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The efficacy and mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Abstract

Background: Subjective cognitive decline (SCD) is related to the decreased ability of memory and cognition in perception, while objective neuropsychological deficits are not observed. It provides an opportunity for dementia prevention. However, there is no effective treatments recommended for individuals with SCD. Acupuncture, as a non-pharmacologic intervention, has been widely employed for patients with cognitive disorders.

Methods/Design: The proposed study is a randomized, assessor-blind and placebo-controlled study to investigate the efficacy and mechanism of acupuncture in SCD. Sixty patients with SCD will be randomly allocated either into an acupuncture group or a sham acupuncture group. They will receive 24 sessions of real acupuncture treatment or identical treatment sessions using the placebo needle. Global cognitive change based on a comprehensive neuropsychological test battery will be evaluated to detect the clinical efficacy of acupuncture treatment at the baseline and the end of treatment. Magnetic Resonance Imaging (MRI) scans will be used to explore acupuncture related neuroplasticity changes. Correlation analyses will be performed to investigate the relationships between the changes in brain function and symptom improvement.

Discussion: This trial will investigate the effect of acupuncture in SCD patients. We will compare real acupuncture treatment with the sham acupuncture treatment by fMRI to verify neuroplasticity changes. The results of this trial may provide relevant evidences for acupuncture treatment on SCD and also reveal the underlying mechanisms of the treatment effect.

Trial registration: ClinicalTrials.gov NCT03444896. Retrospectively registered on 23 February 2018.

Keywords: Subjective cognitive decline; Dementia; Acupuncture; Sham acupuncture; Magnetic Resonance Imaging; Clinical trial

Strengths and limitations of this study

Compared with the neuropsychological tests alone as efficacy evaluations, the combination with in-vivo measures of brain alternations in this study will be more sensitive in detecting acupuncture efficacy.

This study will firstly uncover the efficacy and mechanism of acupuncture treatment on the older people with SCD.

A comprehensive neuropsychological test battery will be employed, which can assess multiple cognitive domains including executive function, attention, visuospatial function, and language.

Sham needles and adhesive pads will be used for better patients blinding.

A potential limitation is that not a large sample size and no sample size estimates were performed in this study.

INTRODUCTION

Dementia is the greatest global challenge for health and social care in the 21st century [1]. Globally, about 47 million people were living with dementia in 2015, and this number is projected to triple by 2050, based on the World Alzheimer Report (2015). Alzheimer's disease (AD) is the most common form of dementia [2]. Currently, there is no effective cure for AD, and the available treatments have only moderately alleviate symptoms [3]. Therefore, prevention is essential to reduce the dementia epidemic [4 5]. The long "preclinical" phase of AD provides an opportunity for targeted dementia prevention [6-8].

Subjective cognitive decline (SCD) usually occurs in healthy older adults, and it refers to the self-perception of cognitive decline, when individuals perform cognitive tests within normal limits and have preserved activities of daily living [9]. A review demonstrated that the prevalence of these complaints in persons aged 65 years or more varied from 25% to 50% [10]. Actually, many older adults who claim cognitive decline may have been regarded by healthcare professionals as hypochondriacs. However, several lines of evidence from longitudinal aging studies suggests that older adults with SCD are more likely than their healthy peers to present AD biomarkers. About 60% SCD individuals will decline to mild cognitive impairment (MCI) and AD over a 15-year period [11 12]. People with SCD have more brain abnormalities such as

hippocampal volume loss and hypometabolism compared with healthy controls [13]. Moreover, cerebrospinal amyloid β is strongly predictive of subsequent clinical progression in patients with SCD [14]. This suggests that, for some older adults, SCD may represent a preclinical phase of AD.

There is still much plasticity in SCD. The patients in this stage may not progress to dementia or even revert to no cognitive impairment after effective treatment. Development of safe and effective interventions in early AD stages is important. Specific criteria to define SCD have been published [9 15], but clinical trials in SCD are in their infancy, and no pharmacological treatment or interventions is currently recommended for individuals with SCD. Despite these difficulties, the development of new treatments must be encouraged [16].

The accumulated evidence suggests that non-pharmacologic intervention may benefit cognitive function in older adults with SCD [17]. Acupuncture, as a non-pharmacologic intervention, that has been widely used for patients with cognitive disorders. The WHO reports that acupuncture treatment can be beneficial for vascular dementia. A number of clinical studies have provided evidence that acupuncture is beneficial for the treatment of dementia or MCI [18]. A systematic review supports the use of acupuncture for MCI [19]. Animal studies showed that acupuncture elicits its roles by mediation of neural plasticity in pathological conditions [20]. However, the efficacy of acupuncture in patients with SCD has not be investigated. An experimental design is needed to test this hypothesis.

By definition, individuals with SCD are within normal range on clinical-neuropsychological tests. It will be difficult to detect response in this population compared with people who already have clinically manifest impairment (e.g., MCI) due to the ceiling effect. As such, neuropsychological tests in conjunction with in-vivo measures of brain function such as electroencephalo-graph, or functional magnetic resonance imaging (fMRI), may be a more sensitive alternative to neuropsychological tests [21-23]. Brain alterations are subtle but measurable to identify individuals at risk for AD well before cognitive symptoms are manifested by using fMRI, allowing researchers to use neuroimaging to ascertain response after intervention sensitivity [24]. For better ascertaining the clinical response and unveiling the mechanism, brain alterations measured by fMRI could also be used to evaluate the efficacy of acupuncture.

OBJECTIVES

This study is a randomized controlled functional brain imaging trial with 12 weeks of treatment, aiming at: (1) evaluating the effects of acupuncture treatment on cognitive function in older adults with SCD; (2) exploring the central mechanism of the long-lasting effect of acupuncture on SCD; and (3) investigating the safety of acupuncture treatment on SCD.

METHODS

Design

This trial is a 1:1 randomized single-blinded, and placebo-controlled study with two parallel groups involving elderly adults. The study is designed to examine the effect and central mechanism of acupuncture treatment on cognitive function before and after 12 weeks intervention period. As shown in Figure 1, the cognitive assessments and MRI scans will be performed at baseline and immediately after the completion of the intervention.

Ethics

General ethical approval has been obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018 (Ref: 2017BL-061-02). The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement and the CONSORT statement for non-pharmacological interventions have been used as a framework for development of the methodology for this project [24 25]. If any modifications or decision are made, amendments will be reviewed and approved by the ethics eommittee, and new protocols would be uploaded to Clinicaltrials.gov.

Participants

The study will mainly take place in Dongcheng, Fengtai, and Shunyi District in Bejing. People with SCD will be recruited from community-dwelling population in those Districts. In order to have better insight into the trial for community residents, science popularization lectures about dementia and advertisement of the trial will be given in those community service centers. The brochures and posters will also be distributed. Besides, advertisement of the trial will be shared in the official account on WeChat (China's most popular social media platform) of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University and Dongfang

Hospital, Beijing University of Chinese Medicine, health web site (www.39.net) or local newspaper for recruiting participants. Interested individuals will be screened in the clinic, community service centers or by phone using the inclusion/exclusion criteria. All those who meet the inclusion criteria will receive a study information sheet including the design, procedure, benefits, and risks of the study. Before the study procedure started, the subjects will have to provide signed written informed consent forms.

Inclusion criteria

- Male and female adults aged 55-75;
- Native Chinese speakers who are right-handed and have at least 8 years of education;
- Self-reported persistent memory decline compared with a previous normal status within the last 5 years, which was confirmed by caregivers;
- Normal age- and education-adjusted performance on neuropsychological test including the Chinese version of Mini-Mental State Examination (MMSE) [27], the auditory verbal learning test (the short-term delayed free recall, the long-term delayed free recall, and the recognition test) [28], Trail Making Test [29], and Animal Fluency Test [30].
- No or minimal impairment in activities of daily living;

Exclusion criteria

- Presence of a positive neurologic history (e.g., traumatic brain injury, stroke, Parkinson's disease, multiple sclerosis);
- Treatments that would affect cognitive function;
- Presence of significant psychiatric history (e.g., bipolar disorder, schizophrenia) and/or severe anxiety and depression (a score of > 24 on the Hamilton depression rating scale or a score of > 29 on Hamilton anxiety scale);
- Presence of serious heart, kidney, liver, gastrointestinal, infectious, endocrine disease or cancer;
- History of alcohol or drug abuse/addiction;
- Any contraindications for MRI scans (e.g., aneurysm clip pacemaker);

- Significant visual and/or auditory impairment uncorrected by aids, and unable to perform neuropsychological evaluations;
- Currently enrolled in another research study;
- Received acupuncture treatment in the preceding month；

Intervention

Patients will receive 24 acupuncture treatment sessions over 12 weeks. Hwato brand single-use acupuncture needles (size 0.35 × 25 mm or 0.35 × 40 mm), pragmatic placebo needles (size 0.30 × 25 mm), and SDZ-V electroacupuncture apparatuses will be used.

For the acupuncture group (Figure 2), acupuncture needles will be placed at acupoints Baihui (DU20), Shengting (DU24), Fengfu (DU16), Fengchi(GB20), Danzhong (RN17), Zhongwan (RN12), Qihai (RN6), Neiguan (PC6), Tongli (HT5), Xuehai (SP10), Zusanli (ST36), Zhaohai (KI6), Xinshu (BL15), and Yixi (BL45). After skin disinfection in patient in the supine position, the adhesive pads are pasted on the acupoints surface except for Baihui (DU20) and Shengting (DU24). Then, the acupuncture needles will be inserted through the adhesive pads approximately 5 to 15 mm into the skin depending on the location of the needle. Manual acupuncture by acupuncturists using a small, equal manipulations of twirling, lifting, and thrusting will performed on all needles to reach *deqi*. The patients will feel the *deqi* sensation, such as soreness, numbness, distention, heaviness, and other sensations. Paired electrodes from the electroacupuncture apparatus will be attached to the needle holders of the DU20 and DU24. A dilatational wave of 2-100 Hz and a current intensity of 1 to 5 mA will be performed following by the degree of needle handle shivering, ignoring the patient's feelings. The needles will be extracted after 20 minutes for each treatment. The acupoints of DU16, BL15 and BL45 will achieve *deqi* in patients with sitting position without retaining needle.

For the sham acupuncture group, sham acupoints at locations away from known acupuncture points will be used to minimize physiological effects, and the location of sham acupoints are showed in Table 1. Patients will receive noninsertive acupuncture using the pragmatic placebo needles (Hwato brand, size 0.35 × 25 mm; supplement figure 1). As same as acupuncture group, the adhesive pads are firstly pasted on the sham acupoints surface except for sham acupoint 1. The pragmatic placebo needles with a blunt tip will be placed on the adhesive pads. In order to

minimize the physiological effect, acupuncturists will be instructed to lightly place the placebo needle on the adhesive pads with no manipulation. The acupuncture needle will be inserted to a shallow depth at the sham acupoint 1, which does not penetrate below the skin, and needle manipulation for *deqi*. Sham acupoint 11 and Sham acupoint 12 will be inserted with placebo needles without retaining needle. Paired electrodes will be attached the needle holders of the bilateral sham acupoint 2 but with no electricity output.

In the treatment session, the concomitant medications which taking by enrolled patients will be record such as aspirin, antihypertensive agents, and lipid-reducing agents. The enrolled patients who use of medications that may affect cognitive functioning, including tranquilizers, anti-anxiolytics, hypnotics, nootropics, and cholinomimetic agents will be discontinued.

Table 1. Location of sham acupoints used in the sham acupuncture group.

Sham Acupoint	Location
Sham acupoint 1	Midpoint of Shuaigu (GB8) and Touwei (ST8)
Sham acupoint 2	Midpoint of Touwei (ST8) and Yangbai(GB14)
Sham acupoint 3	Midpoint between Tianyou (SJ16) and Tianrong(SI17)
Sham acupoint 4	4 cun above the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 5	2 cun below the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 6	1 cun outside the point 1/4 of the line between Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 7	1 cun outside the midpoint of Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 8	6 cun above mediosuperior border of the patella
Sham acupoint 9	3 cun below the Yanglingquan (GB34) and in the middle of the gallbladder and bladder channels
Sham acupoint 10	Midpoint between Jiexi (ST 41) and Qiuxu (GB40)
Sham acupoint 11	2 cun from the lower border of the spinous process of the fifth thoracic vertebra
Sham acupoint 12	2 cun from the lower border of the spinous process of the sixth thoracic vertebra

MRI protocol

Patients will undergo brain MRI at baseline and after treatments. The MRI scan will be performed with a 3.0 Tesla superconductor (Skyra, Siemens, Erlangen, Germany) in the Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University. The parameters of sequences employed in this study are provided by China Association of Brain Imaging (www.abimaging.org). Sagittal structural images will be acquired using a magnetization prepared rapid gradient echo (MP-RAGE) three-dimensional T1-weighted sequence with the following parameters: TR/TE = 2,530/2.98 ms, flip angle = 7◦, inversion time = 1100 ms, matrix = 256 × 256, 1 mm slice thickness without slice gap. T2*-weighted functional images will be collected using a gradient-echo echo-planar imaging sequence with the following parameters: repetition time = 3200 ms, echo time = 407 ms, flip angle = 120°.

Resting state-fMRI and task fMRI will perform using an echo planar imaging (EPI) sequence with the following parameters: TR/TE = 2,000/30 ms, flip angle = 90◦, matrix = 64 × 64, slice thickness = 3.5 mm with 1 mm slice gap. Diffusion tensor imaging (DTI) will use a double spin-echo echo-planar imaging sequence (TR/TE = 12,000/77 ms, flip angle = 90°, Volume interval = 12, 2 mm thick axial slices). ASL imaging of the whole brain is performed by use of a 3D pseudocontinuous ASL sequence (TR/TE = 5,000/ 15.92 ms, flip angle = 180°, Slice thickness = 3.5 mm; labeling duration, number of slices = 40).

All scans will be reviewed qualitatively by a radiologist to screen for possible brain lesions or structural abnormalities. DTI images will be analyzed using the PANDA package [31]. The functional MRI data collected during the memory task and rest, and ASL data will be performed with the Resting-State fMRI (DPARSF) toolbox and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) for MATLAB. Brain activation, connectivity changes, and cerebral blood flow will be compared between two groups before and after treatment.

Outcome measures

Clinical outcome assessments

Global cognitive function based on a composite score will be used to evaluate the clinical efficacy of acupuncture treatment at baseline, and at the end of the treatment. It will be computed by averaging z-scores from a comprehensive neuropsychological test battery that includes 6 tests.

Animal Fluency Test (AFT) that assess the language and executive function by examining categorical verbal fluency [30]. Trail Making Test (TMT) part A and B examines graphomotor speed, attention, and executive function [32]. Digit Symbol Substitution Test (DSST) is used to assess processing speed, attention and concentration [33]. Stroop color word test (SCWT) is a classic instrument for the assessment of selective attention, cognitive flexibility, cognitive inhibition, and information processing speed [34]. Auditory Verbal Learning Test (AVLT) a powerful neuropsychological test to assess episodic memory [35 36].

All patients will do a subjective cognitive decline questionnaire (SCDQ) [37] to assess their degree of subjective cognitive decline. The Santa Barbara Sense of Direction Scale (SBSDS) [38] will be used to measure the ability in facial recognition and direction. Depressive, anxiety symptoms, and sleep quality are measured by Hamilton Rating Scale for Depression (HAMD) [39], Hamilton Rating Scale for Anxiety (HAMA) [40], and Pittsburgh Sleep Quality Index (PSQI) [41].

Neuroplasticity assessments

In this study, neuroplasticity changes between two groups, as measured by fMRI both before and after the acupuncture treatment, will be used as outcome measures (Figure 3). Brain activity and functional connectivity will be assessed under a resting state and a memory task. Besides, group difference on cerebral blood flow and white matter integrity will be assessed by means of arterial spin labeling (ASL) or diffusion tensor imaging (DTI).

Memory task. Before entering the fMRI scanner, patients will complete a practice version of fMRI paradigm. This practice task guarantees that the patients understand the rule of the memory task in the scanner. A blocked periodic design that incorporated alternating 1-back, and 2-back tasks will be used. Patients will view facial pictures, scene pictures or single digits (0–9, black on a white background). In the 1-back task, the patients are required to press a button when a facial picture, scene picture or digit appears that is the same as the one before. In the 2-back task, the patients are asked to press a response button if the current facial picture, scene picture or digit is the same as the one which was presented two trials before. The stimuli consisted of 17 blocks for 236 seconds and are presented on a computer using MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) for 1 second, with an inter-stimulus interval of 1 second. Trial types within blocks are presented in pseudorandom order.

Once in the scanner, anatomical and functional scans will be performed. During the scanning all patients are asked to lie quietly in the scanner with their eyes open, trying to avoid thinking systematically, and moving as little as possible. In resting state fMRI scanning, the patients are instructed to view centrally placed fixation cross (+), and try to not think of anything. Two memory task runs will be conducted after T1 and resting state scans, then following by ASL, and DTI scans.

Apolipoprotein E genotyping

A TIANamp Blood DNA Kit (TIANGEN BIOTECH, Beijing, China) will be used to isolate High-molecular-weight DNA. The genotype of APOE will be detected by APOE Gene Detection Kit (Wuhan YZY Biopharma, Wuhan, China) with E2 and E4 reaction liquid. Genomic DNA and reaction liquid will be place on a 7500 HT Fast Real-Time PCR (Applied Biosystems, Foster City, USA) with the following conditions: Uracil-N-Glycosylase treatment at 37 °C for 10 minutes, denaturation at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 15 s and annealing at 60 °C for 60 s (acquiring the fluorescence signal in this step). According to the amplification plot, the genotype of APOE will be determined.

Safety and Monitoring

Treatment-related adverse events, including hematoma, bleeding, subcutaneous hemorrhage, serious pain, local infection and fatigue will be compared among patients in the acupuncture group and the sham acupuncture group, and will be documented at each treatment session. Severe adverse events will report to the relevant responsible person. The enrolled patients will signature in the diary card after each treatment for monitoring adherence. In order to ensure consistency, the neuropsychological tests and other assessment scales will be performed by the same researcher at baseline and the end of treatment. A professional staff will check the imaging data for quality and protocol conformity after each scanning session. Patients will undergo blood routine test and blood biochemical tests including blood glucose, homocysteine, total cholesterol, etc. before randomization and at the end of treatment.

Sample size and blinding

Task fMRI studies characteristically include small sample size [42] and thus have a low statistical power. The statistically underpowered study by definition means that a study will have

less of a chance for detecting significant effects [43]. Therefore, power-based sample size will be calculated prior to fMRI data collection [44]. Fortunately, approaches for sample size calculations in studies using functional magnetic resonance imaging have been developed.

By using the non-central random field theory, Hayasaka et al estimated that at least 12 subjects would be required to detect signals in either of the auditory cortices with at least 80% power [45]. They also found that approximately 13 subjects would be required to detect signals in the auditory cortices with 80% power, when generated a sample size map based on the mock pilot analysis. Adopting a simulation-based method to calculate statistical power for group-level fMRI studies, Desmond and Glover found a minimum of 12 subjects are required to achieve 80% power at a = 0.05 at the single voxel level [46]. For a more realistic threshold, twice as many subjects are recommended to maintain this level of power after correcting for multiple comparisons. For a stricter alpha of 0.000002, approximately 25 subjects are needed. Mumford and Nichol recommended 20 subjects and a Type I error of $\alpha = 0.005$ should probably be used, and this power calculation is based on a non-central T or F distribution [47]. We used the largest sample size of 25 for each group with an estimated dropout rate and loss of data due to head motion, then we planned to enroll 60 participants in the two groups.

Eligible patients will be randomly assigned into either the acupuncture or sham acupuncture group after signed written informed consent forms via a randomization system for clinical research using a 1:1 ratio with a block size of 6. The randomization list will be stored by a noninvolved investigator and out of reach and sight of the involved investigators. The patients, outcome assessors, and statisticians will be blinded to treatment allocation.

Data collection and management

At baseline, information of patients about age, sex, education, dominant hand, and medical history will be collected (Figure 4). For all patients that met the inclusion criteria, the neuropsychological tests will be performed before and after treatment in a quiet room by evaluators, who have been trained by Alliance of early AD. All of the MRI scans will be conducted in the same machine, and the operators have been technically trained by a professional engineer from the China Association of Brain Imaging. Blood samples will be collected in the morning and handled by 2 persons. After each treatment session, adverse event will be recorded in participant's diary card by acupuncturists.

A case report form (CRF) will be used to collect the clinical data for each patient, labeled by unique numeric identifier and recorded by a trained graduate student. The data in the CRF will be verified for accuracy, missing data, and data consistency by a clinical research associate. Paper-based research data will be entered into an EpiData electronic database. For reducing errors in data entry, data will be entered independently twice by two independent people, and value pairs will then be compared for discordances, followed by resolution of discordances by referral to the original data source. The EpiData will export data in a Microsoft Excel spreadsheet formats. The data will be entered in an electronic Statistical Package SPSS software (SPSS 12.0 KO for Windows ©) for ease of statistical analysis.

