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Corticocortical paired associative stimulation for treating motor dysfunction after stroke: study protocol for a randomised sham-controlled double-blind clinical trial

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ABSTRACT

Introduction Stroke survivors can have a high disability rate with low quality of daily life, resulting in a heavy burden on family and society. Transcranial magnetic stimulation has been widely applied to brain injury repair, neurological disease treatment, cognition and emotion regulation and so on. However, there is still much to be desired in the theories of using these neuromodulation techniques to treat stroke-caused hemiplegia. It is generally recognised that synaptic plasticity is an important basis for functional repair after brain injury. This study protocol aims to examine the corticocortical paired associative stimulation (ccPAS) for inducing synaptic plasticity to rescue the paralysed after stroke.

Methods and analysis The current study is designed as a 14-week double-blind randomised sham-controlled clinical trial, composed of 2-week intervention and 12-week follow-up. For the study, 42 patients who had a stroke aged 40–70 will be recruited, who are randomly assigned either to the ccPAS intervention group, or to the control group at a 1:1 ratio, hence an equal number each. In the intervention group, ccPAS is practised in conjunction with the conventional rehabilitation treatment, and in the control group, the conventional rehabilitation treatment is administered with sham stimulation. A total of 10 interventions will be made, 5 times a week for 2 weeks. The same assessors are supposed to evaluate the participants' motor function at four time points of the baseline (before 10 interventions), treatment ending (after 10 interventions), and two intervals of follow-up (1 and 3 months later, respectively). The Fugl-Meyer Assessment Upper Extremity is used for the primary outcomes. The secondary outcomes include changes in the assessment of Action Research Arm Test (ARAT), Modified Barthel Index (MBI), electroencephalogram (EEG) and functional MRI data. The adverse events are to be recorded throughout the study.

Ethics and dissemination This study was approved by the Medical Ethics Committee of Yueyang Hospital. All ethical work was performed in accordance with the Helsinki declaration. Written informed consent was obtained from all individual participants included in the study. Study findings will be disseminated in the printed media.

Strengths and limitations of this study

- This study provides a novel direction for the future clinical trials in this field, developing more efficient neural regulation model for the rehabilitation of motor dysfunction after a stroke.
- This randomised sham-controlled double-blind clinical trial with stringent concealment of allocation eliminates treatment and allocation bias.
- Due to the individual differences, it may result in slight deviations on the brain regions location.
- The trial will include a single centre; it may result in under-representation of the study.

Trial registration number Chinese Clinical Trial Registry: ChiCTR2000036685.

INTRODUCTION

Stroke is a common cerebrovascular disease with a high disability rate and a high mortality rate.[1 2] Nowadays, it is one of the most important diseases that threaten human health in the world.[3] Although the mortality rate of stroke has been continuously decreased with the improvement of emergency medicine in the recent years, the disability rate of survivors is still as high as 70%–80%, which significantly affects patients' daily activities,[4] and more important, the majority of stroke survivors cannot return to work, placing a heavy burden on family and society.[5] Although many patients can have a spontaneous recovery process at the early stage of a stroke,[6 7] improving functional recovery of patients through the existing therapeutics and rehabilitation strategies is actually far from satisfactory.[8] Stroke is well known to affect brain function and structure[9]; however, so far the repair and functional

reconstruction of brain tissues still remains a challenge for clinical rehabilitation.

Stroke destroys the cortices and the connections between them, with major functional impairments in motor, sensory, language and cognition. One of the most common stroke-caused sequela is a significant decline in motor function and the loss of dexterity caused by the destruction of motor cortex and connection between motor cortices.[10] Therefore, focal brain injury can cause the destruction of the integrity of the motor circuits during the movement process.[11] For the reason that the brain is highly networked, the global network organisation of the brain can also be widely affected by stroke,[12] impairing the flexibility of the functional network in patients who had a stroke.[13] As previously reported, the reconstruction of neural circuits was an important foundation for the reconstruction of functional brain networks after focal brain injury.[14] A number of researchers have tried to stimulate the affected primary motor cortex (M1) for motor function recovery[15]; however, little has been achieved to enhance the integration and collaboration of large-scale brain networks for performing limb movements after stroke. We believe that enhancing specific brain connections to achieve the reconstruction of neural circuits and even brain networks is essential for patients who had a stroke to achieve better motor control of the paralysed limbs and recover functions. In the case of the motor-related cortex, for example, there are rich and close connections between the supplementary motor area (SMA) and M1.[16]

Reconstruction of the brain, which depends on neuroplasticity, is the basis for function recovery. A growing number of evidence have shown that the compensatory ability of the injured brain is of synaptic plasticity on the cellular level, and that the compensatory ability largely depends on the strength of changes in the precise synaptic connections between neurons at different regions.[17] During the compensatory procedure, both structural and functional remodelling rely on the synaptic plasticity, which is regulated by the neuronal activities and various secretory factors.[18] Therefore, the foundation of neural circuit reconstruction is to enhance the intensity of synaptic activity and the regeneration of new synapses.

