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### Supplementary Table 1

Adverse events	cTBS (n=41)	1 Hz rTMS (n=40)	Sham (n=39)	p-value
Transient headache	12/29	9/31	2/37	0.019
Dizziness	7/33	9/31	1/38	0.032
Seizures	0/41	0/40	0/39	>0.999
Manic episodes	0/41	0/40	0/39	>0.999
Syncope	0/41	0/40	0/39	>0.999
Transient hearing or cognitive changes	0/41	0/40	0/39	>0.999

### **Changes in HAM-D<sub>17</sub> scores**

The HAM-D<sub>17</sub> scores also had a significant within-subjects effect in the time  $\times$  group interaction (Wald chi-square=208.07, df=4,  $p<0.001$ ). The cTBS and 1 Hz rTMS groups significantly decreased over time, whereas the sham group showed little change, as shown in Fig 2B. The pairwise comparisons showed that HAM-D<sub>17</sub> scores improved more significantly in the cTBS group than in the other groups following treatment, as shown in Table 2.

Appendix 1 to Li X, Zhang C, Tan J, et al. Clinical effects of continuous theta burst stimulation for generalized anxiety disorder and a mechanism involving  $\alpha$  oscillations: a randomized controlled trial. *J Psychiatry Neurosci* 2022. doi: 10.1503/jpn.210134. Copyright © 2022 The Author(s) or their employer(s).

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## **Clinical effects of continuous theta burst stimulation treatment for generalised anxiety disorder and a mechanism involving alpha oscillations: Protocol to a randomised controlled trial**

### **Clinical sites:**

The Second Hospital of Dalian Medical University

### **Trial registration**

This study has been registered in the Chinese Clinical Trials Registry, with number ChiCTR2000029663.

### **Protocol version**

Version number: 1.1

### **Funding**

This work was supported by the Dalian Science and Technology Innovation Foundation of China (grant number 2020JJ26SN054), and the Liaoning Province Key Research and Development Program of China (grant number 2017225070).

## Introduction and rationale

Generalised anxiety disorder (GAD) is one of the most common mental disorders, with worry, tension and fear as the core symptoms. Repetitive transcranial magnetic stimulation (rTMS), a neuromodulation technique, has been recommended as a potential treatment for anxiety disorders such as GAD, post-traumatic stress disorder and panic disorder.<sup>1</sup> It generates a pulsed magnetic field through the coil, which is converted into an induced current in the cerebral cortex and interferes with the electrical activity of the central nervous system.<sup>2,3</sup> Theta burst stimulation (TBS) is a kind of burst stimulation, applying three 50-Hz pulses as a burst pulse which is repeated five times per second. It was showed that 600-pulse continuous TBS (cTBS) inhibited motor evoked potential (MEP) amplitude and produced a long-term inhibitory effect; 600-pulse intermittent TBS (iTBS) could promote synaptic transmission, produce an excitatory effect and induce a long-term enhancement effect.<sup>4</sup> TBS is gradually being used more widely in clinical treatment because of its short stimulation time and long after-effect time. However, there is a lack of comparative studies on the efficacy of the TBS and rTMS modules and the possible electrophysiological mechanism of TBS treatment. Some studies suggest that long-term potentiation (LTP) and long-term depression (LTD) in hippocampal glutamatergic neurons are monosynaptic events, while MEP changes are polysynaptic transmission processes, which are not ideal to evaluate cortical excitability.<sup>5</sup> Electroencephalography (EEG) is a non-invasive method used to extract rhythmic synchronised neural electrical activities from the human brain, which can directly reflect the excitation and inhibition activities of cortical neurons.<sup>6</sup> The EEG oscillation activity of patients with anxiety disorder has been found to undergo changes related to attention control, cognitive control and anxiety behaviour. Alpha oscillation is a characteristic feature of EEG oscillation activity, prominent in the parietooccipital region.<sup>7</sup> It is a reliable marker of brain dysfunction, can be used as an important

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evaluation index of mental and psychological diseases and plays a key role in the process of cognitive behaviour and emotional regulation.<sup>8</sup> A previous study found that there were differences in the alpha oscillations of high- and low-anxiety trait individuals.<sup>9</sup>

## **Study Objective**

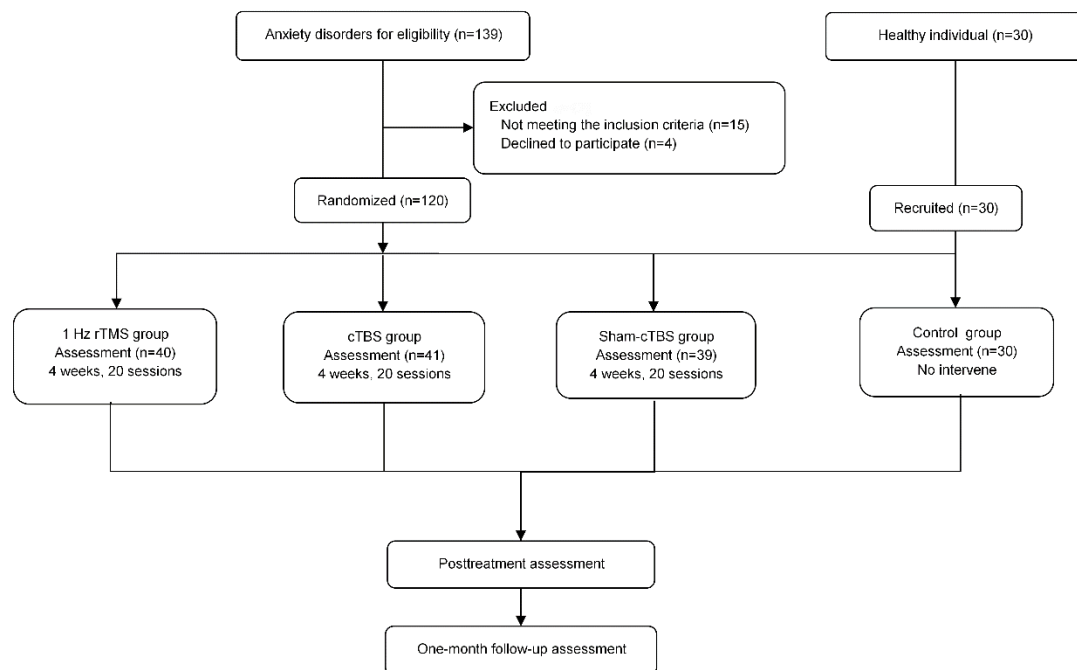
The primary aim was to compare the clinical effects of cTBS versus 1 Hz rTMS in GAD patients and investigate the electrophysiological changes.