Statistical analysis

Clinical data analysis

In this study, per protocol analysis will be used. The per protocol population defined as all randomized patients who did not discontinue prematurely, completed the treatment and had MRI scans before and after treatment. Observation of histograms and normal probability plots and a Shapiro–Wilk test will be performed to determine the data whether followed a normal distribution. Distributed data will be described using means, standard deviations, and 95% confidence intervals (CIs). Clinical outcome analyses will be done with SPSS software (SPSS 12.0 KO for Windows ©), with a significance level of 0.05, and all hypothesis tests are two-tailed.

Baseline demographic characteristics between the two groups will be analyzed with χ^2 test or Fisher's exact test for categorical measures, and with the T-test or Wilcoxon rank-sum test for continuous measures. The clinical outcome of the global cognitive function based on composite scores will be compared between the acupuncture group and sham acupuncture group. The composite scores will be created by converting all individual cognitive scores to standardized z scores. As previously described [48], we computed z-scores by subtracting the baseline group mean and dividing the baseline group standard deviation, and then averaging the standardized z scores across all tests. The general linear model will be used to examine change in cognitive scores. For the other outcomes, SCDQ, SBSDS, HAMD, HAMA, and PSQI will be analyzed with independent T-test if it agreed with normal distribution or Wilcoxon's signed rank test for normal distribution data. The statisticians who analyzed the data are blinded to the test settings.

MRI data analysis

For imaging data will be analyzed using DPABI toolkit performing on MATLAB 8.6 (Mathworks,Inc., Natick, MA, USA) to detect any changes in brain function due to acupuncture treatment. After data preprocessing, some graph theory-based or data driven approaches will be performed to investigate neuroplasticity between two groups, such as regional homogeneity, amplitude of low-frequency fluctuation, and voxel-wise degree centrality. A two-sample t-test will be conducted to investigate the differences in brain regions between acupuncture group and sham acupuncture group in the DPARSF software. Multiple comparisons will be used in order to better control a high inflated false positive rate. Pearson's correlation analysis will be performed to examine the association between the fMRI image data and clinical variables.

Public involvement and dissemination

The participants can achieve the study results via social media and software. The results of the study will be published in a peer-reviewed academic journal and will also be disseminated electronically through conference presentations.

DISCUSSION

SCD, as the initial phase of AD, could serve as a window of opportunity for interventions at early disease stages [23]. This study is planned as a randomized, assessor-blind, placebo-controlled trial to evaluate the efficacy and central mechanism of acupuncture treatment on SCD compared with the sham acupuncture group. To our knowledge, this trial is also the first study to investigate the effect of acupuncture on SCD patients.

This study has several strengths. First, we will assess patients with SCD on multiple levels, including comprehensive neuropsychological tests, functional brain alterations, cerebrovascular risk factors, and AOPE genotyping. This comprehensive assessment will be used to identify possible biomarkers involved in the effects of acupuncture in SCD.

Second, the neuropsychological assessment in the present study is a comprehensive neuropsychological test battery that included six tests, which can assess multiple cognitive domains including executive functions, attention, visuospatial functioning, and language. Memory decline associated with AD risk factors [49 50], and then most instruments were used to

measure SCD focus on memory [51]. However, it might be too restrictive to limit the SCD assessment to memory, in particular for atypical forms of AD [9 52]. In this study, multi-domain cognitive function will be assessed as well.

Additionally, functional brain alterations will be used as an outcome measures. Compared with the neuropsychological tests alone, the combination of in-vivo measures of brain alternations in this study will be more sensitive in detecting acupuncture efficacy. Smaller training effects and ceiling effect will be found when using MRI [53]. Furthermore, it can be used as a tool to understand the mechanism of the acupuncture's effects. Functional MRI can identify whether neural efficiency is improved or the brain connectome is reorganized to achieve cognitive enhancement [54].

A potential limitation is that the patients in this study are recruited from community. Considering the research environment, individuals recruited from a memory clinic may have a higher probability of having preclinical AD. They had specific concerns sufficient to prompt a medical visit [15]. However, community based studies have shown some predictive value of SCD for cognitive decline and dementia [10 55]. Another potential limitation of our study is that it will not include a large sample size, and this problem may lead to a future study. The main feature of our study is to systemically investigate and analyze the central mechanisms of acupuncture treatment in patients with SCD. The findings may provide deeper insight into the benefits and mechanisms of acupuncture for patients with SCD.

Trial status

This trial is currently in the recruitment phase.

Figure Legends

Figure 1. Flow chart.

Figure 2. Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

Figure 3. Magnetic resonance imaging experiment paradigm and Illustrative diagram of the memory task.

Figure 4. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of the trial.

Supplement figure 1. (a) The adhesive pads and sham needles with a blunt tip; (b) The adhesive pad was pasted on the surface of skin; (c) The sham needle was placed on the adhesive pad.

List of abbreviations SCD: Subjective Cognitive Decline; AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; APOE: apolipoprotein E; MRI: Magnetic Resonance Imaging; AFT: Animal Fluency Test; TMT: Trail Making Test; DSST: Digit Symbol Substitution Test; SCWT: Stroop Color Word Test; AVLT: Auditory Verbal Learning Test; SCDQ: Subjective Cognitive Decline Questionnaire; SBSDS: Santa Barbara Sense of Direction Scale; HAMD: Hamilton Rating Scale for Depression; HAMA: Hamilton Rating Scale for Anxiety; PSQI: Pittsburgh Sleep Quality Index.

Ethics approval and consent to participate Ethics approval: The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial. The methods were carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from each patient.

Consent for publication Not applicable.

Availability of data and materials Not applicable. This is a study protocol only and as such no unpublished data are available. The sponsor will access to the final trial dataset.

Competing interests The authors declare that they have no competing interests.

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Authors' contributors Conceived and designed the experiments: CZL and JWH. Performed the experiments: PZ, ZYW, CQY, GXS, SQH JQL, YNZ, XW, JFT. Wrote the paper: CQY, XW and CZL. All authors approved the final manuscript.

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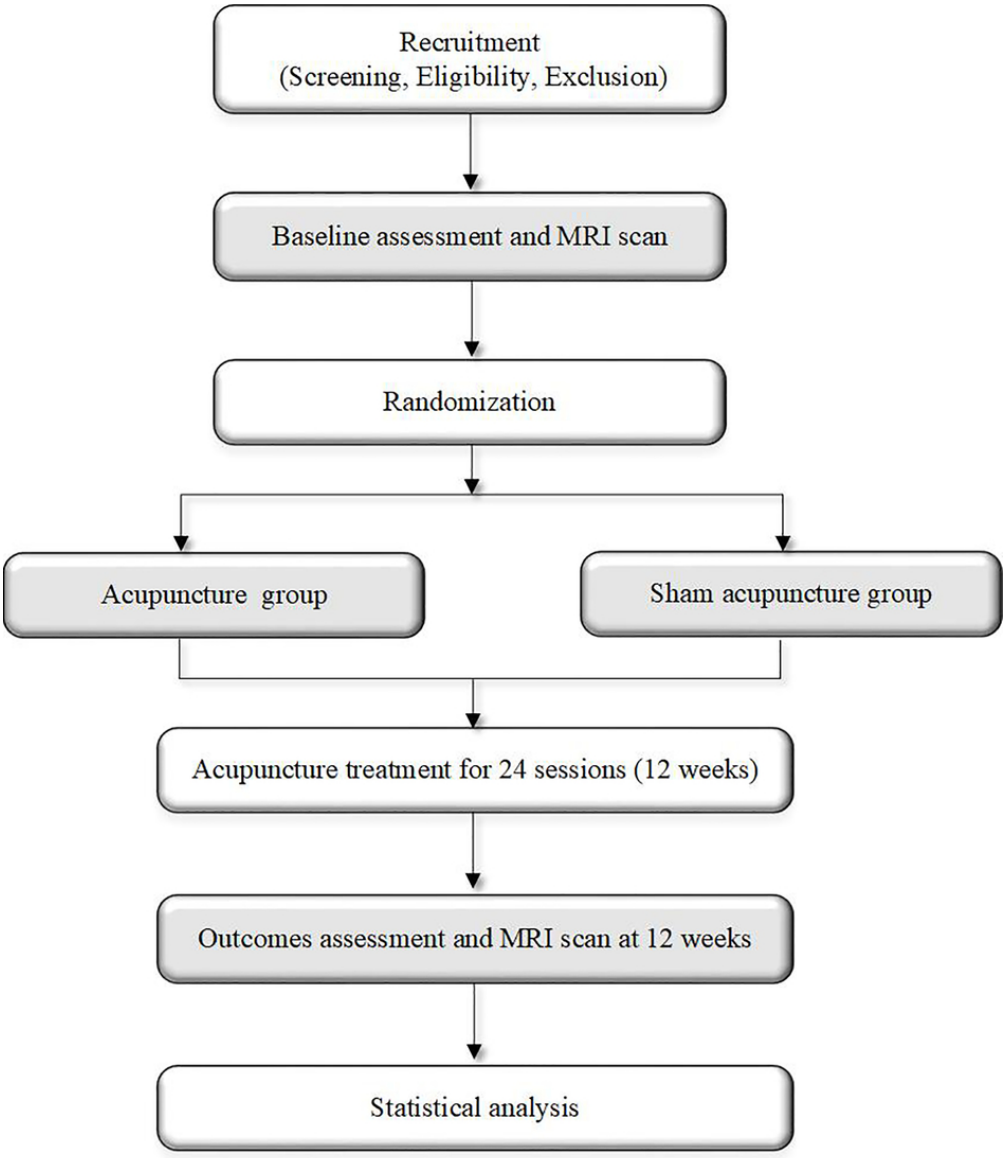

Flow chart.

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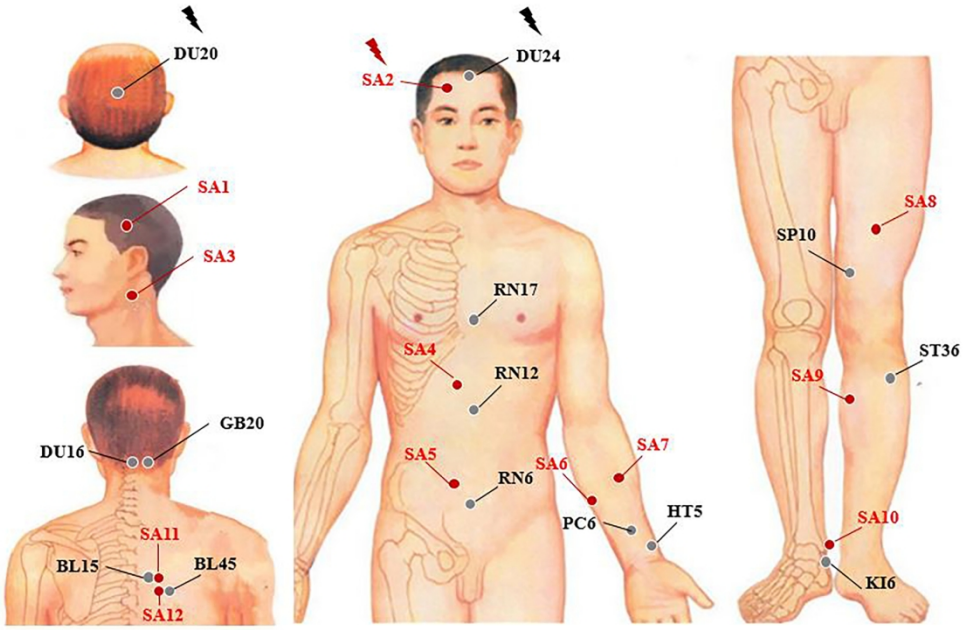

Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

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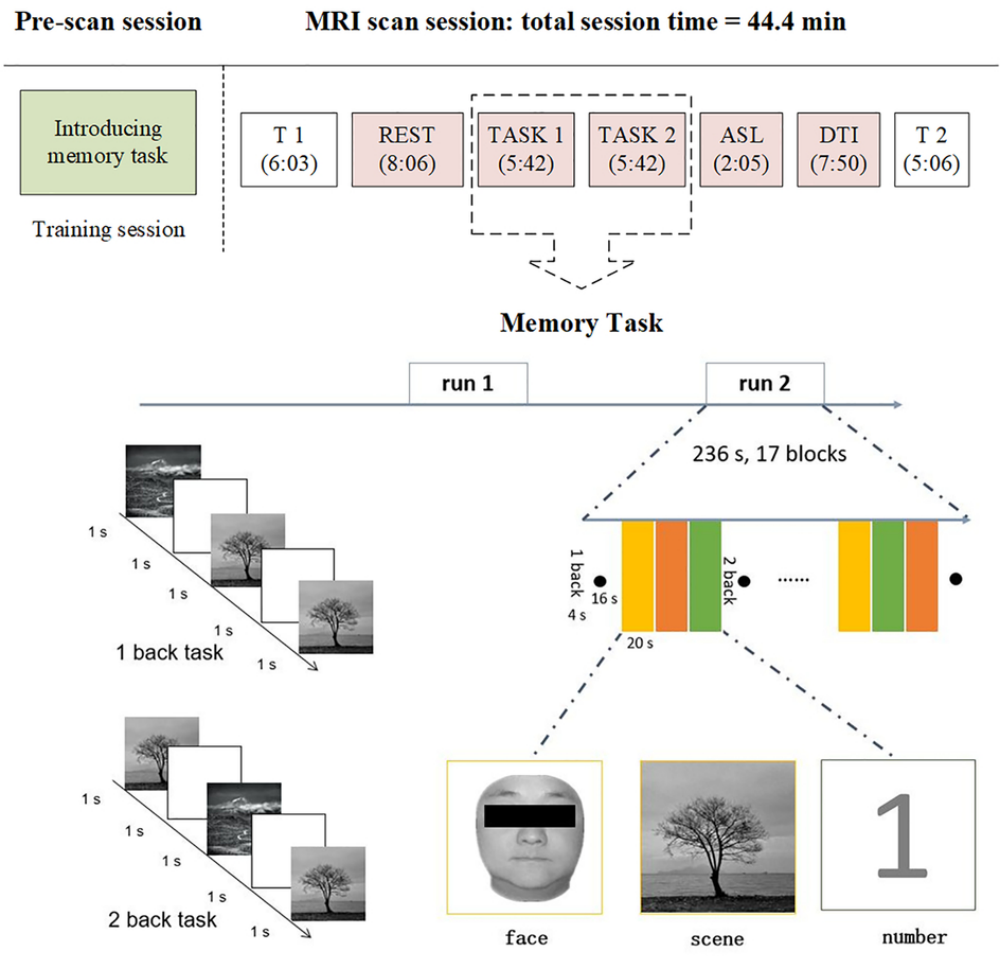

Magnetic resonance imaging experiment paradigm and Illustrative diagram of the memory task.

79x77mm (300 x 300 DPI)

Study period				
Items	Baseline		Treatment phase	Outcome assessment
Time point	-1 week	0 week	1- 12 weeks	13-14 weeks
Enrolment				
Eligibility screen	X			
Informed consent	X			
Examination		X		
Randomization		X		
Intervention				
Acupuncture (n = 30)			X	
Sham acupuncture (n = 30)			X	
MRI scan				
Acupuncture		X		X
Sham acupuncture		X		X
Assessment				
AFT		X		X
TMT		X		X
DSST		X		X
SCWT		X		X
AVLT		X		X
SCDQ		X		X
SBSDS		X		X
HAMD		X		X
HAMA		X		X
PSQI		X		X
Safety				
Laboratory test		X		X
Adverse events			X	X

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of the trial.

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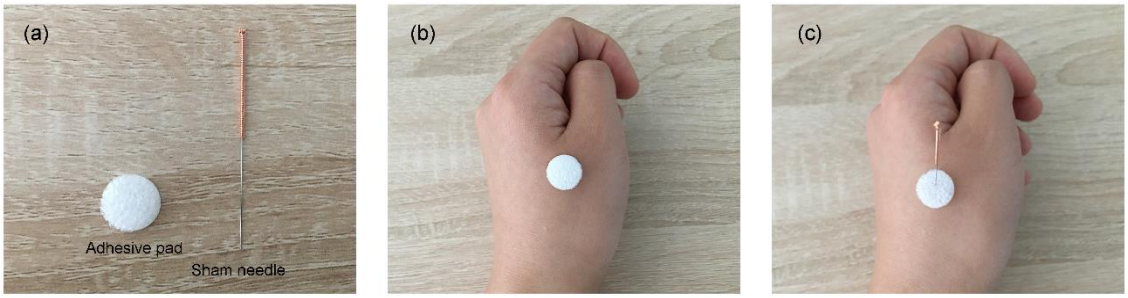
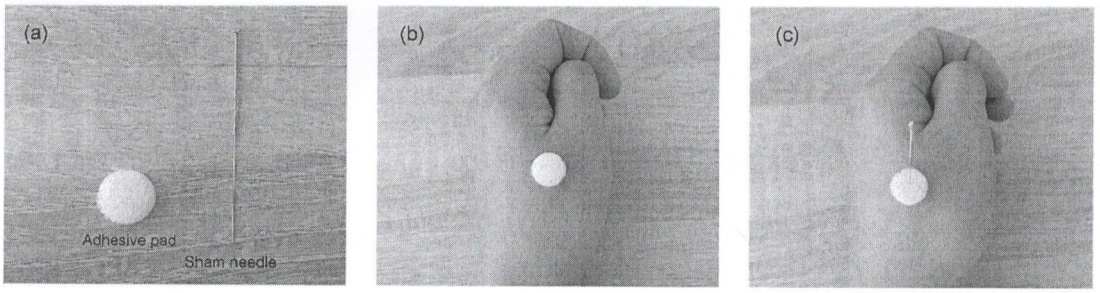


Supplement figure 1. Pictures of sham needle. (a) The adhesive pad and sham needle with a blunt tip; (b) The adhesive pad is pasted on the surface of skin; (c) The sham needle is placed on the adhesive pad.



Supplement figure 1. Pictures of sham needle. (a) The adhesive pad and sham needle with a blunt tip; (b) The adhesive pad is pasted on the surface of skin; (c) The sham needle is placed on the adhesive pad.

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电子邮箱地址： tujianfeng1@126.com *电话号码：* 15210516192

日期： 2018.12.4

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	5
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	Not statement
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	5,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11,12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3–4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9–11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Figure 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11,12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Not statement
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	5

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not offer in the manuscript
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Primary Subject Heading:	Neurology
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The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Abstract

Background: Subjective cognitive decline (SCD) refers to individuals' perceived decline in memory and/or other cognitive abilities relative to their previous level of performance, while objective neuropsychological deficits are not observed. SCD may represent a preclinical phase of Alzheimer's disease. At this very early stage of decline, intervention could slow the rate of incipient decline to prolong and preserve cognitive and functional abilities. However, there is no effective treatments recommended for individuals with SCD. Acupuncture, as a non-pharmacologic intervention, has been widely employed for patients with cognitive disorders.

Methods/Design: The proposed study is a randomized, assessor-blinded and placebo-controlled study to investigate the efficacy and mechanism of acupuncture in SCD. Sixty patients with SCD will be randomly allocated either into an acupuncture group or a sham acupuncture group. They will receive 24 sessions of real acupuncture treatment or identical treatment sessions using a placebo needle. Global cognitive changes based on a multi-domain neuropsychological test battery will be evaluated to detect the clinical efficacy of acupuncture treatment at baseline and end of treatment. Magnetic Resonance Imaging (MRI) scans will be used to explore acupuncture related neuroplasticity changes. Correlation analyses will be performed to investigate the relationships between the changes in brain function and symptom improvement.

Discussion: This trial will investigate the effect of acupuncture in SCD patients. We will compare real acupuncture treatment with the sham acupuncture treatment by fMRI to verify neuroplasticity changes. The results of this trial may provide relevant evidences for acupuncture treatment on SCD and also reveal the underlying mechanisms of the treatment effect.

Trial registration: ClinicalTrials.gov NCT03444896. Retrospectively registered on 23 February 2018.

Keywords: Subjective cognitive decline; Dementia; Acupuncture; Sham acupuncture; Magnetic Resonance Imaging; Clinical trial

Strengths and limitations of this study

Compared with the neuropsychological tests alone as efficacy evaluations, the combination with in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy.

This study will firstly uncover the efficacy and neural mechanism of acupuncture treatment in older adults with SCD.

A multi-domain neuropsychological test battery will be employed, which can assess multiple cognitive domains including executive function, attention, visuospatial function, and language.

Sham needles and adhesive pads will be used for better patient blinding.

A potential limitation is that not a large sample size will be performed in this study.

