registration number: ChiCTR2400084666).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Ya-Qin Li, Zi-Wen Chen and Hui He are co-first authors for this study. The authors declare that they have no competing interests to report for this work.

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