Transcranial magnetic stimulation (TMS), one of the most commonly used non-invasive brain stimulation techniques,[19] has been generally accepted to induce neuroplasticity compensation for brain injury and repair.[20–22] At present, however, most related investigations are based on the regulation of excitability in the local brain regions,[15] and few of them focus on the regulating of the connectivity between key brain regions affiliated to the specific neural circuits of the brain networks. In the current study, we will follow the spike-timing-development plasticity (STDP) based on Hebbian plasticity principle.[23] Hebbian plasticity is the major form of activity-dependent synaptic learning rules that modify neural circuits,[24] proposed by Donald Hebb.[25] It is also the learning rule of how neural activity determines the changes of synaptic strength in a spatiotemporal pattern. Its basic idea is as follows: when an axon of cell A is near enough to excite cell B or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.[25] We will adopt a well-designed novel paired associative stimulation (PAS) method of corticocortical PAS (ccPAS), which is a special paradigm where both stimulation points are located in the cerebral cortex.

In so doing, we pioneer in exploring the reconstruction of neural circuits after focal brain injury, as a targeted rehabilitation opportunity after stroke. This might open up a new direction to the development of a new neural regulation model with a prospective significance for the development and implementation of promising and effective neurorehabilitation programmes.

Objective

The aim of our study is to explore whether ccPAS can strengthen the connection of the motor circuit represented by M1 and SMA to promote the recovery of motor function after stroke, since no randomised controlled clinical researches have been reported to verify the clinical outcomes of the ccPAS in patients who had a stroke, and to delve into the brain remodelling after neuromodulation therapy using electroencephalogram (EEG) and functional MRI (fMRI) technique.

METHODS AND ANALYSIS

Study design

Our study is defined as a prospective single-centre double-blind randomised controlled clinical trial with 2-week intervention and 12-week follow-up (see figures 1 and 2). The protocol is registered with the Chinese Clinical Trial Registry. According to the ratio of 1:1, 42 participants are randomly divided into the ccPAS intervention group and control group, respectively, the former receiving ccPAS therapy combined with the conventional rehabilitation, and the latter receiving the conventional rehabilitation integrated with sham stimulation treatment. The routine rehabilitation treatment is normally performed, and the intervention is conducted 5 times a week, 10 times in total. At the baseline (Pre) of 1 day (Post1), and at the time intervals of 1 (Post2) and 3 months (Post3) after intervention, respectively, the effects are measured using a variety of rating scales such as Fugl-Meyer Assessment-Upper Extremity (FMA-UE), Action Research Arm Test (ARAT), Modified Barthel Index (MBI), EEG as well as fMRI evaluations.

Study setting

The study will be conducted at Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine. All assessments and ccPAS interventions are to be performed at the Department of Rehabilitation Medicine of Yueyang