INTRODUCTION

Dementia is the greatest global challenge for health and social care in the 21st century [1]. Globally, about 47 million people were living with dementia in 2015, and this number is projected to triple by 2050, based on the World Alzheimer Report (2015). Alzheimer's disease (AD) is the most common form of dementia [2]. Currently, there is no effective cure for AD, and the available treatments have only moderately alleviate symptoms [3]. Therefore, prevention is essential to reduce the dementia epidemic [4 5]. The long "preclinical" phase of AD provides an opportunity for individuals to participate in treatment trials to delay or prevent cognitive decline [6-8].

Subjective cognitive decline (SCD) usually occurs in older adults, and it refers to the self-perception of cognitive decline, when individuals perform cognitive tests within normal limits and have preserved activities of daily living [9]. A review demonstrated that the prevalence of these complaints in persons aged 65 years or more varied from 25% to 50% [10]. Actually, many older adults claim cognitive decline might be regarded as hypochondriacs by professional healthy carers. However, several lines of evidence from longitudinal aging studies suggests that older adults with SCD are more likely to present AD biomarkers than their healthy peers. About 60% SCD individuals will decline to mild cognitive impairment (MCI) and AD over a 15-year

period [11 12]. People with SCD have more brain abnormalities such as hippocampal volume loss and hypometabolism compared with healthy controls [13]. Moreover, cerebrospinal amyloid β is strongly predictive of subsequent clinical progression in patients with SCD [14]. This suggests that, for some older adults, SCD may represent a preclinical phase of AD.

There is still much plasticity in SCD. The patients in this stage may not progress to dementia or even revert to no cognitive impairment after effective treatment. Development of safe and effective interventions in early AD stages is important. Specific criteria to define SCD have been published [9 15], but clinical trials in SCD are in their infancy, and no pharmacological treatment or interventions is currently recommended for individuals with SCD. Despite these difficulties, the development of new treatments should be encouraged [16].

The accumulated evidence suggests that non-pharmacologic intervention may benefit cognitive function in older adults with SCD [17]. Acupuncture, as a non-pharmacologic intervention, that has been widely used for patients with cognitive disorders. The WHO reports that acupuncture treatment can be beneficial for vascular dementia. A number of clinical studies have provided evidence that acupuncture is beneficial for the treatment of dementia or MCI [18]. A systematic review supports the use of acupuncture for MCI [19]. Animal studies showed that acupuncture elicits its effects by mediation of neural plasticity in pathological conditions [20]. However, the efficacy of acupuncture in patients with SCD has not be investigated. An experimental study is needed to test this hypothesis.

By definition, individuals with SCD are within the normal range on clinical-neuropsychological tests. It will be difficult to detect response in this population compared with people who already have clinically manifest impairment (e.g., MCI) due to a ceiling effect. As such, neuropsychological tests in conjunction with in-vivo measures of brain function such as electroencephalo-graphy, or functional magnetic resonance imaging (fMRI), may be a more sensitive alternative to neuropsychological tests [21-23]. Brain alterations are subtle but measurable to identify individuals at risk for AD well before cognitive symptoms are manifested by using fMRI, allowing researchers to use neuroimaging to ascertain response after intervention sensitivity [24]. For better ascertaining the clinical response and unveiling the mechanism, brain alterations measured by fMRI could also be used to evaluate the efficacy of acupuncture.

OBJECTIVES

This study is a randomized controlled functional brain imaging trial with 12 weeks of treatment, aiming at: (1) evaluating the effects of acupuncture treatment on cognitive function in older adults with SCD; (2) exploring the central mechanism of the long-lasting effect of acupuncture on SCD; and (3) investigating the safety of acupuncture treatment on SCD.

METHODS

Design

General ethical approval was obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University (Ref: 2017BL-061-03). The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement for non-pharmacological interventions has been used as a framework for development of the methodology for this project [25 26].

This trial is a 1:1 randomized single-blinded, and placebo-controlled study with two parallel groups involving elderly adults. The study is designed to examine the effect and neural mechanism of acupuncture treatment on cognitive function before and after a 12 weeks intervention period. As shown in Figure 1, the cognitive assessments and MRI scans will be performed at baseline and immediately after the completion of the intervention.

Participants

The study will mainly take place in Dongcheng, Fengtai, and Shunyi District in Bejing. People with SCD will be recruited from community-dwelling population in those Districts. In order to have better insight into the trial for community residents, science popularization lectures about dementia and advertisement of the trial will be given in community service centers. The brochures and posters will also be distributed. Besides, advertisement of the trial will be shared in the official account on WeChat (China's most popular social media platform) of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University and health web site (www.39.net) or local newspaper for recruiting participants. Interested individuals will be screened in the clinic, community service centers or by phone using the inclusion/exclusion criteria. All those who meet the inclusion criteria will receive a study information sheet including

the design, procedure, benefits, and risks of the study. Before the study procedure started, the subjects will have to provide signed written informed consent forms. The diagnosis of SCD will be based on the published SCD research criteria proposed by the Subjective Cognitive Decline Initiative (SCD-I) [9].

Inclusion criteria

- Male and female adults aged 55-75;
- Native Chinese speakers who are right-handed and have at least a primary school education;
- Self-reported persistent memory decline compared with a previous normal status within the last 5 years, which is confirmed by caregivers;
- Normal age- and education-adjusted performance on neuropsychological test including the Chinese version of Mini-Mental State Examination (MMSE) [27], the auditory verbal learning test (the short-term delayed free recall, the long-term delayed free recall, and the recognition test) [28], Trail Making Test [29], and Animal Fluency Test [30].
- No or minimal impairment in activities of daily living;

Exclusion criteria

- Presence of a positive neurologic history (e.g., traumatic brain injury, stroke, Parkinson's disease, multiple sclerosis);
- Treatments which would affect cognitive function (e.g., treatment for an acute psychiatric episode, therapy with memantine, rivastigmine and donepezil, traditional Chinese medicines which improve cognitive function such as Bushen capsule);
- Presence of significant psychiatric history (e.g., bipolar disorder, schizophrenia) and/or severe anxiety and depression;
- Presence of serious heart, kidney, liver, gastrointestinal, infectious, endocrine disease or cancer;
- History of alcohol or drug abuse/addiction;
- Any contraindications for MRI scans (e.g., aneurysm clip pacemaker);

- Significant visual and/or auditory impairment uncorrected by aids, and unable to perform neuropsychological evaluations;
- Currently enrolled in another research study;
- Received acupuncture treatment in the preceding month；

During the trial period, patients with SCD who meet the following criteria will be excluded from the study:

- taking medication or receiving additional treatment that is expected to affect the cognitive function (e.g., tranquilizers, antianxiolytics, hypnotics, nootropics, and cholinomimetic agents);
- withdrawal of consent for study participation because the patients does not wish to continue;
- missing more than 5 of 24 acupuncture treatment sessions;
- occurrence of a serious adverse event that the doctors consider should termination;
- critical protocol violation such as violation of eligibility criteria.

Intervention

Patients will receive 24 acupuncture treatment sessions over 12 weeks (twice a week). Hwato brand single-use acupuncture needles (size 0.35 × 25 mm or 0.35 × 40 mm), pragmatic placebo needles (size 0.30 × 25 mm), and SDZ-V electroacupuncture apparatuses will be used.

For the acupuncture group (Figure 2), acupuncture needles will be placed at acupoints Baihui (DU20), Shengting (DU24), Fengfu (DU16), Fengchi(GB20), Danzhong (RN17), Zhongwan (RN12), Qihai (RN6), Neiguan (PC6), Tongli (HT5), Xuehai (SP10), Zusanli (ST36), Zhaohai (KI6), Xinshu (BL15), and Yixi (BL45). After skin disinfection in patient in the supine position, the adhesive pads are pasted on the acupoints surface except for Baihui (DU20) and Shengting (DU24). Then, the acupuncture needles will be inserted through the adhesive pads into the skin depending on the location of the needle. The location is show in Table 1. Manual acupuncture by acupuncturists using a small, equal manipulations of twirling, lifting, and thrusting will performed on all needles to reach deqi. The patients will feel the deqi sensation, such as soreness, numbness, distention, heaviness, and other sensations. Paired electrodes from the electroacupuncture apparatus will be attached to the needle holders of the DU20 and DU24. A dilatational wave of 2-100 Hz and a current intensity of 1 to 5 mA will be performed following

by the degree of needle handle shivering, ignoring the patient's feelings. The needles will be extracted after 20 minutes for each treatment. The acupoints of DU16, BL15 and BL45 will achieve deqi in patients in a sitting position without retaining the needle.

Table 1. Location of Acupoints Used in the Acupuncture Group

Acupoints	Location	Depth
Baihui (DU20)	5 cun directly above the midpoint of the posterior hairline, or at the midpoint of the line connecting the apexes of the two auricles.	0.2 cun
Shengting (DU24)	1.0 cun directly above the midpoint of posterior hairline.	0.2 cun
Fengfu (DU16)	On the back of neck, 1 cun directly above the midpoint of the posterior hairline, directly below the external occipital protuberance.	0.5~0.1 cun
Fengchi(GB20)	On the nape, below the occiput, at the level of Fengfu, in the depression between the upper portion of sternocleidomastoideus and trapezius muscle.	0.5~0.8 cun
Danzhong (RN17)	On the anterior median line of the chest, at the level of the fourth intercostal space, at the midpoint between the two nipples.	0.5 cun
Zhongwan (RN12)	On the the anterior median line of the upper abdomen, 4 cun above the umbilicus.	1~1.5 cun
Qihai (RN6)	On the anterior median line of the lower abdomen, 1.5 cun below the umbilicus.	1~1.5 cun
Neiguan (PC6)	On the palmar aspect of forearm, 2.0 cun above the transverse crease of the wrist, between the tendons of palmaris longus and flexor carpi radialis muscle.	0.5~0.1 cun
Tongli (HT5),	On the radial aspect of the tendon of the ulnar flexor muscle of the wrist, and 1 cun above the carpal crease.	0.3~0.5 cun
Xuehai (SP10)	When the knee is flexed, on the medial aspect of the thigh, the point is 2 cun above the mediosuperior border of the patella, on the bulgs of the medial portion of muscle quadriceps femoris.	0.8~1 cun
Zusanli (ST36)	3 cun directly below Dubi* and one finger-breadth lateral to the anterior border of the tibia.	0.8~1 cun
Zhaohai (KI6)	On the depression below the tip of the medial malleolus.	0.5~0.8 cun
Xinshu (BL15)	1.5 cun from the lower border of the spinous process of the fifth thoracic vertebra.	0.3~0.5 cun
Yixi (BL45)	3 cun from the lower border of the spinous process of the sixth thoracic vertebra.	0.5~0.8 cun

Dubi* location = When the knee is flexed, the point is at the knee, below the patella, in the depression from the patella ligament.

For the sham acupuncture group, sham acupoints at locations away from known acupuncture points will be used to minimize physiological effects, and the location of sham acupoints are showed in Table 2. Patients will receive noninsertive acupuncture using the pragmatic placebo needles (Hwato brand, size 0.35 × 25 mm; supplement figure 1). As same as in the acupuncture group, the adhesive pads are initially pasted on the sham acupoints surface except for sham acupoint 1. The pragmatic placebo needles with a blunt tip will be placed on the adhesive pads.

In order to minimize the physiological effect, acupuncturists will be instructed to lightly place the sham needles with a blunt tip on the adhesive pads with no manipulation. The acupuncture needle will be inserted to a shallow depth at the sham acupoint 1, which does not penetrate below the skin, and needle manipulation for *deqi*. Sham acupoint 11 and Sham acupoint 12 will be inserted with sham needles without retaining the needle. Paired electrodes will be attached the needle holders of the bilateral sham acupoint 2 but with no electricity output.

Table 2. Location of sham acupoints used in the sham acupuncture group.

Sham Acupoints	Location
Sham acupoint 1	Midpoint of Shuaigu (GB8) and Touwei (ST8)
Sham acupoint 2	Midpoint of Touwei (ST8) and Yangbai(GB14)
Sham acupoint 3	Midpoint between Tianyou (SJ16) and Tianrong(SI17)
Sham acupoint 4	4 cun above the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 5	2 cun below the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 6	1 cun outside the point 1/4 of the line between Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 7	1 cun outside the midpoint of Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 8	6 cun above mediosuperior border of the patella
Sham acupoint 9	3 cun below the Yanglingquan (GB34) and in the middle of the gallbladder and bladder channels
Sham acupoint 10	Midpoint between Jiexi (ST 41) and Qiuxu (GB40)
Sham acupoint 11	2 cun from the lower border of the spinous process of the fifth thoracic vertebra
Sham acupoint 12	2 cun from the lower border of the spinous process of the sixth thoracic vertebra

MRI protocol

Patients will undergo brain MRI at baseline and after treatments. The MRI scan will be performed with a 3.0 Tesla superconductor (Skyra, Siemens, Erlangen, Germany) in the Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University. The parameters of sequences employed in this study are provided by China Association of Brain Imaging (www.abimaging.org). Sagittal structural images will be acquired using a magnetization

prepared rapid gradient echo (MP-RAGE) three-dimensional T1-weighted sequence with the following parameters: TR/TE = 2,530/2.98 ms, flip angle = 7◦, inversion time = 1100 ms, matrix = 256 × 256, 1 mm slice thickness without slice gap. T2*-weighted functional images will be collected using a gradient-echo echo-planar imaging sequence with the following parameters: repetition time = 3200 ms, echo time = 407 ms, flip angle = 120°.

Resting state-fMRI and task fMRI will be performed using an echo planar imaging (EPI) sequence with the following parameters: TR/TE = 2,000/30 ms, flip angle = 90◦, matrix = 64 × 64, slice thickness = 3.5 mm with 1 mm slice gap. Diffusion tensor imaging (DTI) will use a double spin-echo echo-planar imaging sequence (TR/TE = 12,000/77 ms, flip angle = 90°, Volume interval = 12, 2 mm thick axial slices). ASL imaging of the whole brain is performed by use of a 3D pseudocontinuous ASL sequence (TR/TE = 5,000/ 15.92 ms, flip angle = 180°, Slice thickness = 3.5 mm; labeling duration, number of slices = 40).

All scans will be reviewed qualitatively by two radiologists to screen for possible brain lesions or structural abnormalities. DTI images will be analyzed using the PANDA package [31].Functional MRI data collected during the memory task and at rest. ASL data will be performed with the Resting-State fMRI (DPARSF) toolbox and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) for MATLAB. Brain activation, connectivity changes, and cerebral blood flow will be compared between two groups before and after treatment.

Outcome measures

Clinical outcome assessments

Global cognitive function based on a composite score will be used to evaluate the clinical efficacy of acupuncture treatment at baseline, and at the end of the 12 week treatment period. It will be computed by averaging z-scores from a multi-domain neuropsychological test battery that includes 8 tests [32]. Animal Fluency Test (AFT) that assess the language and executive function by examining categorical verbal fluency [30]. Trail Making Test (TMT) part A and B examines graphomotor speed, attention, and executive function [33]. Digit Symbol Substitution Test (DSST) is used to assess processing speed, attention and concentration [34]. Clock Drawing Test (CDT) can assess multiple cognitive functions, including verbal understanding, memory, abstract

thinking, and executive functions [35]. Digit-Span Test (DST) is used to measure working memory's number storage capacity. Stroop color word test (SCWT) is a classic instrument for the assessment of selective attention, cognitive flexibility, cognitive inhibition, and information processing speed [36]. Auditory Verbal Learning Test (AVLT) a powerful neuropsychological test to assess episodic memory [37 38].

All patients will fill out a subjective cognitive decline questionnaire (SCDQ) [39] to assess their degree of subjective cognitive decline. The Face recognition questionnaire and Santa Barbara Sense of Direction scale [40] will be used to measure the ability for facial recognition and direction. Depressive symptoms, and sleep quality will be measured by Geriatric Depression Scale and Pittsburgh Sleep Quality Index (PSQI) [41].

Neuroplasticity assessments

In this study, neuroplasticity changes between the two groups will be measured by fMRI. Before the acupuncture treatment, patients will completed the fMRI scan within 3 days. They will also have the follow up fMRI scan within 3 days after the completion of their intervention (Figure 3). Brain activity and functional connectivity will be assessed under a resting state and a memory task. Group difference on cerebral blood flow and white matter integrity will be assessed by means of arterial spin labeling (ASL) or diffusion tensor imaging (DTI).

Memory task. The patients will be trained before entering the fMRI scanner. They will complete a practice version of the memory task paradigm in computer. This practice mock test will show the correct number, and they need to perform the tasks over an accuracy criterion of 80% to ensure that the patients understand how to comlete the memory task in the scanner. A blocked periodic design that incorporated alternating 1-back, and 2-back tasks will be used. Patients will view facial pictures, scene pictures or single digits (0–9, black on a white background). In the 1-back task, the patients are required to press a button when a facial picture, scene picture or digit appears that is the same as the one before. In the 2-back task, the patients are asked to press a response button if the current facial picture, scene picture or digit is the same as the one which was presented two trials before. The stimuli consist of 17 blocks for 236 seconds and are presented on a computer using MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) for 1 second, with an inter-stimulus interval of 1 second. Trial types within blocks are presented in pseudorandomized order.

Once in the scanner, anatomical and functional scans will be performed. During the scanning, all patients are asked to lie quietly in the scanner with their eyes open, trying to avoid thinking systematically, and moving as little as possible. In resting state fMRI scanning, the patients are instructed to view centrally placed fixation cross (+), and try to not think of anything. Two memory task runs will be conducted after T1 and resting state scans, then following by ASL, and DTI scans.

Apolipoprotein E genotyping

A TIANamp Blood DNA Kit (TIANGEN BIOTECH, Beijing, China) will be used to isolate High-molecular-weight DNA. The genotype of APOE will be detected by APOE Gene Detection Kit (Wuhan YZY Biopharma, Wuhan, China) with E2 and E4 reaction liquid. Genomic DNA and reaction liquid will be place on a 7500 HT Fast Real-Time PCR (Applied Biosystems, Foster City, USA) with the following conditions: Uracil-N-Glycosylase treatment at 37 °C for 10 minutes, denaturation at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 15s and annealing at 60 °C for 60s (acquiring the fluorescence signal in this step). According to the amplification plot, the genotype of APOE will be determined.

Safety and Monitoring

Treatment-related adverse events, including hematoma, bleeding, subcutaneous hemorrhage, serious pain, local infection and fatigue will be compared among patients in the acupuncture group and the sham acupuncture group, and will be documented at each treatment session. In order to ensure consistency, the neuropsychological tests and other assessment scales will be performed by the same researcher at baseline and the end of treatment. A professional staff will check the imaging data for quality and protocol conformity after each scanning session. Patients will undergo blood routine test and blood biochemical tests including blood glucose, homocysteine, total cholesterol, etc. before randomization and at the end of treatment.

Sample size and blinding

Task fMRI studies characteristically include small sample size [42] and thus have a low statistical power. The statistically underpowered study by definition means that a study will have less of a chance for detecting significant effects [43]. Therefore, power-based sample size will be

calculated prior to fMRI data collection [44]. Fortunately, approaches for sample size calculations in studies using functional magnetic resonance imaging have been developed.

By using the non-central random field theory, Hayasaka et al estimated that at least 12 subjects would be required to detect signals in either of the auditory cortices with at least 80% power [45]. They also found that approximately 13 subjects would be required to detect signals in the auditory cortices with 80% power, when generated a sample size map based on the mock pilot analysis. Adopting a simulation-based method to calculate statistical power for group-level fMRI studies, Desmond and Glover found a minimum of 12 subjects are required to achieve 80% power at a = 0.05 at the single voxel level [46]. For a more realistic thresholds, twice as many subjects are recommended to maintain this level of power after correcting for multiple comparisons. For a stricter alpha of 0.000002, approximately 25 subjects are needed. Mumford and Nichol recommended 20 subjects and a Type I error of $\alpha = 0.005$ should probably be used, and this power calculation is based on a non-central T or F distribution [47]. We used the largest sample size of 25 for each group with an estimated dropout rate and loss of data due to head motion, then we planned to enroll 60 participants in the two groups.

Eligible patients will randomly assigned into either the acupuncture or sham acupuncture group after signed written informed consent forms via a randomization digital table with a 1:1 ratio. Blocked randomization with a block size of 6 will be employed to ensure balance within the two groups. The randomization sequence will generated by a third-party professional statistician using computer-generated randomization the digital table by using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The randomization list will be stored by a noninvolved investigator and out of reach and sight of the involved investigators. The allocation schedule using a telephone randomization procedure. The randomization list was restricted to this research coordinator and was concealed from other study personnel. The patients, outcome assessors, and statisticians will be blinded to treatment allocation. Patients are told that they will receive one of two effective interventions randomized after enrolment. During the acupuncture treatment, the adhesive pads are pasted on the acupoints or sham acupoints after skin disinfection. The true or sham needles with a blunt tip will place in the adhesive pads. Patients in different groups will be separated into cubicles to refrain from communication.

Data collection and management

At baseline, information of patients about age, sex, education, dominant hand, and medical history will be collected (Figure 4). For all patients that met the inclusion criteria, the neuropsychological tests will be performed before and after treatment in a quiet room by evaluators, who have been trained by the organization of Alliance of early AD. All of the MRI scans will be conducted in the same machine, and the operators have been technically trained by a professional engineer from the China Association of Brain Imaging. Blood samples will be collected in the morning and handled by 2 persons. After each treatment session, adverse event will be recorded in participant's diary card by acupuncturists.

A case report form (CRF) will be used to collect the clinical data for each patient, labeled by unique numeric identifier and recorded by a trained graduate student. The data in the CRF will be verified for accuracy, missing data, and data consistency by a clinical research associate. Paper-based research data will be entered into an EpiData electronic database. For reducing errors in data entry, data will be entered independently twice by two independent people, and value pairs will then be compared for discordances, followed by resolution of discordances by referral to the original data source. The EpiData will export data in a Microsoft Excel spreadsheet formats. The data will be entered in an electronic Statistical Package SPSS software (SPSS 12.0 KO for Windows ©) for ease of statistical analysis.

Statistical analysis

Clinical data analysis

In this study, intent-to-treat analysis and per protocol analysis will be used. In this study, the intent-to-treat population consisted of all randomized patients who received at least one dose of treatment and had a complete baseline assessment. The per protocol population defined as all randomized patients who did not discontinue prematurely, completed the 12 weeks treatment and had MRI scans before and after treatment. Observation of histograms and normal probability plots and a Shapiro–Wilk test will be performed to determine the data whether followed a normal distribution. Distributed data will be described using means, standard deviations, and 95% confidence intervals (CIs). Clinical outcome analyses will be done with SPSS software (SPSS 22.0 KO for Windows ©), with a significance level of 0.05, and all hypothesis tests are two-tailed.

Baseline demographic characteristics between the two groups will be analyzed with χ^2 test or Fisher's exact test for categorical measures, and with the T-test or Wilcoxon rank-sum test for continuous measures. The clinical outcome of the global cognitive function based on composite scores will be compared between the acupuncture group and sham acupuncture group. The composite scores will be created by converting all individual cognitive scores to standardized z scores. As previously described [32], we computed z-scores by subtracting the baseline group mean and dividing the baseline group standard deviation, and then averaging the standardized z scores across all tests. The general linear model will be used to examine change in cognitive scores. For the other outcomes, if it agreed with normal distribution, an independent T-test will used. Otherwise, for abnormal distribution, the data will analyzed with Wilcoxon's test. The statisticians who analyzed the data are blinded to the test settings.

MRI data analysis

For imaging data will be analyzed using DPABI toolkit performing on MATLAB 8.6 (Mathworks,Inc., Natick, MA, USA) to detect any changes in brain function due to acupuncture treatment. After data preprocessing, some graph theory-based or data driven approaches will be performed to investigate neuroplasticity between the two groups, such as regional homogeneity, amplitude of the low-frequency fluctuation, and voxel-wise degree centrality. A two-sample t-test will be conducted to investigate the differences in brain regions between the acupuncture group and the sham acupuncture group in the DPARSF software. Multiple comparisons will be used in order to better control for a highly inflated false positivity rate. Pearson's correlation analysis will be performed to examine the association between the fMRI image data and clinical variables.

Ethics and dissemination

General ethical approval has been obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018 (Ref: 2017BL-061-02). The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement and the CONSORT statement for non-pharmacological interventions have been used as a framework for development of the methodology for this project [24 25].

If any modifications or decision are made, amendments will be reviewed and approved by the ethics eommittee, and new protocols would be uploaded to Clinicaltrials.gov. The results of the study will be published in a peer-reviewed academic journal and will also be disseminated electronically through conference presentations.

Patient and public involvement

Currently, this trial is at the recruitment phase with no patient involved in. The participants will be able to view the study results via social media.

DISCUSSION

SCD, may be the initial phase of AD, and could serve as a window of opportunity for interventions at an early disease stage [23]. This study is planned as a randomized, assessor-blinded, placebo-controlled trial to evaluate the efficacy and central mechanism of acupuncture treatment on SCD compared with a sham acupuncture group. To our knowledge, this trial is also the first study to investigate the effect of acupuncture in SCD patients.

This study has several strengths. First, we will assess patients with SCD on multiple levels, including multi-domain neuropsychological tests, functional brain alterations, cerebrovascular risk factors, and AOPE genotyping. This multi-domain assessment will be used to identify possible biomarkers involved in the effects of acupuncture in SCD.

Second, the neuropsychological assessment in the present study is a multi-domain neuropsychological test battery that included eight tests, which can assess multiple cognitive domains including executive functions, attention, visuospatial functioning, and language. Memory decline associated with AD risk factors [48 49], and then most instruments were used previously to measure SCD focus on memory [50]. However, it might be too restrictive to limit the SCD assessment to memory, in particular for atypical forms of AD [9 51]. In this study, multi-domain cognitive function will be assessed.

Additionally, functional brain alterations will be used as outcome measures. Compared with the neuropsychological tests alone, the combination of in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy. A smaller training effect and ceiling effect may be found when using MRI [52]. Furthermore, it can be used as a tool to

understand the mechanism of the acupuncture's effects. Functional MRI can identify whether neural efficiency is improved or the brain connectome is reorganized to achieve cognitive enhancement [53].

A potential limitation is that the patients in this study will be recruited from the community. Considering the research environment, individuals recruited from a memory clinic may have a higher probability of having preclinical AD. They had specific concerns sufficient to prompt a medical visit [15]. However, community based studies have shown some predictive value of SCD for cognitive decline and dementia [10 54]. Another potential limitation of our study is that it will not include a large sample size, and this may lead to the need for a future study. The main feature of our study is to systemically investigate and analyze the central mechanisms of acupuncture treatment in patients with SCD. The findings may provide deeper insight into the benefits and mechanisms of acupuncture for patients with SCD.

Trial status

This trial is currently in the recruitment phase.

Figure Legends

Figure 1. Flow chart.

Figure 2. Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

Figure 3. Magnetic resonance imaging experimental paradigm and an Illustrative diagram of the memory task.

Figure 4. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the schedule of the trial.

Supplement figure 1. (a) The adhesive pads and sham needles with a blunt tip; (b) The adhesive pad was pasted on the surface of skin; (c) The sham needle was placed on the adhesive pad.

Footnotes

List of abbreviations SCD: Subjective Cognitive Decline; AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; APOE: apolipoprotein E; MRI: Magnetic Resonance Imaging; AFT: Animal Fluency Test; TMT: Trail Making Test; DSST: Digit Symbol Substitution Test; SCWT: Stroop Color Word Test; AVLT: Auditory Verbal Learning Test; SCDQ: Subjective Cognitive Decline Questionnaire; SBSDS: Santa Barbara Sense of Direction Scale; HAMD: Hamilton Rating Scale for Depression; HAMA: Hamilton Rating Scale for Anxiety; PSQI: Pittsburgh Sleep Quality Index.

Ethics approval and consent to participate Ethics approval The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial. The methods were carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from each patient.

Consent for publication Not applicable.

Availability of data and materials Not applicable. This is a study protocol only and as such no unpublished data are available.

Competing interests The authors declare that they have no competing interests.

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Authors' contributors Conceived and designed the experiments: CZL and JWH. Performed the experiments: PZ, ZYW, SQH, CQY, GXS; JQL, YNZ. Analyzed the data: XW, JFT. Wrote the paper: CQY, XW and CZL. All authors approved the final manuscript.

Provenance and peer review Not commissioned; externally peer reviewed.

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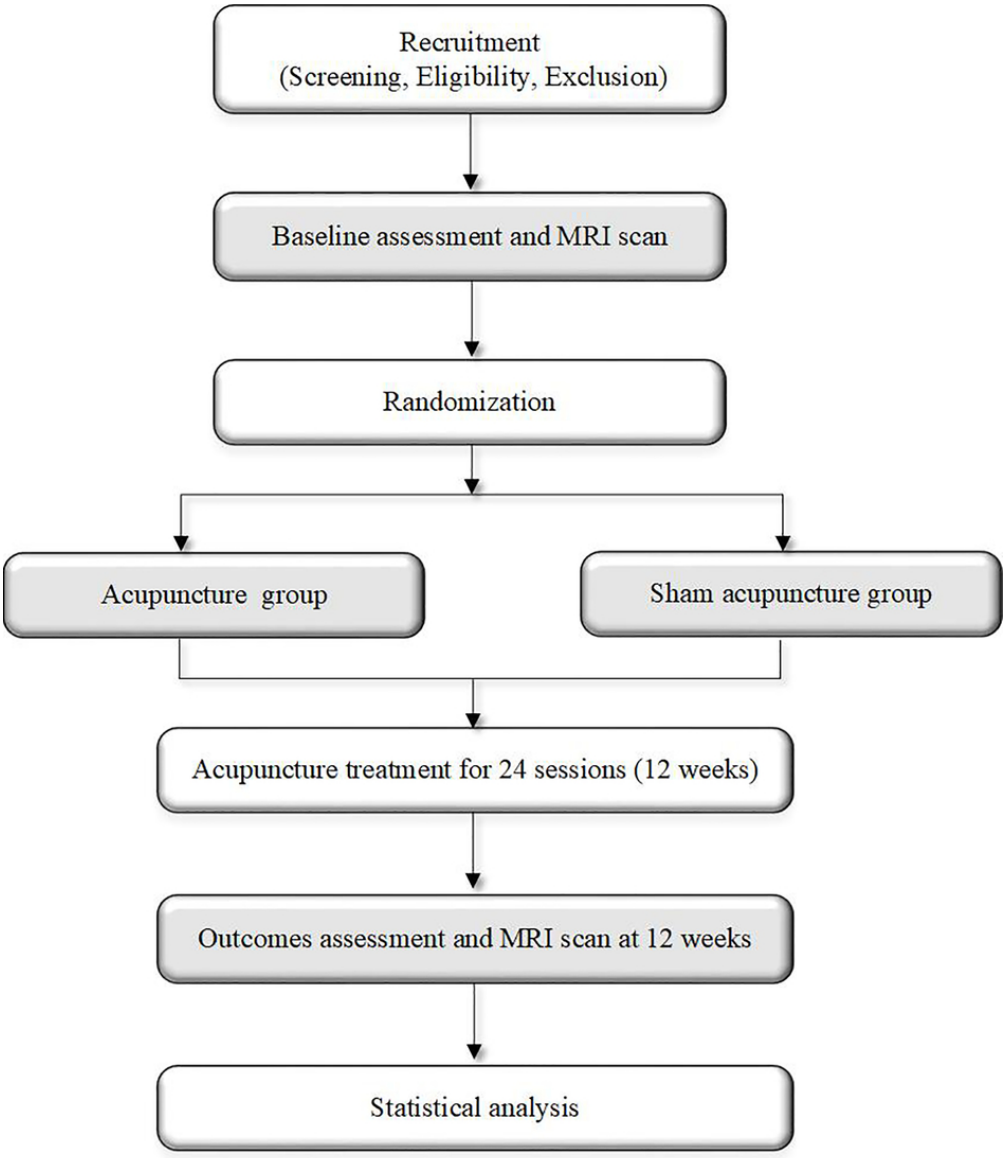

Flow chart.

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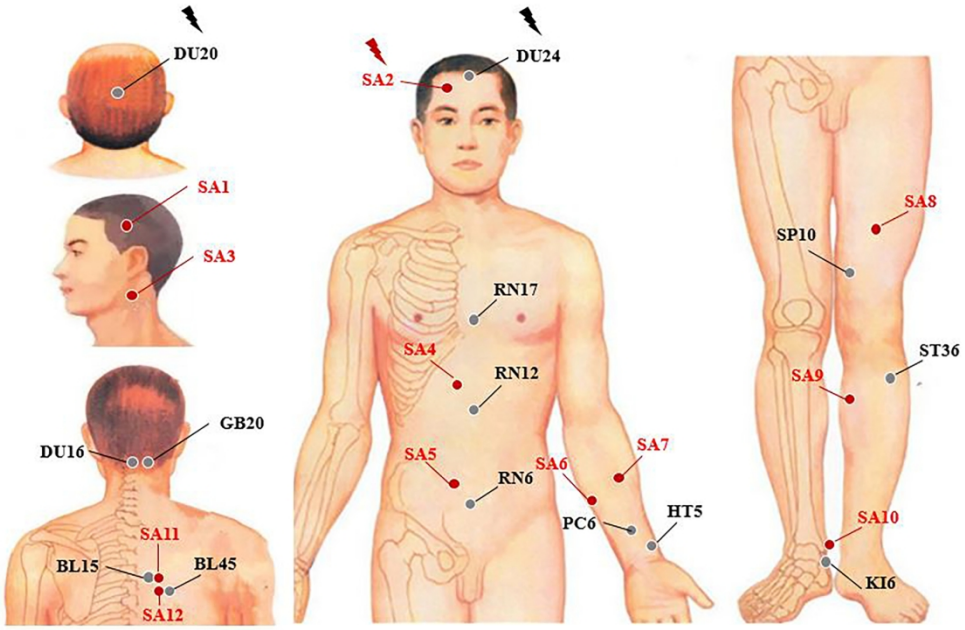

Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

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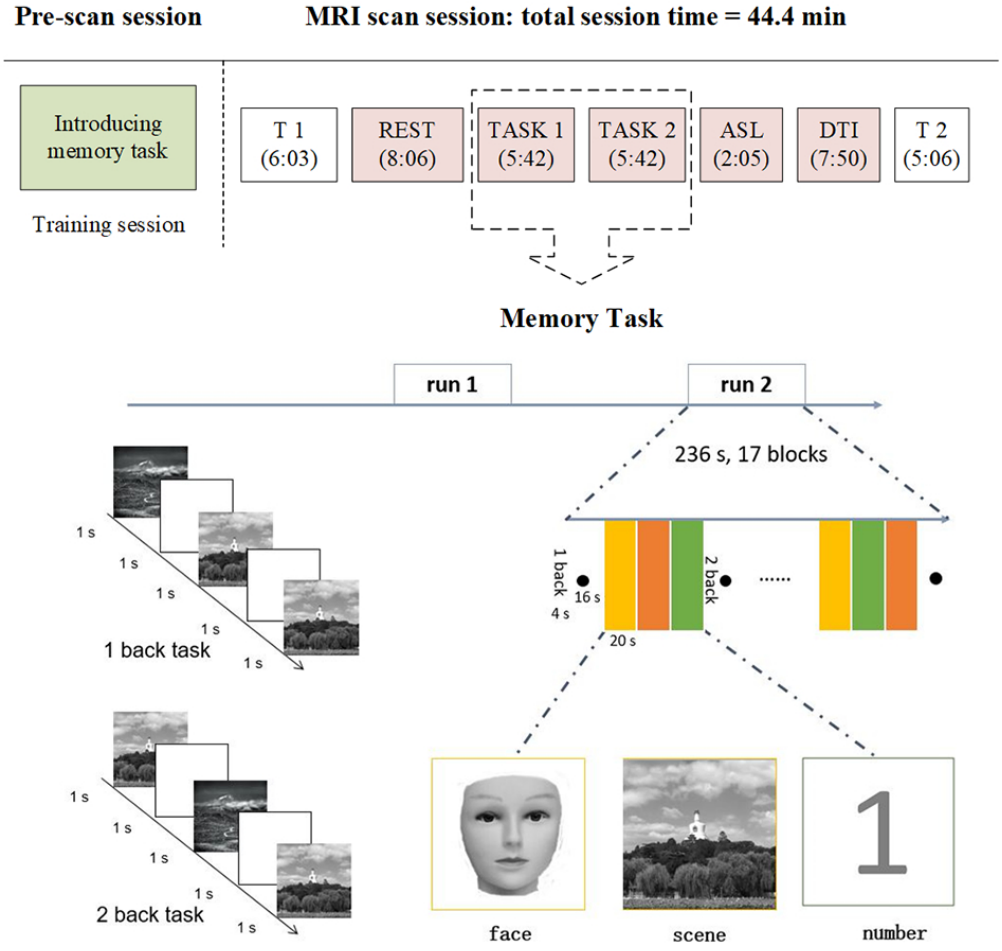
Pre-scan session
MRI scan session: total session time = 44.4 min
Introducing memory task
Training session
T 1 (6:03)
REST (8:06)
TASK 1 (5:42)
TASK 2 (5:42)
ASL (2:05)
DTI (7:50)
T 2 (5:06)
Memory Task
run 1
run 2
236 s, 17 blocks
1 back
2 back
16 s
4 s
20 s
1 s
1 back task
2 back task
face
scene
number

Study period				
Items	Baseline		Treatment phase	Outcome assessment
Time point	-1 week	0 week	1- 12 weeks	13-14 weeks
Enrolment				
Eligibility screen	X			
Informed consent	X			
Examination		X		
Randomization		X		
Intervention				
Acupuncture (n = 30)			X	
Sham acupuncture (n = 30)			X	
MRI scan				
Acupuncture		X		X
Sham acupuncture		X		X
Assessment				
AFT		X		X
TMT		X		X
DSST		X		X
SCWT		X		X
AVLT		X		X
SCDQ		X		X
SBSDS		X		X
HAMD		X		X
HAMA		X		X
PSQI		X		X
Safety				
Laboratory test		X		X
Adverse events			X	X

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of the trial.

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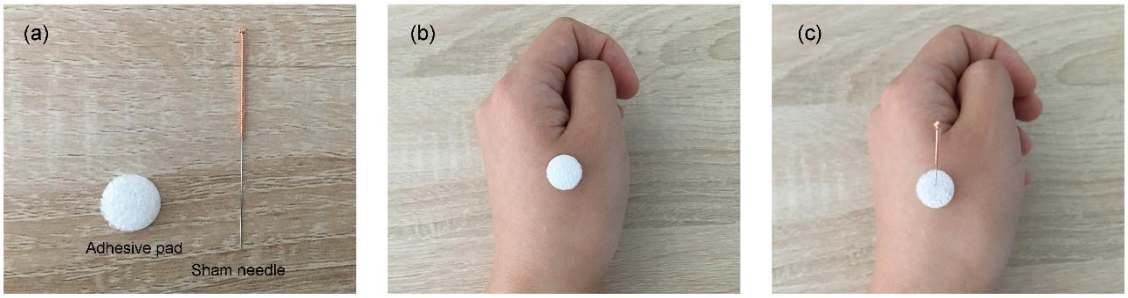

Supplement figure 1. Pictures of sham needle. (a) The adhesive pad and sham needle with a blunt tip; (b) The adhesive pad is pasted on the surface of skin; (c) The sham needle is placed on the adhesive pad.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	5
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	Not statement
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	5,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11,12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3–4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9–11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Figure 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11,12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Not statement
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	5

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not offer in the manuscript
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	Subjective cognitive decline, Dementia < NEUROLOGY, Acupuncture, Sham acupuncture, Magnetic resonance imaging < RADIOLOGY & IMAGING, Clinical trial

The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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ABSTRACT

Background: Subjective cognitive decline (SCD) refers to individuals' perceived decline in memory and/or other cognitive abilities relative to their previous level of performance, while objective neuropsychological deficits are not observed. SCD may represent a preclinical phase of Alzheimer's disease. At this very early stage of decline, intervention could slow the rate of incipient decline to prolong and preserve cognitive and functional abilities. However, there is no effective treatments recommended for individuals with SCD. Acupuncture, as a non-pharmacologic intervention, has been widely employed for patients with cognitive disorders.

Methods/Design: The proposed study is a randomized, assessor-blinded and placebo-controlled study to investigate the efficacy and mechanism of acupuncture in SCD. Sixty patients with SCD will be randomly allocated either into an acupuncture group or a sham acupuncture group. They will receive 24 sessions of real acupuncture treatment or identical treatment sessions using a placebo needle. Global cognitive changes based on a multi-domain neuropsychological test battery will be evaluated to detect the clinical efficacy of acupuncture treatment at baseline and end of treatment. Magnetic Resonance Imaging (MRI) scans will be used to explore acupuncture related neuroplasticity changes. Correlation analyses will be performed to investigate the relationships between the changes in brain function and symptom improvement.

Discussion: This trial will investigate the effect of acupuncture in SCD patients. We will compare real acupuncture treatment with the sham acupuncture treatment by fMRI to verify neuroplasticity changes. The results of this trial may provide relevant evidences for acupuncture treatment on SCD and also reveal the underlying mechanisms of the treatment effect.

Trial registration: ClinicalTrials.gov NCT03444896. Retrospectively registered on 23 February 2018.

Keywords: Subjective cognitive decline; Dementia; Acupuncture; Sham acupuncture; Magnetic Resonance Imaging; Clinical trial

Strengths and limitations of this study

Compared with the neuropsychological tests alone as efficacy evaluations, the combination with in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy.

This study will firstly uncover the efficacy and neural mechanism of acupuncture treatment in older adults with SCD.