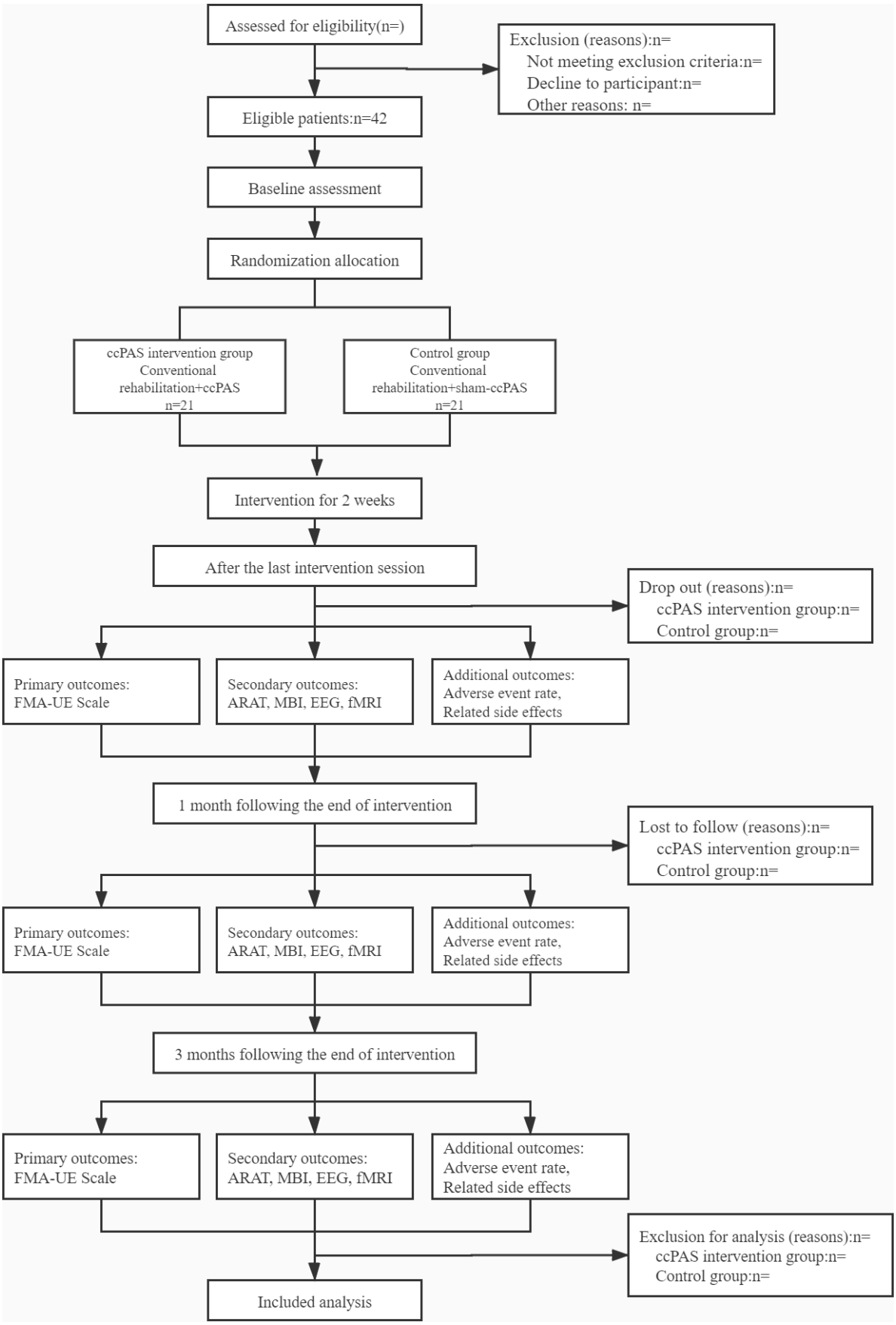

Figure 1 Flow diagram of study design. ARAT, Action Research Arm Test; ccPAS, corticocortical paired associative stimulation; EEG, electroencephalogram; fMRI, functional MRI; FMA-UE, Fugl-Meyer Assessment-Upper Extremity (scale); MBI, Modified Barthel Index.

		STUDY PERIOD						
TIMEPOINT		Enrollment	Baseline	Allocation	Treatment period		Treatment-free follow-up period	
					W1	W2	W6	W10
Eligibility screen		×						
Medical information		×						
Informed consent		×						
Allocation				×				
Intervention								
Active ccPAS					×	×		
Sham ccPAS					×	×		
Assessment								
Primary outcome	FMA-UE		×			×	×	×
Secondary outcomes	ARAT		×			×	×	×
	MBI		×			×	×	×
	EEG		×			×	×	×
	fMRI		×			×	×	×
Additional outcome	Adverse event rate		×			×	×	×
	Related side effects		×			×	×	×

Figure 2 Flow diagram of study design. ARAT, Action Research Arm Test; ccPAS, corticocortical paired associative stimulation; EEG, electroencephalogram; fMRI, functional MRI; FMA-UE, Fugl-Meyer Assessment-Upper Extremity (scale); MBI, Modified Barthel Index; W, week.

Hospital, and the fMRI acquisitions, at the Department of Neuroimaging Medicine of Yueyang Hospital.

Recruitment and sample selection

The participants will be recruited from the Department of Rehabilitation Medicine of Yueyang Hospital, Shanghai University of Traditional Chinese Medicine in Shanghai, China. For the clinical trials the voluntary participants are carefully screened based on the inclusion and exclusion criteria. All participants, who are required to obtain verbal and written information about the purpose and process of the study, ought to sign a written informed consent form.

Stroke diagnosis[26]

Clinically, the WHO defines stroke as an acute episode of vascular marginal neurological dysfunction associated with focal cerebral symptoms that lasts for more than 24 hours.

Inclusion criteria

a. A diagnosis of a stroke by clinical evaluation and comprehensive imaging examination according to the definition of the International Classification of Diseases.
b. Aged between 40 and 70, regardless of gender.
c. A primary stroke, 2 weeks to 6 months from the onset.
d. A lesion distributed in the brain region supplied by middle cerebral artery, and accompanied by unilateral upper limb motor dysfunction.
e. Right-handedness.
f. Scoring over 27 by the Minimum Mental State Examination.
g. A written informed consent submitted.