A multi-domain neuropsychological test battery will be employed, which can assess multiple cognitive domains including executive function, attention, visuospatial function, and language.

Sham needles and adhesive pads will be used for better patient blinding.

A potential limitation is that not a large sample size will be performed in this study.

INTRODUCTION

Dementia is the greatest global challenge for health and social care in the 21st century [1]. Globally, about 47 million people were living with dementia in 2015, and this number is projected to triple by 2050, based on the World Alzheimer Report (2015). Alzheimer's disease (AD) is the most common form of dementia [2]. Currently, there is no effective cure for AD, and the available treatments have only moderately alleviate symptoms [3]. Therefore, prevention is essential to reduce the dementia epidemic [4 5]. The long "preclinical" phase of AD provides an opportunity for individuals to participate in treatment trials to delay or prevent cognitive decline [6-8].

Subjective cognitive decline (SCD) usually occurs in older adults, and it refers to the self-perception of cognitive decline, when individuals perform cognitive tests within normal limits and have preserved activities of daily living [9]. A review demonstrated that the prevalence of these complaints in persons aged 65 years or more varied from 25% to 50% [10]. Actually, many older adults claim cognitive decline might be regarded as hypochondriacs by professional healthy carers. However, several lines of evidence from longitudinal aging studies suggests that older adults with SCD are more likely to present AD biomarkers than their healthy peers. About 60% SCD individuals will decline to mild cognitive impairment (MCI) and AD over a 15-year

period [11 12]. People with SCD have more brain abnormalities such as hippocampal volume loss and hypometabolism compared with healthy controls [13]. Moreover, cerebrospinal amyloid β is strongly predictive of subsequent clinical progression in patients with SCD [14]. This suggests that, for some older adults, SCD may represent a preclinical phase of AD.

There is still much plasticity in SCD. The patients in this stage may not progress to dementia or even revert to no cognitive impairment after effective treatment. Development of safe and effective interventions in early AD stages is important. Specific criteria to define SCD have been published [9 15], but clinical trials in SCD are in their infancy, and no pharmacological treatment or interventions is currently recommended for individuals with SCD. Despite these difficulties, the development of new treatments should be encouraged [16].

The accumulated evidence suggests that non-pharmacologic intervention may benefit cognitive function in older adults with SCD [17]. Acupuncture, as a non-pharmacologic intervention, that has been widely used for patients with cognitive disorders. The WHO reports that acupuncture treatment can be beneficial for vascular dementia. A number of clinical studies have provided evidence that acupuncture is beneficial for the treatment of dementia or MCI [18]. A systematic review supports the use of acupuncture for MCI [19]. Animal studies showed that acupuncture elicits its effects by mediation of neural plasticity in pathological conditions [20]. However, the efficacy of acupuncture in patients with SCD has not be investigated. An experimental study is needed to test this hypothesis.

By definition, individuals with SCD are within the normal range on clinical-neuropsychological tests. It will be difficult to detect response in this population compared with people who already have clinically manifest impairment (e.g., MCI) due to a ceiling effect. As such, neuropsychological tests in conjunction with in-vivo measures of brain function such as electroencephalo-graphy, or functional magnetic resonance imaging (fMRI), may be a more sensitive alternative to neuropsychological tests [21-23]. Brain alterations are subtle but measurable to identify individuals at risk for AD well before cognitive symptoms are manifested by using fMRI, allowing researchers to use neuroimaging to ascertain response after intervention sensitivity [24]. For better ascertaining the clinical response and unveiling the mechanism, brain alterations measured by fMRI could also be used to evaluate the efficacy of acupuncture.

OBJECTIVES

This study is a randomized controlled functional brain imaging trial with 12 weeks of treatment, aiming at: (1) evaluating the effects of acupuncture treatment on cognitive function in older adults with SCD; (2) exploring the central mechanism of the long-lasting effect of acupuncture on SCD; and (3) investigating the safety of acupuncture treatment on SCD.

METHODS

Design

General ethical approval was obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018. The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement for non-pharmacological interventions has been used as a framework for development of the methodology for this project [25 26].

This trial is a 1:1 randomized single-blinded, and placebo-controlled study with two parallel groups involving elderly adults. The study is designed to examine the effect and neural mechanism of acupuncture treatment on cognitive function before and after a 12 weeks intervention period. As shown in Figure 1, the cognitive assessments and MRI scans will be performed at baseline and immediately after the completion of the intervention.

Participants

The study will mainly take place in Dongcheng, Fengtai, and Shunyi District in Bejing. People with SCD will be recruited from community-dwelling population in those Districts. In order to have better insight into the trial for community residents, science popularization lectures about dementia and advertisement of the trial will be given in community service centers. The brochures and posters will also be distributed. Besides, advertisement of the trial will be shared in the official account on WeChat (China's most popular social media platform) of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University and health web site (www.39.net) or local newspaper for recruiting participants. Interested individuals will be screened in the clinic, community service centers or by phone using the inclusion/exclusion criteria. All those who meet the inclusion criteria will receive a study information sheet including

the design, procedure, benefits, and risks of the study. Before the study procedure started, the subjects will have to provide signed written informed consent forms. The diagnosis of SCD will be based on the published SCD research criteria proposed by the Subjective Cognitive Decline Initiative (SCD-I) [9]. In this study, the patients who answer "yes" to the question "Do you have problem in memory?" in the initial screening by phone or face-to-face interviews, will be selected to the next step. Besides, the Subjective Cognitive Decline questionnaire 9 (SCD-Q9) are further used for SCD screening. Only the patients with the SCD-Q9 score more than 5.0 will pass the screening.

Inclusion criteria

- Male and female adults aged 55-75;
- Native Chinese speakers who are right-handed and have at least a primary school education;
- Self-reported persistent memory decline compared with a previous normal status within the last 5 years, which is confirmed by caregivers;
- Normal age- and education-adjusted performance on neuropsychological test including the Chinese version of Mini-Mental State Examination (MMSE) [27], the auditory verbal learning test (the short-term delayed free recall, the long-term delayed free recall, and the recognition test) [28], Trail Making Test [29], and Animal Fluency Test [30].
- No or minimal impairment in activities of daily living;

Exclusion criteria

- Presence of a positive neurologic history (e.g., traumatic brain injury, stroke, Parkinson's disease, multiple sclerosis);
- Treatments that would affect cognitive function (e.g., treatment for an acute psychiatric episode, therapy with memantine, rivastigmine and donepezil);
- Presence of significant psychiatric history (e.g., bipolar disorder, schizophrenia) and/or severe anxiety and depression;
- Presence of serious heart, kidney, liver, gastrointestinal, infectious, endocrine disease or cancer;

- History of alcohol or drug abuse/addiction;
- Any contraindications for MRI scans (e.g., aneurysm clip pacemaker);
- Significant visual and/or auditory impairment uncorrected by aids, and unable to perform neuropsychological evaluations;
- Currently enrolled in another research study;
- Received acupuncture treatment in the preceding month；

During the trial period, patients with SCD who meet the following criteria will be excluded from the study:

- taking medication or receiving additional treatment that is expected to affect the cognitive function (e.g., tranquilizers, antianxiolytics, hypnotics, nootropics, and cholinomimetic agents);
- withdrawal of consent for study participation because the patients does not wish to continue;
- missing more than 5 of 24 acupuncture treatment sessions;
- occurrence of a serious adverse event that the doctors consider the treatment should be termination;
- critical protocol violation such as violation of eligibility criteria.

Intervention

Patients will receive 24 acupuncture treatment sessions over 12 weeks (twice a week). Hwato brand single-use acupuncture needles (size 0.35 × 25 mm or 0.35 × 40 mm), pragmatic placebo needles (size 0.30 × 25 mm), and SDZ-V electroacupuncture apparatuses will be used.

For the acupuncture group (Figure 2), acupuncture needles will be placed at acupoints Baihui (DU20), Shengting (DU24), Fengfu (DU16), Fengchi(GB20), Danzhong (RN17), Zhongwan (RN12), Qihai (RN6), Neiguan (PC6), Tongli (HT5), Xuehai (SP10), Zusanli (ST36), Zhaohai (KI6), Xinshu (BL15), and Yixi (BL45). After skin disinfection in patient in the supine position, the adhesive pads are pasted on the acupoints surface except for Baihui (DU20) and Shengting (DU24). Then, the acupuncture needles will be inserted through the adhesive pads into the skin depending on the location of the needle. The location is show in Table 1. Manual acupuncture by acupuncturists using a small, equal manipulations of twirling, lifting, and thrusting will be

performed on all needles to reach deqi. The patients will feel the deqi sensation, such as soreness, numbness, distention, heaviness, and other sensations. Paired electrodes from the electroacupuncture apparatus will be attached to the needle holders of the DU20 and DU24. A dilatational wave of 2-100 Hz and a current intensity of 1 to 5 mA will be performed following by the degree of needle handle shivering, ignoring the patient's feelings. The needles will be extracted after 20 minutes for each treatment. The acupoints of DU16, BL15 and BL45 will achieve deqi in patients in a sitting position without retaining the needle.

Table 1. Location of Acupoints Used in the Acupuncture Group

Acupoints	Location	Depth
Baihui (DU20)	5 cun directly above the midpoint of the posterior hairline, or at the midpoint of the line connecting the apexes of the two auricles.	0.2 cun
Shengting (DU24)	1.0 cun directly above the midpoint of posterior hairline.	0.2 cun
Fengfu (DU16)	On the back of neck, 1 cun directly above the midpoint of the posterior hairline, directly below the external occipital protuberance.	0.5~0.1 cun
Fengchi(GB20)	On the nape, below the occiput, at the level of Fengfu, in the depression between the upper portion of sternocleidomastoideus and trapezius muscle.	0.5~0.8 cun
Danzhong (RN17)	On the anterior median line of the chest, at the level of the fourth intercostal space, at the midpoint between the two nipples.	0.5 cun
Zhongwan (RN12)	On the the anterior median line of the upper abdomen, 4 cun above the umbilicus.	1~1.5 cun
Qihai (RN6)	On the anterior median line of the lower abdomen, 1.5 cun below the umbilicus.	1~1.5 cun
Neiguan (PC6)	On the palmar aspect of forearm, 2.0 cun above the transverse crease of the wrist, between the tendons of palmaris longus and flexor carpi radialis muscle.	0.5~0.1 cun
Tongli (HT5),	On the radial aspect of the tendon of the ulnar flexor muscle of the wrist, and 1 cun above the carpal crease.	0.3~0.5 cun
Xuehai (SP10)	When the knee is flexed, on the medial aspect of the thigh, the point is 2 cun above the mediosuperior border of the patella, on the bulgs of the medial portion of muscle quadriceps femoris.	0.8~1 cun
Zusanli (ST36)	3 cun directly below Dubi* and one finger-breadth lateral to the anterior border of the tibia.	0.8~1 cun
Zhaohai (KI6)	On the depression below the tip of the medial malleolus.	0.5~0.8 cun
Xinshu (BL15)	1.5 cun from the lower border of the spinous process of the fifth thoracic vertebra.	0.3~0.5 cun
Yixi (BL45)	3 cun from the lower border of the spinous process of the sixth thoracic vertebra.	0.5~0.8 cun

Dubi* location = When the knee is flexed, the point is at the knee, below the patella, in the depression from the patella ligament.

For the sham acupuncture group, sham acupoints at locations away from known acupuncture points will be used to minimize physiological effects, and the location of sham acupoints are

showed in Table 2. Patients will receive non-insertive acupuncture using the pragmatic placebo needles (Hwato brand, size 0.35 × 25 mm; supplement figure 1). As same as in the acupuncture group, the adhesive pads are initially pasted on the sham acupoints surface except for sham acupoint 1. The pragmatic placebo needles with a blunt tip will be placed on the adhesive pads. In order to minimize the physiological effect, acupuncturists will be instructed to lightly place the sham needles with a blunt tip on the adhesive pads with no manipulation. The acupuncture needle will be inserted to a shallow depth at the sham acupoint 1, which does not penetrate below the skin, and needle manipulation for *deqi*. Sham acupoint 11 and Sham acupoint 12 will be inserted with sham needles without retaining the needle. Paired electrodes will be attached the needle holders of the bilateral sham acupoint 2 but with no electricity output.

Table 2. Location of sham acupoints used in the sham acupuncture group.

Sham Acupoints	Location
Sham acupoint 1	Midpoint of Shuaigu (GB8) and Touwei (ST8)
Sham acupoint 2	Midpoint of Touwei (ST8) and Yangbai(GB14)
Sham acupoint 3	Midpoint between Tianyou (SJ16) and Tianrong(SI17)
Sham acupoint 4	4 cun above the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 5	2 cun below the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 6	1 cun outside the point 1/4 of the line between Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 7	1 cun outside the midpoint of Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 8	6 cun above mediosuperior border of the patella
Sham acupoint 9	3 cun below the Yanglingquan (GB34) and in the middle of the gallbladder and bladder channels
Sham acupoint 10	Midpoint between Jiexi (ST 41) and Qiuxu (GB40)
Sham acupoint 11	2 cun from the lower border of the spinous process of the fifth thoracic vertebra
Sham acupoint 12	2 cun from the lower border of the spinous process of the sixth thoracic vertebra

MRI protocol

Patients will undergo brain MRI at baseline and after treatments. The MRI scan will be performed with a 3.0 Tesla superconductor (Skyra, Siemens, Erlangen, Germany) in the Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University. The parameters of sequences employed in this study are provided by China Association of Brain Imaging (www.abimaging.org). Sagittal structural images will be acquired using a magnetization prepared rapid gradient echo (MP-RAGE) three-dimensional T1-weighted sequence with the following parameters: TR/TE = 2,530/2.98 ms, flip angle = 7◦, inversion time = 1100 ms, matrix = 256 × 256, 1 mm slice thickness without slice gap. T2*-weighted functional images will be collected using a gradient-echo echo-planar imaging sequence with the following parameters: repetition time = 3200 ms, echo time = 407 ms, flip angle = 120°.

Resting state-fMRI and task fMRI will be performed using an echo planar imaging (EPI) sequence with the following parameters: TR/TE = 2,000/30 ms, flip angle = 90◦, matrix = 64 × 64, slice thickness = 3.5 mm with 1 mm slice gap. Diffusion tensor imaging (DTI) will use a double spin-echo echo-planar imaging sequence (TR/TE = 12,000/77 ms, flip angle = 90°, Volume interval = 12, 2 mm thick axial slices). ASL imaging of the whole brain is performed by use of a 3D pseudocontinuous ASL sequence (TR/TE = 5,000/ 15.92 ms, flip angle = 180°, Slice thickness = 3.5 mm; labeling duration, number of slices = 40).

All scans will be reviewed qualitatively by two radiologists to screen for possible brain lesions or structural abnormalities. DTI images will be analyzed using the PANDA package [31]. Functional MRI data collected during the memory task and at rest. ASL data will be performed with the Resting-State fMRI (DPARSF) toolbox and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) for MATLAB. Brain activation, connectivity changes, and cerebral blood flow will be compared between two groups before and after treatment.

Outcome measures

Clinical outcome assessments

Global cognitive function based on a composite score will be used to evaluate the clinical efficacy of acupuncture treatment at baseline, and at the end of the 12 weeks treatment period. It will be computed by averaging z-scores from a multi-domain neuropsychological test battery that

includes 8 tests. Animal Fluency Test (AFT) that assess the language and executive function by examining categorical verbal fluency [30]. Trail Making Test (TMT) part A and B examines graphomotor speed, attention, and executive function [32]. Digit Symbol Substitution Test (DSST) is used to assess processing speed, attention and concentration [33]. Clock Drawing Test (CDT) can assess multiple cognitive functions, including verbal understanding, memory, abstract thinking, and executive functions [34]. Digit-Span Test (DST) is used to measure working memory's number storage capacity. Stroop color word test (SCWT) is a classic instrument for the assessment of selective attention, cognitive flexibility, cognitive inhibition, and information processing speed [35]. Auditory Verbal Learning Test (AVLT) a powerful neuropsychological test to assess episodic memory [36 37].

All patients will fill out a subjective cognitive decline questionnaire (SCDQ) [38] to assess their degree of subjective cognitive decline. The Face recognition questionnaire and Santa Barbara Sense of Direction scale [39] will be used to measure the ability for facial recognition and direction. Depressive symptoms, and sleep quality will be measured by Geriatric Depression Scale and Pittsburgh Sleep Quality Index (PSQI) [40].

Neuroplasticity assessments

In this study, neuroplasticity changes between the two groups will be measured by fMRI. Before the acupuncture treatment, patients will complete the fMRI scan within 3 days. They will also have the follow up fMRI scan within 3 days after the completion of their intervention (Figure 3). Brain activity and functional connectivity will be assessed under a resting state and a memory task. Group difference on cerebral blood flow and white matter integrity will be assessed by means of arterial spin labeling (ASL) or diffusion tensor imaging (DTI).

Memory task. The patients will be trained before entering the fMRI scanner. They will complete a practice version of the memory task paradigm in computer. This practice mock test will show the correct number, and they need to perform the tasks with an accuracy criterion of 80% to ensure that the patients understand how to do the memory task in the scanner. A blocked periodic design that incorporated alternating 1-back, and 2-back tasks will be used. Patients will view facial pictures, scene pictures or single digits (0–9, black on a white background). In the 1-back task, the patients are required to press a button when a facial picture, scene picture or digit appears that is the same as the one before. In the 2-back task, the patients are asked to press a

response button if the current facial picture, scene picture or digit is the same as the one which was presented two trials before. The stimuli consist of 17 blocks for 236 seconds and are presented on a computer using MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) for 1 second, with an inter-stimulus interval of 1 second. Trial types within blocks are presented in pseudorandomized order.

Once in the scanner, anatomical and functional scans will be performed. During the scanning, all patients are asked to lie quietly in the scanner with their eyes open, trying to avoid thinking systematically, and moving as little as possible. In resting state fMRI scanning, the patients are instructed to view centrally placed fixation cross (+), and try to not think of anything. Two memory task runs will be conducted after T1 and resting state scans, then following by ASL, and DTI scans.

Apolipoprotein E genotyping

A TIANamp Blood DNA Kit (TIANGEN BIOTECH, Beijing, China) will be used to isolate High-molecular-weight DNA. The genotype of APOE will be detected by APOE Gene Detection Kit (Wuhan YZY Biopharma, Wuhan, China) with E2 and E4 reaction liquid. Genomic DNA and reaction liquid will be place on a 7500 HT Fast Real-Time PCR (Applied Biosystems, Foster City, USA) with the following conditions: Uracil-N-Glycosylase treatment at 37 °C for 10 minutes, denaturation at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 15s and annealing at 60 °C for 60s (acquiring the fluorescence signal in this step). According to the amplification plot, the genotype of APOE will be determined.

Safety and Monitoring

Treatment-related adverse events, including hematoma, bleeding, subcutaneous hemorrhage, serious pain, local infection and fatigue will be compared among patients in the acupuncture group and the sham acupuncture group, and will be documented at each treatment session. In order to ensure consistency, the neuropsychological tests and other assessment scales will be performed by the same researcher at baseline and the end of treatment. A professional staff will check the imaging data for quality and protocol conformity after each scanning session. Patients will undergo blood routine test and blood biochemical tests including blood glucose, homocysteine, total cholesterol, etc. before randomization and at the end of treatment.

Sample size and blinding

Task fMRI studies characteristically include small sample size [41] and thus have a low statistical power. The statistically underpowered study by definition means that a study will have less of a chance for detecting significant effects [42]. Therefore, power-based sample size will be calculated prior to fMRI data collection [43]. Fortunately, approaches for sample size calculations in studies using functional magnetic resonance imaging have been developed.

By using the non-central random field theory, Hayasaka et al estimated that at least 12 subjects would be required to detect signals in either of the auditory cortices with at least 80% power [44]. They also found that approximately 13 subjects would be required to detect signals in the auditory cortices with 80% power, when generated a sample size map based on the mock pilot analysis. Adopting a simulation-based method to calculate statistical power for group-level fMRI studies, Desmond and Glover found a minimum of 12 subjects are required to achieve 80% power at a = 0.05 at the single voxel level [45]. For a more realistic thresholds, twice as many subjects are recommended to maintain this level of power after correcting for multiple comparisons. For a stricter alpha of 0.000002, approximately 25 subjects are needed. Mumford and Nichol recommended 20 subjects and a Type I error of $\alpha = 0.005$ should probably be used, and this power calculation is based on a non-central T or F distribution [46]. We used the largest sample size of 25 for each group with an estimated dropout rate and loss of data due to head motion, then we planned to enroll 60 participants in the two groups.