Exclusion criteria[27 28]

a. Ferromagnetic metal in the head, neck or chest.
b. Microprocessor implants in the body such as cochlear implants, cardiac pacemaker, prosthetic cardiac valves, vagus nerve stimulator, spinal pumps and stimulators.
c. History of epilepsy.
d. History of neurological or psychiatric illness.
e. History of medications known to affect central nervous system excitability.
f. Suffering from tumorous, infectious or a metabolic disease that affects the brain, even without history of seizure and of therapy with anticonvulsants.
g. Pregnancy.

Dropout criteria

a. Withdraw from the study.
b. Violation of the treatment plan.
c. Receiving other therapeutic options during the trial.

d. Blind failure.

Sample size calculation

The calculation of the sample size is based on preliminary experiment results. This study is made up of two groups, and the two-sample mean comparison estimation formula is used as follows:

$$n_C = \frac{\left(Z_{1-\alpha}+Z_{1-\beta}\right)^2\sigma^2\left(1+\frac{1}{k}\right)}{\left(\mu_C-\mu_T-\Delta\right)^2}$$

k=1; α=0.025; β=0.1; $Z_{1-\alpha}$=1.96; $Z_{1-\beta}$=1.28; Δ=0

μ_T corresponding to the mean of ccPAS intervention group; μ_C corresponding to the sham intervention group; Δ as the optimality bounds; σ as the SD; the ratio of cases in the ccPAS intervention and sham intervention group represented by K; assuming a 2.5% one-tailed significance level (α=0.025) and 90% power (β=0.10), the calculated required sample size being 17 participants in each group; allowing for a 20% dropout rate to make up for subsequent losses, a minimum total of 42 participants needed to reach the recruitment target of 21 participants per group.

Randomisation and allocation concealment

This is a double-blind study, for which are enrolled 42 inpatients who had a stroke who meet the eligibility criteria during the baseline enrolment visit. Based on the computer-generated random number to prevent selection bias, the participants are randomly divided into the ccPAS intervention and control group with a ratio of 1:1. Each participant is identified with a particular number to replace the real name. The allocation sequence is concealed from the therapists, evaluator, statistical analyst as well as from the participants' relatives. The allocation sequence is placed in opaque and sealed envelopes with restrictions to access, and the envelope is delivered to the researcher responsible for implementing the intervention the day before.

Blinding

This process is completed independently and remotely by the implementing staff who will not participate in the procedures of information collection, evaluation and data analysis. Their relatives are also blind to the information regarding group assignment. The participant is to be removed from the research protocol in case of blinding failure. The evaluator is required to identify a particular participant by number. An independent researcher is arranged to complete the data analysis, who is not involved in recruitment, screening, evaluation and intervention.

For proper blinding, we plan to follow the schemes of the related studies using sham TMS in the control group. For the sham TMS, we use a sham coil, which is similar to the real coil in terms of appearance, sound and feeling.[29] During the experiment, the sham stimulation is performed with the same TMS procedure at the same location. The parameters on the equipment display are identical in the ccPAS intervention and sham settings.

Safety considerations and adverse events[30–32]

Possible study-related adverse events during the period of follow-ups are listed in the informed consent, such as seizures, syncope, muscle twitches, muscle soreness, headaches, light-headedness, dizziness, neck pain, tooth pain, nausea, transient changes in hearing and abnormal sensations in the stimulation area. After each intervention, the participants are required to complete a questionnaire on adverse reactions. During the period of follow-ups, any accidental injury and sudden illness are recorded as an adverse event for safety assessment. Any symptom is required to be recorded in the observation table in terms of occurrence time, duration, treatment measurement and so on. The relevance to the clinical intervention training is carefully evaluated and completely recorded by the evaluator based on comprehensive consideration. The formula for calculating the incidence of adverse events is as follows: adverse event rate% = (number of adverse events ÷ total number of cases in the group)×100%.

Intervention

Research groups

ccPAS intervention group: conventional exercise rehabilitation+ccPAS.

Control group

Conventional exercise rehabilitation+sham−ccPAS.

Routine sports rehabilitation

Equally, each participant receives exercise therapy, occupational therapy, physical factor therapy and so on.

The assigned ccPAS intervention

To the ccPAS intervention are applied two figure-of-eight coils with 7 cm diameter wings with a Magstim 200 stimulator (Magstim Co., Whitland, UK).