Eligible patients will be randomly assigned into either the acupuncture or sham acupuncture group after signed written informed consent forms via a randomization digital table with a 1:1 ratio. Blocked randomization with a block size of 6 will be employed to ensure balance within the two groups. The randomization sequence will be generated by a third-party professional statistician using computer-generated randomization the digital table by using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The randomization list will be stored by a noninvolved investigator and out of reach and sight of the involved investigators. The allocation schedule using a telephone randomization procedure. The randomization list was restricted to this research coordinator and was concealed from other study personnel. The patients, outcome assessors, and statisticians will be blinded to treatment allocation. Patients are told that they will receive one of two effective interventions randomized after enrolment. During the acupuncture treatment, the

adhesive pads are pasted on the acupoints or sham acupoints after skin disinfection. The true or sham needles with a blunt tip will place in the adhesive pads. Patients in different groups will be separated into cubicles to refrain from communication.

Data collection and management

At baseline, information of patients about age, sex, education, dominant hand, and medical history will be collected (Figure 4). For all patients that met the inclusion criteria, the neuropsychological tests will be performed before and after treatment in a quiet room by evaluators, who have been trained by the organization of Alliance of early AD. All of the MRI scans will be conducted in the same machine, and the operators have been technically trained by a professional engineer from the China Association of Brain Imaging. Blood samples will be collected in the morning and handled by 2 persons. After each treatment session, adverse event will be recorded in participant's diary card by acupuncturists.

A case report form (CRF) will be used to collect the clinical data for each patient, labeled by unique numeric identifier and recorded by a trained graduate student. The data in the CRF will be verified for accuracy, missing data, and data consistency by a clinical research associate. Paper-based research data will be entered into an EpiData electronic database. For reducing errors in data entry, data will be entered independently twice by two independent people, and value pairs will then be compared for discordances, followed by resolution of discordances by referral to the original data source. The EpiData will export data in a Microsoft Excel spreadsheet formats. The data will be entered in an electronic Statistical Package SPSS software (SPSS 12.0 KO for Windows ©) for ease of statistical analysis.

Statistical analysis

Clinical data analysis

In this study, intent-to-treat analysis and per protocol analysis will be used. In this study, the intent-to-treat population consisted of all randomized patients who received at least one dose of treatment and had a complete baseline assessment. The per protocol population defined as all randomized patients who did not discontinue prematurely, completed the 12 weeks treatment and had MRI scans before and after treatment. Observation of histograms and normal probability plots and a Shapiro–Wilk test will be performed to determine the data whether followed a normal

distribution. Distributed data will be described using means, standard deviations, and 95% confidence intervals (CIs). Clinical outcome analyses will be done with SPSS software (SPSS 22.0 KO for Windows ©), with a significance level of 0.05, and all hypothesis tests are two-tailed.

Baseline demographic characteristics between the two groups will be analyzed with χ^2 test or Fisher's exact test for categorical measures, and with the T-test or Wilcoxon rank-sum test for continuous measures. The clinical outcome of the global cognitive function based on composite scores will be compared between the acupuncture group and sham acupuncture group. The composite scores will be created by converting all individual cognitive scores to standardized z scores. As previously described [47], we computed z-scores by subtracting the baseline group mean and dividing the baseline group standard deviation, and then averaging the standardized z scores across all tests. The general linear model will be used to examine change in cognitive scores. For the other outcomes, if it agreed with normal distribution, an independent T-test will used. Otherwise, for abnormal distribution, the data will be analyzed with Wilcoxon's test. The statisticians who analyzed the data are blinded to the test settings.

MRI data analysis

For imaging data will be analyzed using DPABI toolkit performing on MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) to detect any changes in brain function due to acupuncture treatment. After data preprocessing, some graph theory-based or data driven approaches will be performed to investigate neuroplasticity between the two groups, such as regional homogeneity, amplitude of the low-frequency fluctuation, and voxel-wise degree centrality. A two-sample t-test will be conducted to investigate the differences in brain regions between the acupuncture group and the sham acupuncture group in the DPARSF software. Multiple comparisons will be used in order to better control for a highly inflated false positivity rate. Pearson's correlation analysis will be performed to examine the association between the fMRI image data and clinical variables.

Ethics and dissemination

General ethical approval has been obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018

(Ref: 2017BL-061-02). The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement and the CONSORT statement for non-pharmacological interventions have been used as a framework for development of the methodology for this project [24 25].

If any modifications or decision are made, amendments will be reviewed and approved by the ethics committee, and new protocols would be uploaded to Clinicaltrials.gov. The results of the study will be published in a peer-reviewed academic journal and will also be disseminated electronically through conference presentations.

Patient and public involvement

Currently, this trial is at the recruitment phase with no patient involved in. The participants will be able to view the study results via social media.

DISCUSSION

SCD, may be the initial phase of AD, and could serve as a window of opportunity for interventions at an early disease stage [23]. This study is planned as a randomized, assessor-blinded, placebo-controlled trial to evaluate the efficacy and central mechanism of acupuncture treatment on SCD compared with a sham acupuncture group. To our knowledge, this trial is also the first study to investigate the effect of acupuncture in SCD patients.

This study has several strengths. First, we will assess patients with SCD on multiple levels, including multi-domain neuropsychological tests, functional brain alterations, cerebrovascular risk factors, and AOPE genotyping. This multi-domain assessment will be used to identify possible biomarkers involved in the effects of acupuncture in SCD.

Second, the neuropsychological assessment in the present study is a multi-domain neuropsychological test battery that included eight tests, which can assess multiple cognitive domains including executive functions, attention, visuospatial functioning, and language. Memory decline associated with AD risk factors [48 49], and then most instruments were used previously to measure SCD focus on memory [50]. However, it might be too restrictive to limit the SCD assessment to memory, in particular for atypical forms of AD [9 51]. In this study, multi-domain cognitive function will be assessed.

Additionally, functional brain alterations will be used as outcome measures. Compared with the neuropsychological tests alone, the combination of in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy. A smaller training effect and ceiling effect may be found when using MRI [52]. Furthermore, it can be used as a tool to understand the mechanism of the acupuncture's effects. Functional MRI can identify whether neural efficiency is improved or the brain connectome is reorganized to achieve cognitive enhancement [53].

A potential limitation is that the patients in this study will be recruited from the community. Considering the research environment, individuals recruited from a memory clinic may have a higher probability of having preclinical AD. They had specific concerns sufficient to prompt a medical visit [15]. However, community based studies have shown some predictive value of SCD for cognitive decline and dementia [10 54]. Another potential limitation of our study is that it will not include a large sample size, and this may lead to the need for a future study. The main feature of our study is to systemically investigate and analyze the central mechanisms of acupuncture treatment in patients with SCD. The findings may provide deeper insight into the benefits and mechanisms of acupuncture for patients with SCD.

Trial status

This trial is currently in the recruitment phase.

Figure Legends

Figure 1. Flow chart.

Figure 2. Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

Figure 3. Magnetic resonance imaging experimental paradigm and an Illustrative diagram of the memory task.

Figure 4. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the schedule of the trial.

Supplement figure 1. (a) The adhesive pads and sham needles with a blunt tip; (b) The adhesive pad was pasted on the surface of skin; (c) The sham needle was placed on the adhesive pad.

Footnotes

List of abbreviations SCD: Subjective Cognitive Decline; AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; APOE: apolipoprotein E; MRI: Magnetic Resonance Imaging; AFT: Animal Fluency Test; TMT: Trail Making Test; DSST: Digit Symbol Substitution Test; SCWT: Stroop Color Word Test; AVLT: Auditory Verbal Learning Test; SCDQ: Subjective Cognitive Decline Questionnaire; SBSDS: Santa Barbara Sense of Direction Scale; HAMD: Hamilton Rating Scale for Depression; HAMA: Hamilton Rating Scale for Anxiety; PSQI: Pittsburgh Sleep Quality Index.

Ethics approval and consent to participate Ethics approval The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial. The methods were carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from each patient.

Consent for publication Not applicable.

Availability of data and materials Not applicable. This is a study protocol only and as such no unpublished data are available.

Competing interests The authors declare that they have no competing interests.

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Authors' contributors Conceived and designed the experiments: CZL and JWH. Performed the experiments: PZ, ZYW, SQH, CQY, GXS, JQL, YNZ. Analyzed the data: XW, JW, JFT. Wrote the paper: CQY, XW and CZL. All authors approved the final manuscript.

Provenance and peer review Not commissioned; externally peer reviewed.

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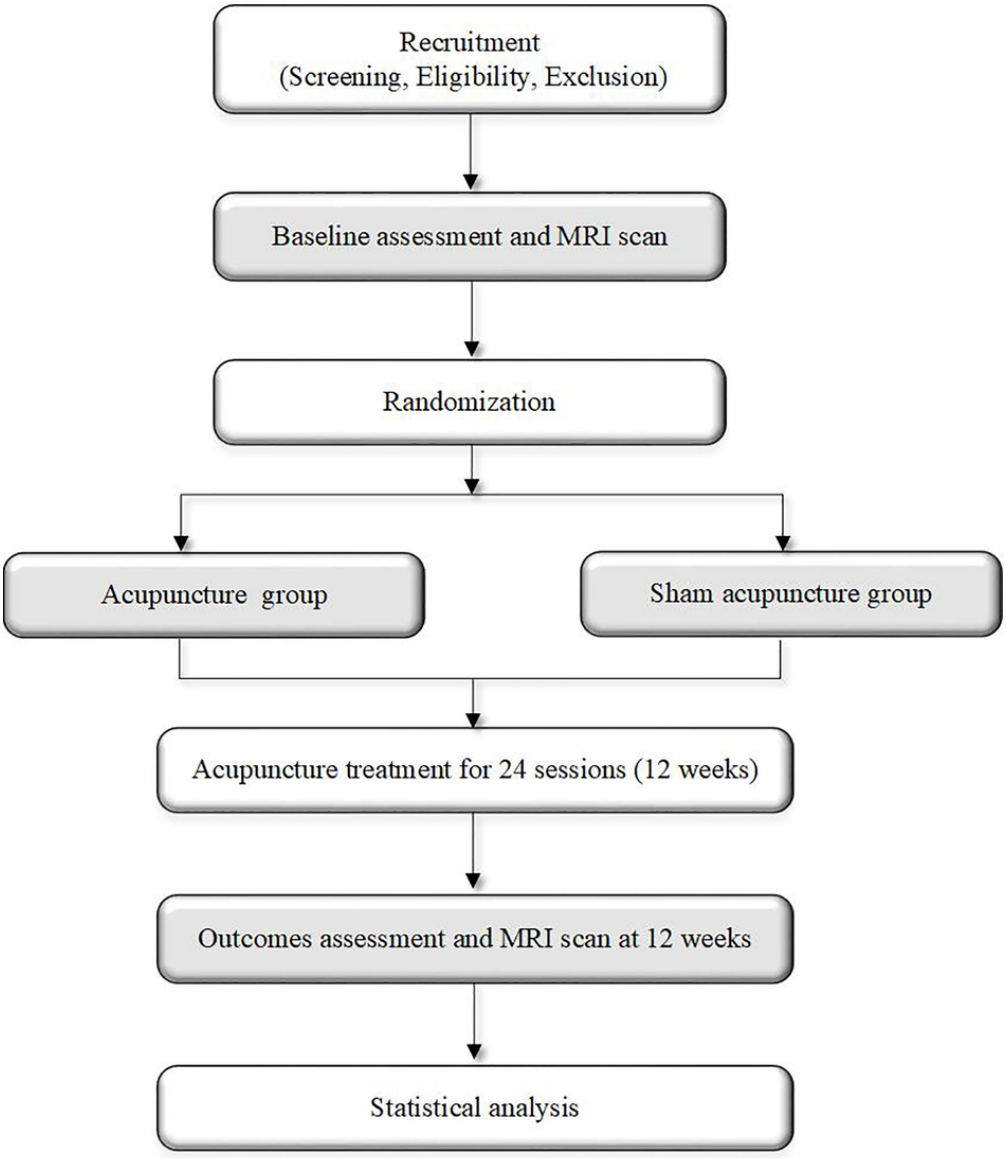

Flow chart.

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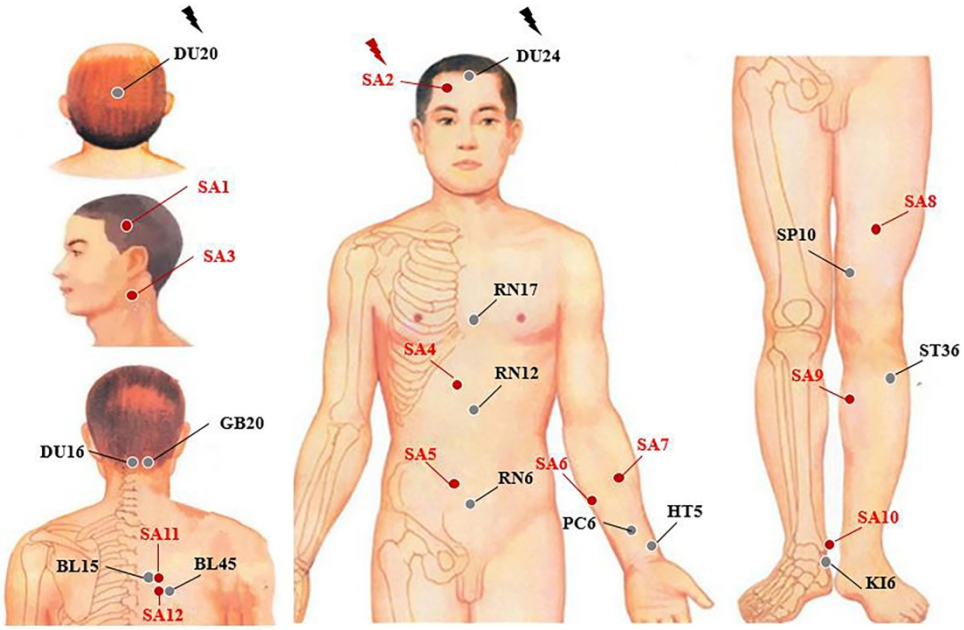

Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

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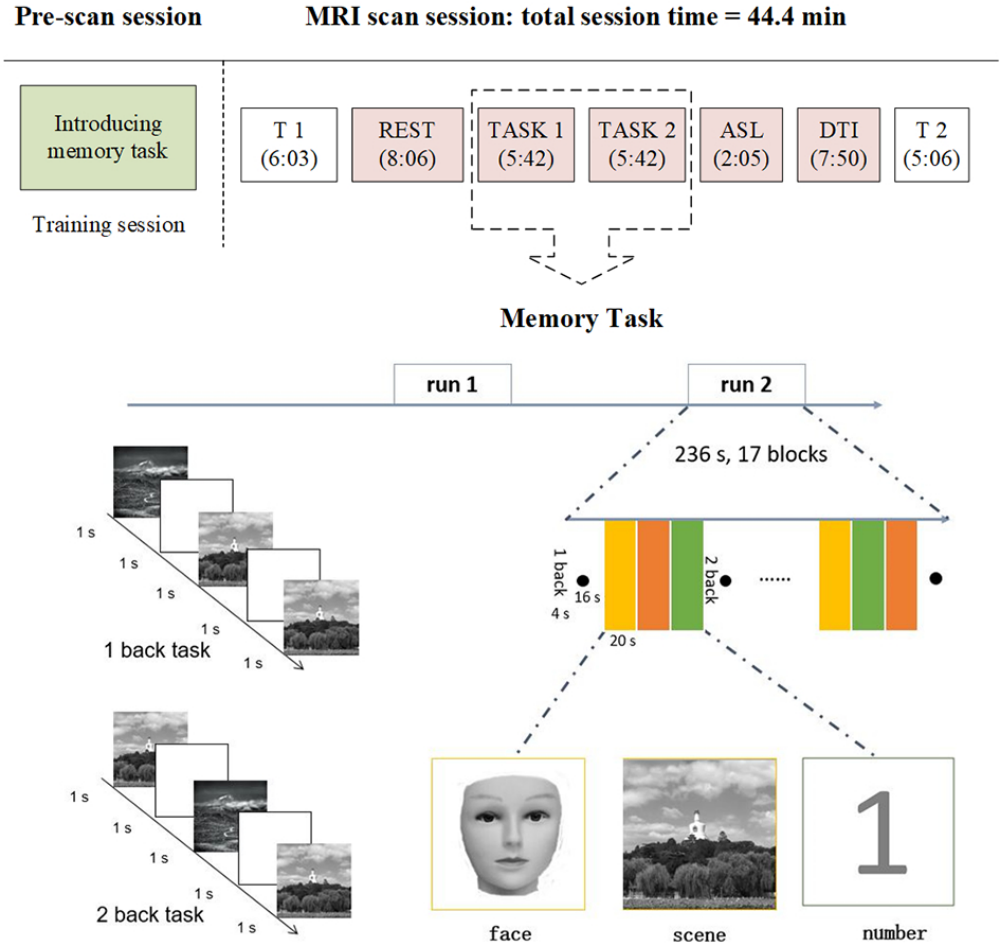
Pre-scan session
MRI scan session: total session time = 44.4 min
Introducing memory task
Training session
T 1 (6:03)
REST (8:06)
TASK 1 (5:42)
TASK 2 (5:42)
ASL (2:05)
DTI (7:50)
T 2 (5:06)
Memory Task
run 1
run 2
236 s, 17 blocks
1 back
2 back
16 s
4 s
20 s
1 s
1 back task
2 back task
face
scene
number

Study period				
Items	Baseline		Treatment phase	Outcome assessment
Time point	-1 week	0 week	1- 12 weeks	13-14 weeks
Enrolment				
Eligibility screen	X			
Informed consent	X			
Examination		X		
Randomization		X		
Intervention				
Acupuncture (n = 30)			X	
Sham acupuncture (n = 30)			X	
MRI scan				
Acupuncture		X		X
Sham acupuncture		X		X
Assessment				
AFT		X		X
TMT		X		X
DSST		X		X
SCWT		X		X
AVLT		X		X
SCDQ		X		X
SBSDS		X		X
HAMD		X		X
HAMA		X		X
PSQI		X		X
Safety				
Laboratory test		X		X
Adverse events			X	X

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of the trial.

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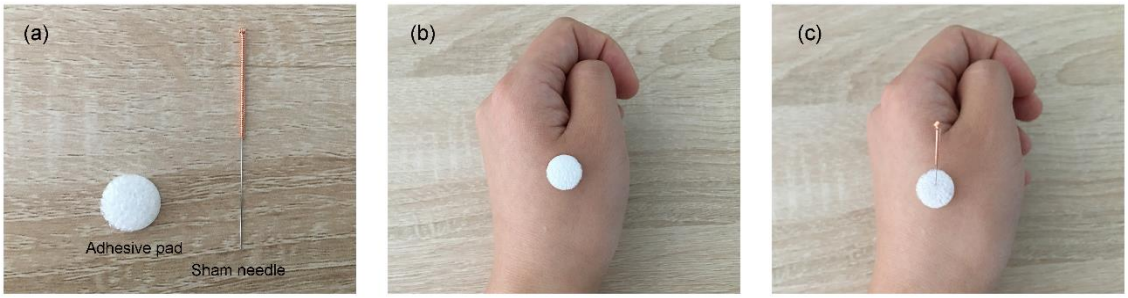

Supplement figure 1. Pictures of sham needle. (a) The adhesive pad and sham needle with a blunt tip; (b) The adhesive pad is pasted on the surface of skin; (c) The sham needle is placed on the adhesive pad.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	5
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	Not statement
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	5,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11,12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3–4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9–11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Figure 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11,12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Not statement
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	5

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not offer in the manuscript
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	Subjective cognitive decline, Dementia < NEUROLOGY, Acupuncture, Sham acupuncture, Magnetic resonance imaging < RADIOLOGY & IMAGING, Clinical trial

The efficacy and neural mechanism of acupuncture treatment in older adults with Subjective Cognitive Decline: study protocol for a randomized controlled clinical trial

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Word count：4588

ABSTRACT

INTRODUCTION: Subjective cognitive decline (SCD) refers to individuals' perceived decline in memory and/or other cognitive abilities relative to their previous level of performance, while objective neuropsychological deficits are not observed. SCD may represent a preclinical phase of Alzheimer's disease. At this very early stage of decline, intervention could slow the rate of incipient decline to prolong and preserve cognitive and functional abilities. However, there is no effective treatments recommended for individuals with SCD. Acupuncture, as a non-pharmacologic intervention, has been widely employed for patients with cognitive disorders.

METHODS AND ANALYSIS: The proposed study is a randomized, assessor-blinded and placebo-controlled study to investigate the efficacy and mechanism of acupuncture in SCD. Sixty patients with SCD will be randomly allocated either into an acupuncture group or a sham acupuncture group. They will receive 24 sessions of real acupuncture treatment or identical treatment sessions using a placebo needle. Global cognitive changes based on a multi-domain neuropsychological test battery will be evaluated to detect the clinical efficacy of acupuncture treatment at baseline and end of treatment. Magnetic Resonance Imaging (MRI) scans will be used to explore acupuncture related neuroplasticity changes. Correlation analyses will be performed to investigate the relationships between the changes in brain function and symptom improvement.