The first figure-of-eight coil is placed on the SMA (3 cm anterior to Cz of the 10–20 EEG system in the sagittal midline),[33] and the second figure-of-eight coil, above the representational field of the first dorsal interosseous (FDI) muscle at the optimal stimulation position. The motor hot spot of right/left affected hand FDI muscle can be detected by moving the coil in steps of 0.5 cm around the assumed motor hand area using a stimulus slightly higher than the threshold. This location, as a hot spot, is the optimal coil position at the affected side, where stimulation can evoke the largest motor-evoked potential (MEP) from the contralateral FDI muscle consistently. The position is then marked to ensure that the position of each stimulation is consistent.

In total, there will be 10 interventions, once a day, 5 days each week, and lasting 2 weeks. The researchers are to be trained to be capable of performing ccPAS and sham interventions before recruiting the first participant.

ccPAS intervention

The participant sits on a comfortable recliner, with the arms and hands kept relaxed, and the eyes kept open to stay awake. The first coil is placed on the SMA, the induced current flowing in the front and back direction. The repetitive TMS (rTMS) pulses are made to SMA with the intensity of 140% active motor threshold (AMT).[34 35] The AMT is determined as the lowest stimulus intensity which produces MEPs of >200 µV in at least 3 of 5 consecutive stimuli during the isometric contraction (10% of maximum voluntary contraction).[36] AMT is determined at the optimal stimulation site for M1 with the coil used for SMA stimulation.

The second coil is held tangentially to the representational field of FDI muscle on the affected side with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane to induce the current from the posterior-lateral to anterior-medial direction.[37] The maximum magnetic field produced by the stimulator is 3.5 T. The intensity of the repetitive single TMS pulses that will be set at 120% resting motor threshold (RMT).[38 39] The RMT is defined as the minimal intensity (an intensity with ≥50 µV peak-to-peak amplitude) that is required to induce a MEP in at least 5 of 10 stimuli.[40] The stimulation intensity is recorded as a percentage of the maximum stimulator output of the magnetic stimulator. Both SMA and the representational fields of FDI muscle will be given 100 pairs of TMS pulses in total at a frequency of 0.2 Hz. The SMA pulses precede the M1 ones by 6 ms.[41] Approximately, each ccPAS intervention takes 8.3 min.

Sham intervention

For a sham stimulation, the sham coil is placed in the same position as in the intervention group. The sham coil can conduct in the similar parameter and manner as in the case of the real stimulation, simulating the sensation produced by the real coil without induction of a magnetic field.[30] Each sham intervention takes the same amount of time (approximately 8.3 min).

Discontinuity criteria

(a) Terminated by the joint decision of the subject and researcher if there are other diseases or serious adverse reactions during the treatment, which may have an impact on the study protocol or safety judgement.

(b) Other medical measures which may interfere with the results of this study.

(c) A major deviation during the implementation.

To reduce the dropout rate

In order to strengthen the compliance of treatment and minimise participants' withdrawal from the study, it is important that the doctor contact the participants regularly by phone to confirm the appointments, assessing the effect of the treatment and discussing the subsequent treatment and the issues that may interfere with adherence.

Assessment

The clinical assessments are performed at the day of enrolment as a baseline, on ccPAS intervention or sham ccPAS intervention and at the time intervals of 1 month and 3 months after intervention, respectively. When entering the groups, the variables are documented as sociodemographic data (age, sex, education, marital status and occupation), medical history (course of diseases), complication (somatic and psychiatric symptoms). Such variables are regularly evaluated as FMA-UE, ARAT, MBI, EEG and fMRI at the baseline, after ccPAS intervention or sham ccPAS intervention and at the follow-ups. All significant side effects or adverse events are to be reported by the research team in the extenuating circumstances request form.

Primary outcome measures

The primary outcomes are the scores of FMA-UE. The FMA-UE Scale is a widely used, which is a strongly recommended as the performance-based method of measurement for people who suffered from different levels of motor function impairment after stroke.[42–44] It is also commonly used instrument for monitoring the recovery process of hemiplegic stroke.[43] It is designed to evaluate the performance of the upper extremity in patients who had a stroke with hemiplegia, including reflex activity, muscle strength and movement control.[45]

As to the evaluation of the upper extremity function, the FMA-UE contains 33 items, which cover the measurements of the reflex actions, locomotion and coordination of the shoulders, elbows, forearms, wrists and hands.[46]The standardisation of scoring is ensured with an administration manual.[46] The function is divided into five levels, scoring from 0 to 66 points. This indicates that the higher the score patients acquire, the better the functional recovery they have.[47]

Secondary outcome measures

Action Research Arm Test

As one of most commonly used measurements for stroke survivors,[48 49] ARAT has been used as a standardised assessment of motor functional limitations of poststroke hemiplegic upper extremity, especially the fine motor function of the hand,[48 50] which has been proven to be reliable and valid.[51–53] The original ARAT is a 15-item scale includes four domains such movement.[54] Each item scored on an ordinal scale of 0, 1, 2 or 3. Item scores are summed to a total score ranging from 0 to 57 (the higher the score, the greater the arm motor function).[53 55]

Modified Barthel Index

MBI is often used in clinical assessment of disability or dependence on the level of activities of daily living (ADL) in patients who had a stroke.[56] It also belongs to the group of tools with the best potential for responsive measuring in ADL function.[57] MBI consists of 10 items to assess the independence of basic life activities: grooming, bathing, feeding, toileting, stair climbing, dressing, bowel

management and bladder management, ambulation and chair–bed transfers.[58] The full score 100 points, higher scores indicate ADL increased.[59 60]

Electroencephalogram

Coherence

One of the most commonly measurements of functional connectivity (FC) in patients who had a stroke is based on EEG coherence between electrodes covering brain regions.[61] EEG activity is acquired with subjects sit on the comfortable armchair during relaxed awake resting-state. With the preprocessed resting-state EEG data acquired, the frequency domain analyses are performed for the electrodes: the trials are used for each subject for fast Fourier transformation within frequency bands of interest. After that we will compute the coherence between pretreatment and post treatment for each participant using the coherence equation with HERMES TOOLBOX (http://hermes.ctb.upm.es/).

fMRI

An important tool for monitoring the various neural activities and behaviours non-invasively,[62] fMRI is used to demonstrate the feasibility of the FC reconstruction in the brain regions through the resting-state fMRI and the task-related fMRI.

Resting-state fMRI

Throughout the process, every patient has their head fixed with foam pads, being told to keep relaxed, awake and mind-blank with their eyes closed. With the preprocessed resting-state fMRI images acquired, we will analyse the data to examine the alternations of FC between pretreatment and post treatment.

SMA and M1 of damaged hemisphere are defined as regions of interest (ROIs). From the BOLD signals, we extract a FC map by correlating the BOLD signal time courses (measured as the Pearson correlation coefficient) of each two ROIs. FC can represent the connectedness of two brain regions by the FC map.[63]

Task-related fMRI

With the task-induced fMRI, we can visualise the brain activities in the brain regions which are related to neural recovery.[64] All participants are required to complete one cycle in a block design with alternating 30-s finger tapping onset and 30-s rest blocks.[65] We design 10 cycles for one stimulation session.[66] During the tapping onset, the participants are asked to tap their affected index finger at 2 Hz; during the scan, they are told to avoid unnecessary movements. The task-related data will be managed into their activation maps. According to our protocol, the SMA and M1 are extracted as ROIs. The extent and peak values of the activated clusters can show the degree of activation in the specific brain regions. Additionally, we will assess the significant difference of activation between the ccPAS intervention group and the control group in certain brain regions.

Data management and confidentiality

Data will be recorded on paper during the study before entering the electronic case report form. To protect the confidentiality, each participant is identified with a particular number to replace their real name in the file. The paper documentation will be kept in a locked cabinet, and the electronic data, stored in a password-protected computer. Only the relevant researcher can access the database, which is required to be kept confidential. To ensure the integrity and authenticity, The Clinical Research Center of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine are responsible for monitoring the study and its data, with the final decision to terminate the trial. Surely, all procedures will comply with the confidentiality standards for medical data. When the study is completed, all documents and collected data will be kept for 3 years before eliminated.

Additional consent provisions for collection and use of participant data and biological specimens

This trial does not involve collecting biological specimens for storage.

Quality control programme

Quality control is an important step to ensure the authenticity and reliability of data. Because of the working characteristics of fMRI, the scanning-generated images are susceptible to many factors. It is important, therefore, that the participants be required to keep awake and remain emotionally stable while keeping the head upright without unnecessary movements, so that the impact of confounding factors can be minimised on image quality. At the end of each scan, the image should be examined to ensure its quality. The adverse events should be recorded and reviewed in details, and the possible impact on the study results should be evaluated.

Statistical analysis

The descriptive analysis will be performed on each patient's data collected throughout the assessment period. The methods of statistical analysis to choose depend on the data type.

Statistical analysis of behavioural data

The Statistical Package for Social Sciences (SPSS) V.22.0 software package (SPSS) is used for statistical analysis.