ETHICS AND DISSEMINATION: The trial was approved by the Research Ethical Committee. The results of the study will be published in a peer-reviewed academic journal and will also be disseminated electronically through conference presentations.

TRIAL REGISTRATION NUMBER: ClinicalTrials.gov NCT03444896. Retrospectively registered on 23 February 2018.

Keywords: Subjective cognitive decline; Dementia; Acupuncture; Sham acupuncture; Magnetic Resonance Imaging; Clinical trial

Strengths and limitations of this study

Compared with the neuropsychological tests alone as efficacy evaluations, the combination with in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy.

This study will firstly uncover the efficacy and neural mechanism of acupuncture treatment in older adults with SCD.

A multi-domain neuropsychological test battery will be employed, which can assess multiple cognitive domains including executive function, attention, visuospatial function, and language.

Sham needles and adhesive pads will be used for better patient blinding.

A potential limitation is that not a large sample size will be performed in this study.

INTRODUCTION

Dementia is the greatest global challenge for health and social care in the 21st century [1]. Globally, about 47 million people were living with dementia in 2015, and this number is projected to triple by 2050, based on the World Alzheimer Report (2015). Alzheimer's disease (AD) is the most common form of dementia [2]. Currently, there is no effective cure for AD, and the available treatments have only moderately alleviate symptoms [3]. Therefore, prevention is essential to reduce the dementia epidemic [4 5]. The long "preclinical" phase of AD provides an opportunity for individuals to participate in treatment trials to delay or prevent cognitive decline [6-8].

Subjective cognitive decline (SCD) usually occurs in older adults, and it refers to the self-perception of cognitive decline, when individuals perform cognitive tests within normal limits and have preserved activities of daily living [9]. A review demonstrated that the prevalence of these complaints in persons aged 65 years or more varied from 25% to 50% [10]. Actually, many older adults claim cognitive decline might be regarded as hypochondriacs by professional healthy carers. However, several lines of evidence from longitudinal aging studies suggests that older adults with SCD are more likely to present AD biomarkers than their healthy peers. About 60% SCD individuals will decline to mild cognitive impairment (MCI) and AD over a 15-year

period [11 12]. People with SCD have more brain abnormalities such as hippocampal volume loss and hypometabolism compared with healthy controls [13]. Moreover, cerebrospinal amyloid β is strongly predictive of subsequent clinical progression in patients with SCD [14]. This suggests that, for some older adults, SCD may represent a preclinical phase of AD.

There is still much plasticity in SCD. The patients in this stage may not progress to dementia or even revert to no cognitive impairment after effective treatment. Development of safe and effective interventions in early AD stages is important. Specific criteria to define SCD have been published [9 15], but clinical trials in SCD are in their infancy, and no pharmacological treatment or interventions is currently recommended for individuals with SCD. Despite these difficulties, the development of new treatments should be encouraged [16].

The accumulated evidence suggests that non-pharmacologic intervention may benefit cognitive function in older adults with SCD [17]. Acupuncture, as a non-pharmacologic intervention, that has been widely used for patients with cognitive disorders. The WHO reports that acupuncture treatment can be beneficial for vascular dementia. A number of clinical studies have provided evidence that acupuncture is beneficial for the treatment of dementia or MCI [18]. A systematic review supports the use of acupuncture for MCI [19]. Animal studies showed that acupuncture elicits its effects by mediation of neural plasticity in pathological conditions [20]. However, the efficacy of acupuncture in patients with SCD has not be investigated. An experimental study is needed to test this hypothesis.

By definition, individuals with SCD are within the normal range on clinical-neuropsychological tests. It will be difficult to detect response in this population compared with people who already have clinically manifest impairment (e.g., MCI) due to a ceiling effect. As such, neuropsychological tests in conjunction with in-vivo measures of brain function such as electroencephalo-graphy, or functional magnetic resonance imaging (fMRI), may be a more sensitive alternative to neuropsychological tests [21-23]. Brain alterations are subtle but measurable to identify individuals at risk for AD well before cognitive symptoms are manifested by using fMRI, allowing researchers to use neuroimaging to ascertain response after intervention sensitivity [24]. For better ascertaining the clinical response and unveiling the mechanism, brain alterations measured by fMRI could also be used to evaluate the efficacy of acupuncture.

OBJECTIVES

This study is a randomized controlled functional brain imaging trial with 12 weeks of treatment, aiming at: (1) evaluating the effects of acupuncture treatment on cognitive function in older adults with SCD; (2) exploring the central mechanism of the long-lasting effect of acupuncture on SCD; and (3) investigating the safety of acupuncture treatment on SCD.

METHODS

Design

General ethical approval was obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018. The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement for non-pharmacological interventions has been used as a framework for development of the methodology for this project [25 26].

This trial is a 1:1 randomized single-blinded, and placebo-controlled study with two parallel groups involving elderly adults. The study is designed to examine the effect and neural mechanism of acupuncture treatment on cognitive function before and after a 12 weeks intervention period. As shown in Figure 1, the cognitive assessments and MRI scans will be performed at baseline and immediately after the completion of the intervention.

Participants

The study will mainly take place in Dongcheng, Fengtai, and Shunyi District in Bejing. People with SCD will be recruited from community-dwelling population in those Districts. In order to have better insight into the trial for community residents, science popularization lectures about dementia and advertisement of the trial will be given in community service centers. The brochures and posters will also be distributed. Besides, advertisement of the trial will be shared in the official account on WeChat (China's most popular social media platform) of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University and health web site (www.39.net) or local newspaper for recruiting participants. Interested individuals will be screened in the clinic, community service centers or by phone using the inclusion/exclusion criteria. All those who meet the inclusion criteria will receive a study information sheet including

the design, procedure, benefits, and risks of the study. Before the study procedure started, the subjects will have to provide signed written informed consent forms. The diagnosis of SCD will be based on the published SCD research criteria proposed by the Subjective Cognitive Decline Initiative (SCD-I) [9]. In this study, the patients who answer "yes" to the question "Do you have problem in memory?" in the initial screening by phone or face-to-face interviews, will be selected to the next step. Besides, the Subjective Cognitive Decline questionnaire 9 (SCD-Q9) are further used for SCD screening. Only the patients with the SCD-Q9 score more than 5.0 will pass the screening.

Inclusion criteria

- Male and female adults aged 55-75;
- Native Chinese speakers who are right-handed and have at least a primary school education;
- Self-reported persistent memory decline compared with a previous normal status within the last 5 years, which is confirmed by caregivers;
- Normal age- and education-adjusted performance on neuropsychological test including the Chinese version of Mini-Mental State Examination (MMSE) [27], the auditory verbal learning test (the short-term delayed free recall, the long-term delayed free recall, and the recognition test) [28], Trail Making Test [29], and Animal Fluency Test [30].
- No or minimal impairment in activities of daily living;

Exclusion criteria

- Presence of a positive neurologic history (e.g., traumatic brain injury, stroke, Parkinson's disease, multiple sclerosis);
- Treatments that would affect cognitive function (e.g., treatment for an acute psychiatric episode, therapy with memantine, rivastigmine and donepezil);
- Presence of significant psychiatric history (e.g., bipolar disorder, schizophrenia) and/or severe anxiety and depression;
- Presence of serious heart, kidney, liver, gastrointestinal, infectious, endocrine disease or cancer;

- History of alcohol or drug abuse/addiction;
- Any contraindications for MRI scans (e.g., aneurysm clip pacemaker);
- Significant visual and/or auditory impairment uncorrected by aids, and unable to perform neuropsychological evaluations;
- Currently enrolled in another research study;
- Received acupuncture treatment in the preceding month；

During the trial period, patients with SCD who meet the following criteria will be excluded from the study:

- taking medication or receiving additional treatment that is expected to affect the cognitive function (e.g., tranquilizers, antianxiolytics, hypnotics, nootropics, and cholinomimetic agents);
- withdrawal of consent for study participation because the patients does not wish to continue;
- missing more than 5 of 24 acupuncture treatment sessions;
- occurrence of a serious adverse event that the doctors consider the treatment should be termination;
- critical protocol violation such as violation of eligibility criteria.

Intervention

Patients will receive 24 acupuncture treatment sessions over 12 weeks (twice a week). Hwato brand single-use acupuncture needles (size 0.35 × 25 mm or 0.35 × 40 mm), pragmatic placebo needles (size 0.30 × 25 mm), and SDZ-V electroacupuncture apparatuses will be used.

For the acupuncture group (Figure 2), acupuncture needles will be placed at acupoints Baihui (DU20), Shengting (DU24), Fengfu (DU16), Fengchi(GB20), Danzhong (RN17), Zhongwan (RN12), Qihai (RN6), Neiguan (PC6), Tongli (HT5), Xuehai (SP10), Zusanli (ST36), Zhaohai (KI6), Xinshu (BL15), and Yixi (BL45). After skin disinfection in patient in the supine position, the adhesive pads are pasted on the acupoints surface except for Baihui (DU20) and Shengting (DU24). Then, the acupuncture needles will be inserted through the adhesive pads into the skin depending on the location of the needle. The location is show in Table 1. Manual acupuncture by acupuncturists using a small, equal manipulations of twirling, lifting, and thrusting will be

performed on all needles to reach deqi. The patients will feel the deqi sensation, such as soreness, numbness, distention, heaviness, and other sensations. Paired electrodes from the electroacupuncture apparatus will be attached to the needle holders of the DU20 and DU24. A dilatational wave of 2-100 Hz and a current intensity of 1 to 5 mA will be performed following by the degree of needle handle shivering, ignoring the patient's feelings. The needles will be extracted after 20 minutes for each treatment. The acupoints of DU16, BL15 and BL45 will achieve deqi in patients in a sitting position without retaining the needle.

Table 1. Location of Acupoints Used in the Acupuncture Group

Acupoints	Location	Depth
Baihui (DU20)	5 cun directly above the midpoint of the posterior hairline, or at the midpoint of the line connecting the apexes of the two auricles.	0.2 cun
Shengting (DU24)	1.0 cun directly above the midpoint of posterior hairline.	0.2 cun
Fengfu (DU16)	On the back of neck, 1 cun directly above the midpoint of the posterior hairline, directly below the external occipital protuberance.	0.5~0.1 cun
Fengchi(GB20)	On the nape, below the occiput, at the level of Fengfu, in the depression between the upper portion of sternocleidomastoideus and trapezius muscle.	0.5~0.8 cun
Danzhong (RN17)	On the anterior median line of the chest, at the level of the fourth intercostal space, at the midpoint between the two nipples.	0.5 cun
Zhongwan (RN12)	On the the anterior median line of the upper abdomen, 4 cun above the umbilicus.	1~1.5 cun
Qihai (RN6)	On the anterior median line of the lower abdomen, 1.5 cun below the umbilicus.	1~1.5 cun
Neiguan (PC6)	On the palmar aspect of forearm, 2.0 cun above the transverse crease of the wrist, between the tendons of palmaris longus and flexor carpi radialis muscle.	0.5~0.1 cun
Tongli (HT5),	On the radial aspect of the tendon of the ulnar flexor muscle of the wrist, and 1 cun above the carpal crease.	0.3~0.5 cun
Xuehai (SP10)	When the knee is flexed, on the medial aspect of the thigh, the point is 2 cun above the mediosuperior border of the patella, on the bulgs of the medial portion of muscle quadriceps femoris.	0.8~1 cun
Zusanli (ST36)	3 cun directly below Dubi* and one finger-breadth lateral to the anterior border of the tibia.	0.8~1 cun
Zhaohai (KI6)	On the depression below the tip of the medial malleolus.	0.5~0.8 cun
Xinshu (BL15)	1.5 cun from the lower border of the spinous process of the fifth thoracic vertebra.	0.3~0.5 cun
Yixi (BL45)	3 cun from the lower border of the spinous process of the sixth thoracic vertebra.	0.5~0.8 cun

Dubi* location = When the knee is flexed, the point is at the knee, below the patella, in the depression from the patella ligament.

For the sham acupuncture group, sham acupoints at locations away from known acupuncture points will be used to minimize physiological effects, and the location of sham acupoints are

showed in Table 2. Patients will receive non-insertive acupuncture using the pragmatic placebo needles (Hwato brand, size 0.35 × 25 mm; supplement figure 1). As same as in the acupuncture group, the adhesive pads are initially pasted on the sham acupoints surface except for sham acupoint 1. The pragmatic placebo needles with a blunt tip will be placed on the adhesive pads. In order to minimize the physiological effect, acupuncturists will be instructed to lightly place the sham needles with a blunt tip on the adhesive pads with no manipulation. The acupuncture needle will be inserted to a shallow depth at the sham acupoint 1, which does not penetrate below the skin, and needle manipulation for *deqi*. Sham acupoint 11 and Sham acupoint 12 will be inserted with sham needles without retaining the needle. Paired electrodes will be attached the needle holders of the bilateral sham acupoint 2 but with no electricity output.

Table 2. Location of sham acupoints used in the sham acupuncture group.

Sham Acupoints	Location
Sham acupoint 1	Midpoint of Shuaigu (GB8) and Touwei (ST8)
Sham acupoint 2	Midpoint of Touwei (ST8) and Yangbai(GB14)
Sham acupoint 3	Midpoint between Tianyou (SJ16) and Tianrong(SI17)
Sham acupoint 4	4 cun above the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 5	2 cun below the umbilicus and 1 cun right of the umbilical midline
Sham acupoint 6	1 cun outside the point 1/4 of the line between Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 7	1 cun outside the midpoint of Shenmen (HT7) and Shaohai (HT3)
Sham acupoint 8	6 cun above mediosuperior border of the patella
Sham acupoint 9	3 cun below the Yanglingquan (GB34) and in the middle of the gallbladder and bladder channels
Sham acupoint 10	Midpoint between Jiexi (ST 41) and Qiuxu (GB40)
Sham acupoint 11	2 cun from the lower border of the spinous process of the fifth thoracic vertebra
Sham acupoint 12	2 cun from the lower border of the spinous process of the sixth thoracic vertebra

MRI protocol

Patients will undergo brain MRI at baseline and after treatments. The MRI scan will be performed with a 3.0 Tesla superconductor (Skyra, Siemens, Erlangen, Germany) in the Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University. The parameters of sequences employed in this study are provided by China Association of Brain Imaging (www.abimaging.org). Sagittal structural images will be acquired using a magnetization prepared rapid gradient echo (MP-RAGE) three-dimensional T1-weighted sequence with the following parameters: TR/TE = 2,530/2.98 ms, flip angle = 7◦, inversion time = 1100 ms, matrix = 256 × 256, 1 mm slice thickness without slice gap. T2*-weighted functional images will be collected using a gradient-echo echo-planar imaging sequence with the following parameters: repetition time = 3200 ms, echo time = 407 ms, flip angle = 120°.

Resting state-fMRI and task fMRI will be performed using an echo planar imaging (EPI) sequence with the following parameters: TR/TE = 2,000/30 ms, flip angle = 90◦, matrix = 64 × 64, slice thickness = 3.5 mm with 1 mm slice gap. Diffusion tensor imaging (DTI) will use a double spin-echo echo-planar imaging sequence (TR/TE = 12,000/77 ms, flip angle = 90°, Volume interval = 12, 2 mm thick axial slices). ASL imaging of the whole brain is performed by use of a 3D pseudocontinuous ASL sequence (TR/TE = 5,000/ 15.92 ms, flip angle = 180°, Slice thickness = 3.5 mm; labeling duration, number of slices = 40).

All scans will be reviewed qualitatively by two radiologists to screen for possible brain lesions or structural abnormalities. DTI images will be analyzed using the PANDA package [31]. Functional MRI data collected during the memory task and at rest. ASL data will be performed with the Resting-State fMRI (DPARSF) toolbox and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) for MATLAB. Brain activation, connectivity changes, and cerebral blood flow will be compared between two groups before and after treatment.

Outcome measures

Clinical outcome assessments

Global cognitive function based on a composite score will be used to evaluate the clinical efficacy of acupuncture treatment at baseline, and at the end of the 12 weeks treatment period. It will be computed by averaging z-scores from a multi-domain neuropsychological test battery that

includes 8 tests. Animal Fluency Test (AFT) that assess the language and executive function by examining categorical verbal fluency [30]. Trail Making Test (TMT) part A and B examines graphomotor speed, attention, and executive function [32]. Digit Symbol Substitution Test (DSST) is used to assess processing speed, attention and concentration [33]. Clock Drawing Test (CDT) can assess multiple cognitive functions, including verbal understanding, memory, abstract thinking, and executive functions [34]. Digit-Span Test (DST) is used to measure working memory's number storage capacity. Stroop color word test (SCWT) is a classic instrument for the assessment of selective attention, cognitive flexibility, cognitive inhibition, and information processing speed [35]. Auditory Verbal Learning Test (AVLT) a powerful neuropsychological test to assess episodic memory [36 37].

All patients will fill out a subjective cognitive decline questionnaire (SCDQ) [38] to assess their degree of subjective cognitive decline. The Face recognition questionnaire and Santa Barbara Sense of Direction scale [39] will be used to measure the ability for facial recognition and direction. Depressive symptoms, and sleep quality will be measured by Geriatric Depression Scale and Pittsburgh Sleep Quality Index (PSQI) [40].

Neuroplasticity assessments

In this study, neuroplasticity changes between the two groups will be measured by fMRI. Before the acupuncture treatment, patients will complete the fMRI scan within 3 days. They will also have the follow up fMRI scan within 3 days after the completion of their intervention (Figure 3). Brain activity and functional connectivity will be assessed under a resting state and a memory task. Group difference on cerebral blood flow and white matter integrity will be assessed by means of arterial spin labeling (ASL) or diffusion tensor imaging (DTI).

Memory task. The patients will be trained before entering the fMRI scanner. They will complete a practice version of the memory task paradigm in computer. This practice mock test will show the correct number, and they need to perform the tasks with an accuracy criterion of 80% to ensure that the patients understand how to do the memory task in the scanner. A blocked periodic design that incorporated alternating 1-back, and 2-back tasks will be used. Patients will view facial pictures, scene pictures or single digits (0–9, black on a white background). In the 1-back task, the patients are required to press a button when a facial picture, scene picture or digit appears that is the same as the one before. In the 2-back task, the patients are asked to press a

response button if the current facial picture, scene picture or digit is the same as the one which was presented two trials before. The stimuli consist of 17 blocks for 236 seconds and are presented on a computer using MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) for 1 second, with an inter-stimulus interval of 1 second. Trial types within blocks are presented in pseudorandomized order.

Once in the scanner, anatomical and functional scans will be performed. During the scanning, all patients are asked to lie quietly in the scanner with their eyes open, trying to avoid thinking systematically, and moving as little as possible. In resting state fMRI scanning, the patients are instructed to view centrally placed fixation cross (+), and try to not think of anything. Two memory task runs will be conducted after T1 and resting state scans, then following by ASL, and DTI scans.

Apolipoprotein E genotyping

A TIANamp Blood DNA Kit (TIANGEN BIOTECH, Beijing, China) will be used to isolate High-molecular-weight DNA. The genotype of APOE will be detected by APOE Gene Detection Kit (Wuhan YZY Biopharma, Wuhan, China) with E2 and E4 reaction liquid. Genomic DNA and reaction liquid will be place on a 7500 HT Fast Real-Time PCR (Applied Biosystems, Foster City, USA) with the following conditions: Uracil-N-Glycosylase treatment at 37 °C for 10 minutes, denaturation at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 15s and annealing at 60 °C for 60s (acquiring the fluorescence signal in this step). According to the amplification plot, the genotype of APOE will be determined.

Safety and Monitoring

Treatment-related adverse events, including hematoma, bleeding, subcutaneous hemorrhage, serious pain, local infection and fatigue will be compared among patients in the acupuncture group and the sham acupuncture group, and will be documented at each treatment session. In order to ensure consistency, the neuropsychological tests and other assessment scales will be performed by the same researcher at baseline and the end of treatment. A professional staff will check the imaging data for quality and protocol conformity after each scanning session. Patients will undergo blood routine test and blood biochemical tests including blood glucose, homocysteine, total cholesterol, etc. before randomization and at the end of treatment.

Sample size and blinding

Task fMRI studies characteristically include small sample size [41] and thus have a low statistical power. The statistically underpowered study by definition means that a study will have less of a chance for detecting significant effects [42]. Therefore, power-based sample size will be calculated prior to fMRI data collection [43]. Fortunately, approaches for sample size calculations in studies using functional magnetic resonance imaging have been developed.