A descriptive analysis is applied to all the data acquired during the clinical assessment, the time points of which are at the baseline, after ccPAS intervention or sham ccPAS intervention, and at the time interval of one and 3 months after intervention, respectively. Changes in the data compared with those at the baseline will be used for analysis. The obtained measurement data are evaluated by Kolmogorov-Smirnov test for assessing normal distribution. Homogeneity of variance is assessed by Levene's test. For the data following a normal distribution, Paired t-test will be performed for the comparison of control values between pretreatment and post treatment, and

two-way repeated measures analysis of variance (ANOVA) (double-factor, 2×4), to study the main effect of condition (ccPAS intervention and sham stimulation). The data for the measurement, which follow a normal distribution, are presented as mean±SD (x±s), and for the data which do not present a normal distribution, the Wilcoxon post-hoc test follows to compare the control values before intervention with those after ccPAS intervention and to analyse the interaction between the two groups. The significance level will be set at p<0.05, one-tailed test. The measurement data which do not pass the normality test will be presented as medians, maximum and minimum.

Statistical analysis of EEG data

The SPSS V.22.0 software package (SPSS) is used for statistical analysis. We wanted to identify the relevant differences in coherence values, that could be associated with the ccPAS intervention group and control group at four time points of the baseline (before 10 interventions), treatment ending (after 10 interventions), and two intervals of follow-up (1 and 3 months later, respectively). Thus, the between-group differences in coherence values were examined by t-test for independent samples (p<0.05) across frequencies. Significant coherence values variations among four time points were also examined in each group by one-way repeated measures ANOVA (p<0.05).

Statistical analysis of fMRI data

The Statistical Parametric Mapping V.12 (SPM V.12) toolbox (http://www.fil.ion.ucl.ac.uk/spm/) software is used to analyse the MRI of each participant based on Matlab V.7.1 (Mathworks).

Resting-state fMRI

FC measures are computed between each two ROIs. We obtain the remaining BOLD time courses from the given seeds before calculating the Pearson correlation coefficients between every two ROIs, thus acquiring individual FC maps (ROI-wise analysis). And then we use Fisher's Z transformation to transform these maps into Z-score maps (zFC), the seed-to-seed FC estimated for each participant. After that, we will apply the post-hoc two sample t-test to the comparison of the ccPAS intervention group with the control group and employ the one-way repeated measures ANOVA to the comparison of the four time points. The corrected significance level of ROI-wise FC is set as p<0.05 with a false discovery rate (FDR) correction.

Task-related fMRI

We use a mass-univariate approach, a first-level analysis based on General Linear Models (GLMs), to analyse the fMRI data of motor task. According to our experimental paradigm, we make a GLM design matrix. Then we will take the classical or Bayesian approach to estimate the GLM parameters, having the SPMs produced by the interrogation of the results with contrast vectors, and the corrections for multiple comparisons performed.

A T-map of group analysis for each motor task is to be generated by the sample t-test group analysis for each session across the participants. We will perform the second-level analysis of the variability of the effects on a group of participants with one-way repeated measures ANOVA or between the ccPAS intervention group and the control group with the two sample t-test. The corrected significance level of ROI-wise FC is set as p<0.05 with FDR correction, and the CI is 95%.

DISCUSSION

Neuromodulation technology is a commonly used brain remodelling method with significant effects in rehabilitation. The existing neuromodulation technologies, such as TMS, have been widely used in neurorehabilitation and cognitive neuroscience research.

At present, the two common methods are to directly regulate the excitability of a single brain region, or to indirectly regulate the excitability of the brain area, as manifested in the interhemispheric inhibition (IHI) theory, which does not depend on brain connectivity. Since stroke reduces the excitability of the damaged area,[67] a large number of clinical studies focus on stimulating a single brain area to strengthen excitability, to enhance the rate of skill acquisition in stroke rehabilitation. In the case where the excitability of a single brain region is directly regulated, the accurateness of distinguishing the left-versus-right movement direction, which displayed by moving dots, was enhanced by stimulating the motion-sensitive area V5 with the high-frequency rTMS.[68] The other study showed that the patients with major depression disorder acquired significant remission rates after a high-frequency TMS was applied to the left prefrontal, which proved the significant antidepressant therapeutic effect of the treatment on the acute phase of depressed patients.[69]

In the other case where the excitability of the brain area is indirectly regulated, the theory of IHI indicates the common modulation method of enhancing excitability.[70] The activities of each cerebral hemisphere are inhibited by the neuronal excitability of the contralateral one so that the brain can usually function in balance between the hemispheres.[71 72] However, the hyperactivity of the normal hemisphere can occur in patients who had a stroke, thereby increasing the IHI of the affected hemispheres and restricting the excitability of the affected hemisphere at a low level.[1 73] A quite number of TMS intervention studies have been conducted using the theory of IHI on the motor cortex to regulate the excitability and correct the imbalance between the hemispheres. It was reported that 1-Hz rTMS over the M1 of the affected side was compared with the combination of 1-Hz rTMS over the M1 of the affected side and 10-Hz rTMS over the M1 of the unaffected side, which produced the results that the bilateral use of rTMS had a more positive effect on motor improvement than the unilateral use of 1-Hz affected side rTMS alone.[74]

However, these two methods still focus on the regulation of brain excitability in the damaged brain regions. We design a new regulation method to restore the brain function by focusing on the FC between brain regions.