By using the non-central random field theory, Hayasaka et al estimated that at least 12 subjects would be required to detect signals in either of the auditory cortices with at least 80% power [44]. They also found that approximately 13 subjects would be required to detect signals in the auditory cortices with 80% power, when generated a sample size map based on the mock pilot analysis [44]. Adopting a simulation-based method to calculate statistical power for group-level fMRI studies, Desmond and Glover found a minimum of 12 subjects are required to achieve 80% power at a = 0.05 at the single voxel level [45]. For a more realistic thresholds, twice as many subjects are recommended to maintain this level of power after correcting for multiple comparisons. For a stricter alpha of 0.000002, approximately 25 subjects are needed. Mumford and Nichol recommended 20 subjects and a Type I error of $\alpha = 0.005$ should probably be used, and this power calculation is based on a non-central T or F distribution [46]. We used the largest sample size of 25 for each group with an estimated dropout rate and loss of data due to head motion, then we planned to enroll 60 participants in the two groups.

Eligible patients will be randomly assigned into either the acupuncture or sham acupuncture group after signed written informed consent forms via a randomization digital table with a 1:1 ratio. Blocked randomization with a block size of 6 will be employed to ensure balance within the two groups. The randomization sequence will be generated by a third-party professional statistician using computer-generated randomization the digital table by using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The randomization list will be stored by a noninvolved investigator and out of reach and sight of the involved investigators. The allocation schedule using a telephone randomization procedure. The randomization list was restricted to this research coordinator and was concealed from other study personnel. The patients, outcome assessors, and statisticians will be blinded to treatment allocation. Patients are told that they will receive one of two effective interventions randomized after enrolment. During the acupuncture treatment, the

adhesive pads are pasted on the acupoints or sham acupoints after skin disinfection. The true or sham needles with a blunt tip will place in the adhesive pads. Patients in different groups will be separated into cubicles to refrain from communication.

Data collection and management

At baseline, information of patients about age, sex, education, dominant hand, and medical history will be collected (Figure 4). For all patients that met the inclusion criteria, the neuropsychological tests will be performed before and after treatment in a quiet room by evaluators, who have been trained by the organization of Alliance of early AD. All of the MRI scans will be conducted in the same machine, and the operators have been technically trained by a professional engineer from the China Association of Brain Imaging. Blood samples will be collected in the morning and handled by 2 persons. After each treatment session, adverse event will be recorded in participant's diary card by acupuncturists.

A case report form (CRF) will be used to collect the clinical data for each patient, labeled by unique numeric identifier and recorded by a trained graduate student. The data in the CRF will be verified for accuracy, missing data, and data consistency by a clinical research associate. Paper-based research data will be entered into an EpiData electronic database. For reducing errors in data entry, data will be entered independently twice by two independent people, and value pairs will then be compared for discordances, followed by resolution of discordances by referral to the original data source. The EpiData will export data in a Microsoft Excel spreadsheet formats. The data will be entered in an electronic Statistical Package SPSS software (SPSS 12.0 KO for Windows ©) for ease of statistical analysis.

Statistical analysis

Clinical data analysis

In this study, intent-to-treat analysis and per protocol analysis will be used. In this study, the intent-to-treat population consisted of all randomized patients who received at least one dose of treatment and had a complete baseline assessment. The per protocol population defined as all randomized patients who did not discontinue prematurely, completed the 12 weeks treatment and had MRI scans before and after treatment. Observation of histograms and normal probability plots and a Shapiro–Wilk test will be performed to determine the data whether followed a normal

distribution. Distributed data will be described using means, standard deviations, and 95% confidence intervals (CIs). Clinical outcome analyses will be done with SPSS software (SPSS 22.0 KO for Windows ©), with a significance level of 0.05, and all hypothesis tests are two-tailed.

Baseline demographic characteristics between the two groups will be analyzed with χ^2 test or Fisher's exact test for categorical measures, and with the T-test or Wilcoxon rank-sum test for continuous measures. The clinical outcome of the global cognitive function based on composite scores will be compared between the acupuncture group and sham acupuncture group. The composite scores will be created by converting all individual cognitive scores to standardized z scores. As previously described [47], we computed z-scores by subtracting the baseline group mean and dividing the baseline group standard deviation, and then averaging the standardized z scores across all tests. The general linear model will be used to examine change in cognitive scores. For the other outcomes, if it agreed with normal distribution, an independent T-test will used. Otherwise, for abnormal distribution, the data will be analyzed with Wilcoxon's test. The statisticians who analyzed the data are blinded to the test settings.

MRI data analysis

For imaging data will be analyzed using DPABI toolkit performing on MATLAB 8.6 (Mathworks, Inc., Natick, MA, USA) to detect any changes in brain function due to acupuncture treatment. After data preprocessing, some graph theory-based or data driven approaches will be performed to investigate neuroplasticity between the two groups, such as regional homogeneity, amplitude of the low-frequency fluctuation, and voxel-wise degree centrality. A two-sample t-test will be conducted to investigate the differences in brain regions between the acupuncture group and the sham acupuncture group in the DPARSF software. Multiple comparisons will be used in order to better control for a highly inflated false positivity rate. Pearson's correlation analysis will be performed to examine the association between the fMRI image data and clinical variables.

Ethics and dissemination

General ethical approval has been obtained from the ethics committee of the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University on 29 January, 2018

(Ref: 2017BL-061-02). The study was registered under clinicaltrials.gov (NCT03444896). The CONSORT statement and the CONSORT statement for non-pharmacological interventions have been used as a framework for development of the methodology for this project [24 25].

If any modifications or decision are made, amendments will be reviewed and approved by the ethics committee, and new protocols would be uploaded to Clinicaltrials.gov. The results of the study will be published in a peer-reviewed academic journal and will also be disseminated electronically through conference presentations.

Patient and public involvement

Currently, this trial is at the recruitment phase with no patient involved in. The participants will be able to view the study results via social media.

DISCUSSION

SCD, may be the initial phase of AD, and could serve as a window of opportunity for interventions at an early disease stage [23]. This study is planned as a randomized, assessor-blinded, placebo-controlled trial to evaluate the efficacy and central mechanism of acupuncture treatment on SCD compared with a sham acupuncture group. To our knowledge, this trial is also the first study to investigate the effect of acupuncture in SCD patients.

This study has several strengths. First, we will assess patients with SCD on multiple levels, including multi-domain neuropsychological tests, functional brain alterations, cerebrovascular risk factors, and AOPE genotyping. This multi-domain assessment will be used to identify possible biomarkers involved in the effects of acupuncture in SCD.

Second, the neuropsychological assessment in the present study is a multi-domain neuropsychological test battery that included eight tests, which can assess multiple cognitive domains including executive functions, attention, visuospatial functioning, and language. Memory decline associated with AD risk factors [48 49], and then most instruments were used previously to measure SCD focus on memory [50]. However, it might be too restrictive to limit the SCD assessment to memory, in particular for atypical forms of AD [9 51]. In this study, multi-domain cognitive function will be assessed.

Additionally, functional brain alterations will be used as outcome measures. Compared with the neuropsychological tests alone, the combination of in-vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy. A smaller training effect and ceiling effect may be found when using MRI [52]. Furthermore, it can be used as a tool to understand the mechanism of the acupuncture's effects. Functional MRI can identify whether neural efficiency is improved or the brain connectome is reorganized to achieve cognitive enhancement [53].

A potential limitation is that the patients in this study will be recruited from the community. Considering the research environment, individuals recruited from a memory clinic may have a higher probability of having preclinical AD. They had specific concerns sufficient to prompt a medical visit [15]. However, community based studies have shown some predictive value of SCD for cognitive decline and dementia [10 54]. Another potential limitation of our study is that it will not include a large sample size, and this may lead to the need for a future study. The main feature of our study is to systemically investigate and analyze the central mechanisms of acupuncture treatment in patients with SCD. The findings may provide deeper insight into the benefits and mechanisms of acupuncture for patients with SCD.

Trial status

This trial is currently in the recruitment phase.

Figure Legends

Figure 1. Flow chart.

Figure 2. Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

Figure 3. Magnetic resonance imaging experimental paradigm and an Illustrative diagram of the memory task.

Figure 4. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the schedule of the trial.

Supplement figure 1. (a) The adhesive pads and sham needles with a blunt tip; (b) The adhesive pad was pasted on the surface of skin; (c) The sham needle was placed on the adhesive pad.

Footnotes

List of abbreviations SCD: Subjective Cognitive Decline; AD: Alzheimer's disease; MCI: Mild Cognitive Impairment; APOE: apolipoprotein E; MRI: Magnetic Resonance Imaging; AFT: Animal Fluency Test; TMT: Trail Making Test; DSST: Digit Symbol Substitution Test; SCWT: Stroop Color Word Test; AVLT: Auditory Verbal Learning Test; SCDQ: Subjective Cognitive Decline Questionnaire; SBSDS: Santa Barbara Sense of Direction Scale; HAMD: Hamilton Rating Scale for Depression; HAMA: Hamilton Rating Scale for Anxiety; PSQI: Pittsburgh Sleep Quality Index.

Contributors Conceived and designed the experiments: CZL and JWH. Performed the experiments: PZ, ZYW, SQH, CQY, GXS, JQL, YNZ. Analyzed the data: XW, JW, JFT. Wrote the paper: CQY, XW and CZL. All authors approved the final manuscript.

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Consent for publication Not applicable.

Patient consent for publication Not required.

Ethics approval Ethics approval The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Not applicable. This is a study protocol and no unpublished data are available. Further information unaddressed can be obtained from the corresponding author.

Competing interests The authors declare that they have no competing interests.

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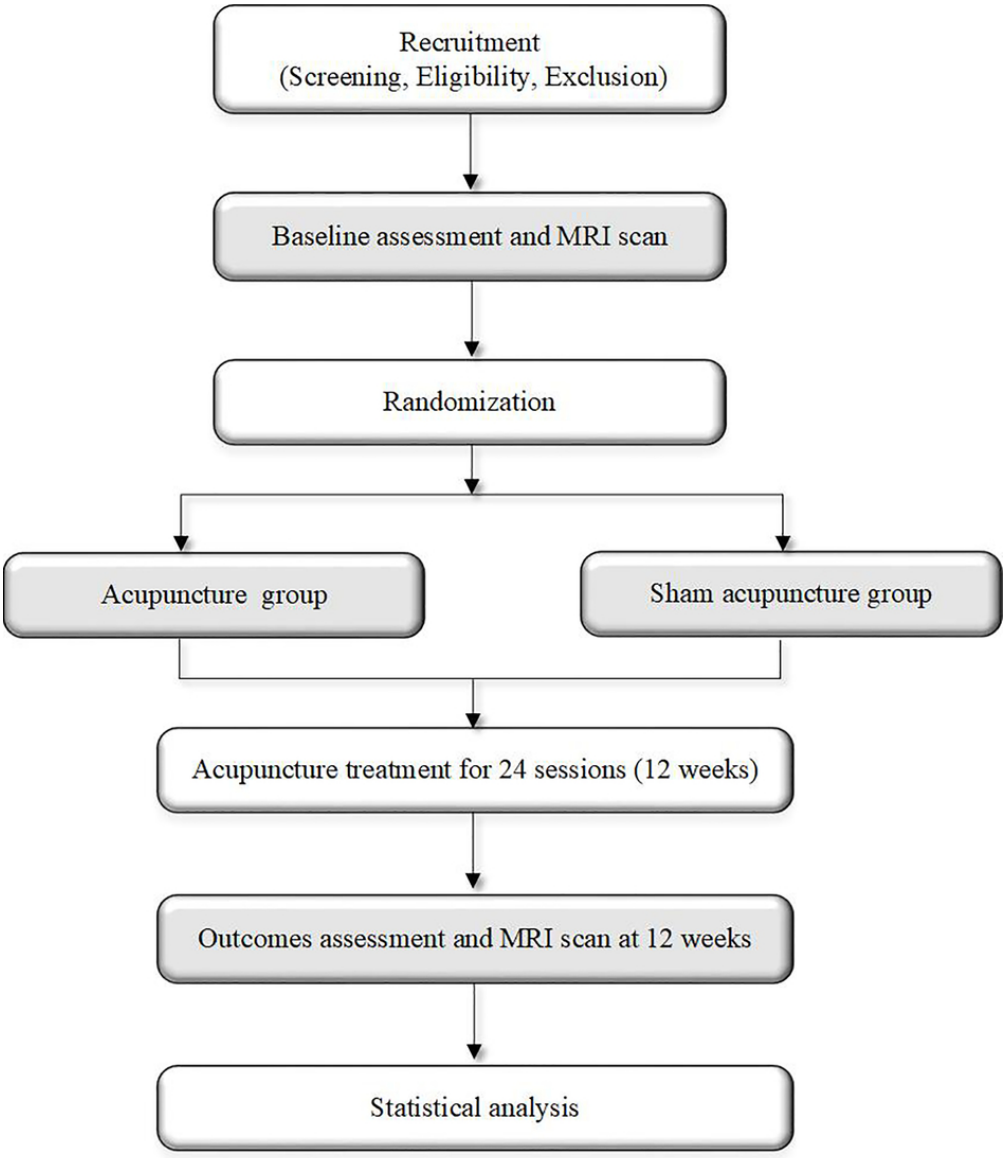

Flow chart.

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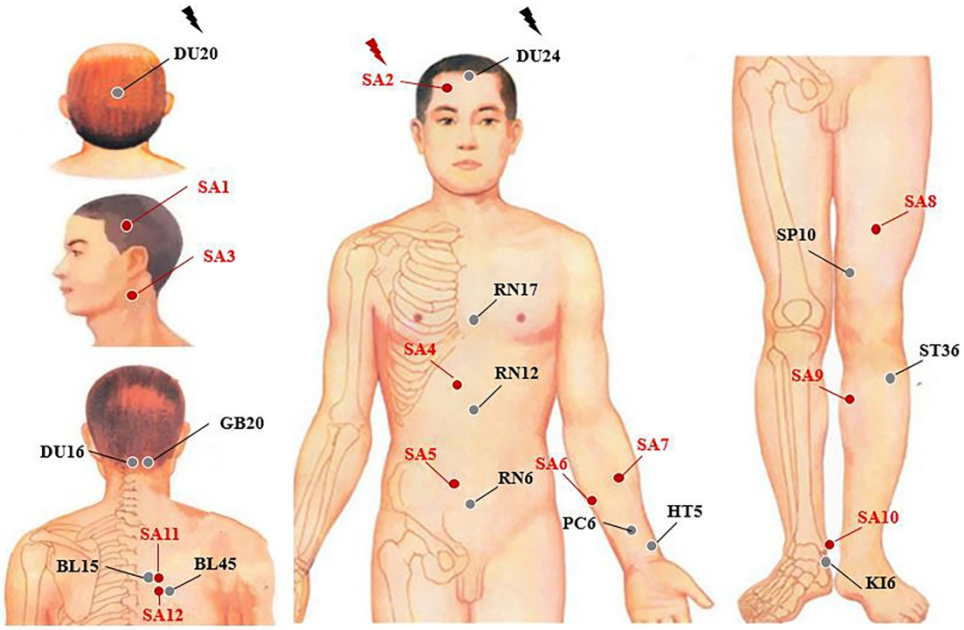

Location of acupoints or sham acupoints in the trial. Abbreviation: SA Sham acupoint, DU20 Baihui, DU24 Shengting, DU16 Fengfu, GB20 Fengchi, RN17 Danzhong, RN12 Zhongwan, RN6 Qihai, PC6 Neiguan, HT5 Tongli, SP10 Xuehai, ST36 Zusanli, KI6 Zhaohai , BL15 Xinshu, BL45 Yixi.

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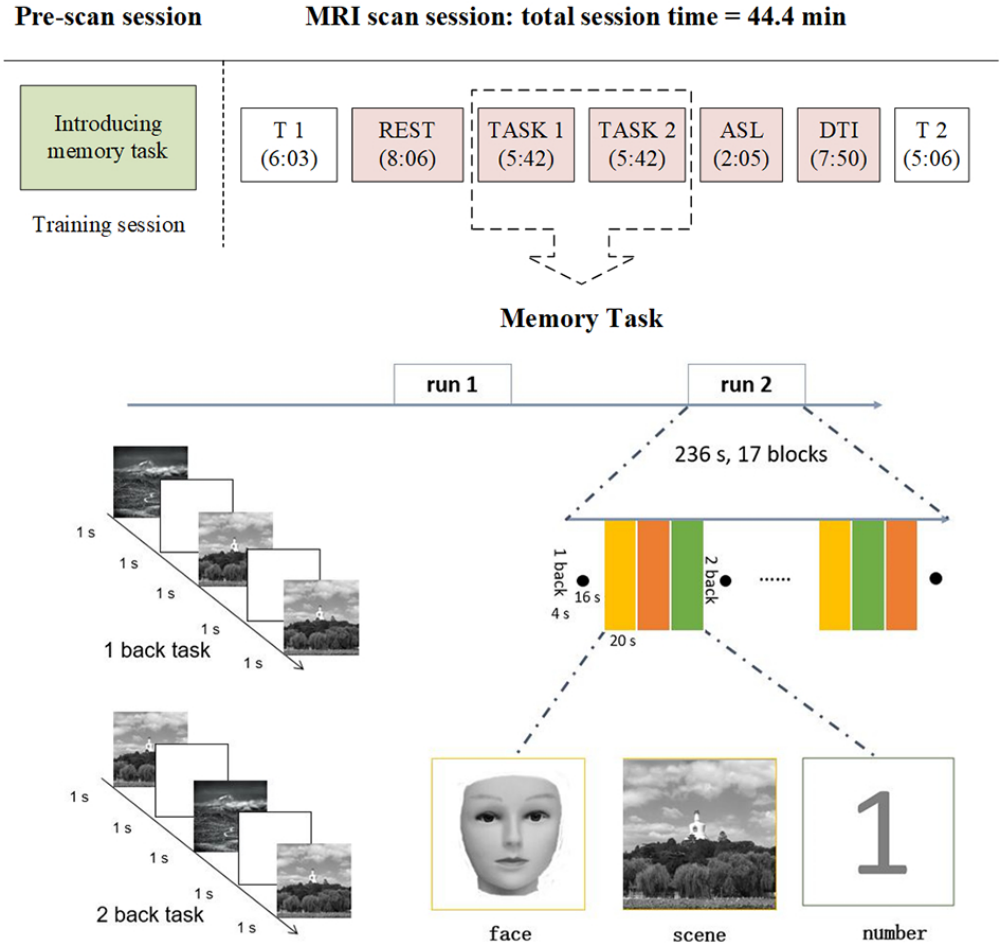
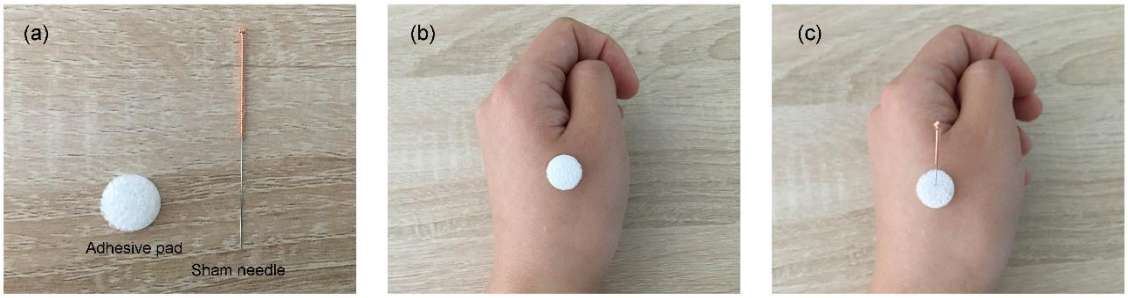

Pre-scan session
MRI scan session: total session time = 44.4 min
Introducing memory task
Training session
T 1 (6:03)
REST (8:06)
TASK 1 (5:42)
TASK 2 (5:42)
ASL (2:05)
DTI (7:50)
T 2 (5:06)
Memory Task
run 1
run 2
236 s, 17 blocks
1 back
2 back
16 s
4 s
20 s
1 s
1 back task
2 back task
face
scene
number

Study period				
Items	Baseline		Treatment phase	Outcome assessment
Time point	-1 week	0 week	1- 12 weeks	13-14 weeks
Enrolment				
Eligibility screen	X			
Informed consent	X			
Examination		X		
Randomization		X		
Intervention				
Acupuncture (n = 30)			X	
Sham acupuncture (n = 30)			X	
MRI scan				
Acupuncture		X		X
Sham acupuncture		X		X
Assessment				
AFT		X		X
TMT		X		X
DSST		X		X
SCWT		X		X
AVLT		X		X
SCDQ		X		X
SBSDS		X		X
HAMD		X		X
HAMA		X		X
PSQI		X		X
Safety				
Laboratory test		X		X
Adverse events			X	X

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of the trial.

80x73mm (300 x 300 DPI)



Supplement figure 1. Pictures of sham needle. (a) The adhesive pad and sham needle with a blunt tip; (b) The adhesive pad is pasted on the surface of skin; (c) The sham needle is placed on the adhesive pad.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	5
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	Not statement
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	5,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11,12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3–4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9–11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Figure 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11,12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Not statement
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	5

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*(continued)

Section/item	Item No	Description	Page
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not offer in the manuscript
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.