The neural circuit is well known to be the basis of function realisation. Each part of the neural circuit has its own specific function, working together to control the movement behaviour completely and smoothly. If the connectivity of any part of the neural circuit is damaged, it will affect the function represented by each component, thus affecting the motor function. Therefore, the reconstruction of the neural circuit is essential for the functional recovery. By targeting the pathways related to specific functions between brain regions, the synaptic efficiency of linking the two interconnected brain regions in the neural circuits, together with the specificity of plasticity, can be enhanced.[75 76]

We try to prove that the reconstruction of functional connection between brain regions is feasible by stimulating SMA and M1 consistently. SMA and M1 affiliate to the motor planning region and motor execution in the neural circuit region, respectively. Studies have found that reduced activity caused by M1 regional brain damage can affect the connectivity in the motor network.[13] SMA directly projects to M1,[77] both of which are the crucial parts of the motor neural circuit.[78 79] When M1 and SMA were stimulated in healthy people, the increases of cortical excitability in the brain regions were thought to be enhanced by changing the amplitude of MEP in M1.[80] The changes in amplitude can be achieved by adjusting interstimulus interval. The connection between the two stimulation points in the brain regions supposed to increase as well.[80]

SMA has shown to be movement related, playing a central role in the motor network during patients' who had a stroke upper limb activities.[81 82] Functional neuroimaging and electrophysiology studies provide evidence for a significant positive connection between SMA and M1,[83 84] showing that the integration of external instructions and internal needs could be located in SMA.[85] SMA is crucial for motor planning, initiation, executing and regulation of voluntary movements,[81 86–89] and clarify some of the characteristics of general motor performance as part of the neural circuit.[81] Thus, the only way to achieve a recovery may be to rebuild the damaged circuit and to compensate through the remaining or reconstructive loops.

The mechanism of neural circuit reconstruction is closely related to the synaptic plasticity. Recent studies have shown that the reason for the recovery of damaged central nervous system is the continuous remodelling in the human central nervous system, which uses synaptic plasticity.[90] Therefore, synaptic plasticity plays a crucial role in normal brain function and works as an important mechanism for compensation.[91] Synaptic plasticity follows certain learning rules to establish neuronal synaptic connections. The learnt motor task is in motor cortex and depends on the formation of new synapses.[92–94] Long-term potentiation induced by the Hebbian mechanism is also related to the newly formed spines,[95 96] so the loss and acquisition of motor capacity are closely related to Hebbian plasticity. We will pay special attention to using the mechanism of the Hebbian plasticity of synaptic learning rules so that the connection between M1 and SMA can be strengthened. This approach can also be taken to rebalance the functional connections between brain regions by establishing behaviour-related compensatory circuits, so as to achieve the neural circuits reconstruction.

We assess the effectiveness of ccPAS intervention in the convalescent stage of patients who had a stroke, through the change of behavioural, EEG and fMRI data. fMRI studies have demonstrated that greater motor deficits result in reduced connectivity in cortical motor regions.[97] In addition, temporary synchrony of neuronal firing is considered to be an effective means of explicitly connecting and widely distributed neuronal clusters.[98] A previous research shown that individual differences in brain states highly associated with subsequent behavioural learning can be acquired from resting-state EEG connectivity measurements.[61] Based on the present research, precise regulation for different targets can be extended to the improvement and evaluation of more functional disorders, and even can be applied to different diseases.

Although there has been several studies that focus on the reconstruction between brain regions, the mechanism is still unclear. Compared with the previous studies, the current prospective well-designed PAS method of ccPAS is our pioneering protocol which uses the theory of synaptic plasticity for neural circuit reconstruction in patients who had a stroke. Our promising results may confirm the connection between brain regions and even the possibility of having the entire motor neural circuit strengthened. Furthermore, such a research may provide a novel direction for the future clinical trials in this field, developing more efficient treatment options for the rehabilitation of motor dysfunction after a stroke.

Contributors Y-JD, X-YH and M-XZ contributed equally to this work. Y-JD and X-YH conceived and designed the study protocol. X-YH is the coordinator of the study. M-XZ is the project manager, helped with general organisation and sought ethical and regulatory approval. Y-JD, X-YH and J-GX wrote the manuscript. X-XX and Y-LL contributed to the ongoing data collection. J-JW is responsible for statistical power calculation and analysis. J-GX wrote the review. All authors read and approved the final manuscript.

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