The secondary objective was to observe the therapeutic effect and explore the possible electrophysiological mechanism from EEG in clinical practice to provide new insights.

## **Study Design**

This was a prospective, randomised controlled clinical trial. All patients were admitted to the Second Hospital of Dalian Medical University, and randomly allocated in an equal ratio to the cTBS, 1 Hz rTMS, or sham-cTBS groups. Meanwhile, 30 healthy volunteers, matched by sex and age, were recruited as the control group. The outcomes were evaluated at baseline, post-treatment and one-month follow-up.

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Supplementary Figure 1. CONSORT trial flowchart.

### Inclusion criteria

- All participants were aged 20-60 years;
- Met the diagnostic criteria of GAD of the International Classification of Disease, 11th revision;
- Hamilton Anxiety Rating Scale (HAM-A) >14 points and <17 points according to the 17-item Hamilton Rating Scale for Depression (HAM-D<sub>17</sub>); and
- Participants who provided written informed consent and met the ethical requirements that were outlined.

### Exclusion criteria

- History of depression, bipolar disorder and/or schizophrenia;
- Drug abuse and/or dependence in the past year;

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- Taking benzodiazepine receptor agonists;
- Having implantable metal devices in the body;
- Pregnancy or lactation;
- History of central nervous system diseases such as cerebral infarction, haemorrhage, encephalitis and epilepsy;
- Underwent craniocerebral operation;
- A history of serious arrhythmia, hypertension (significantly high risk), myocardial infarction and other serious heart diseases;
- Receiving transcranial magnetic stimulation or electroconvulsive therapy in the past year;
- Receiving psychotherapy or cognitive behavioural therapy during the study; or
- Intolerance to provided treatment.

### **Randomisation**

SPSS version 23.0 was used to generate a random table. Participants were randomly allocated using equal percentiles. When clinical details and neurophysiological data had been collected, staff outside the research team assigned participants accordingly using opaque, sealed envelopes containing the allocation codes.

### **Blinding**

The envelopes were given to therapists, aware of the treatment condition, who were not blinded to group allocation but were warned not to disclose any details of the patients' treatment information. An administrator supervised the allocation concealment and treatment process to ensure that the procedure was blinded. None of the participants declared that they knew to which group they had been assigned at the end of treatment. The raters, EEG programmers and statisticians were masked during the whole study process.

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## **Recruitment**

All patients were recruited from the outpatient clinics of the participating hospitals by local advertising, using the hospitals' WeChat platforms.

## **Interventions**

We used TMS machines (YRD CCY-II) made in Wuhan, China, with figure-of-eight coils. All technicians received the same training. The target was localised in the right DLPFC (electrode F4). Resting motor thresholds (RMTs) were determined at the beginning of the treatment according to a MEP  $\geq 50$   $\mu$ V in the right dorsal interosseous muscle.

Patients in Group 1, the 1 Hz rTMS group, received the following intervention: right DLPFC, stimulation frequency 1 Hz, 1200 pulses with 100% RMT stimulation intensity for 20 min.

Patients in Group 2, the cTBS group, received the following intervention: 600 pulses in the right DLPFC with cTBS for 40 s with 100% RMT stimulation intensity. In Groups 1 and 2, the coil was positioned tangentially to the scalp so that the electrical current flowed to the cerebral cortex.

Patients in Group 3, the sham-cTBS group, treated identically to those in the cTBS group, but with the coil held perpendicularly (at 90°) to the scalp, resulting in less than 20% of the electric current affecting the central nervous system.

Group 4, 30 healthy volunteers matched for age, sex and education with the GAD patients, was recruited as a control group for neurophysiological assessment.

The patients of Groups 1 to 3 received 20 therapy sessions (5 days a week for 1 month). A certain proportion of patients had taken medications such as serotonin-noradrenaline reuptake inhibitor for at least three weeks before participating in this

trial and continued a stable dose of their medications throughout the study period. In order to exclude the interference of EEG signals, no subjects were allowed to use benzodiazepine sedative drugs during the study period.

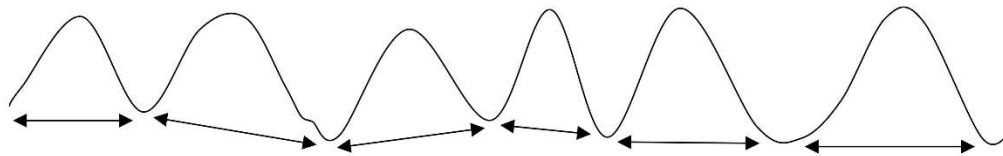
### **EEG signal processing**

At the end of the study, the EEG signal was processed and analysed by a programmer who was blinded to patient information and groups. Stable EEG signals with a duration of 1 min after the eyes were opened and closed were selected, and signals with severe interference (e.g. blinking and EMG artifacts) were deleted after visual examination. The original data of regions of interest were processed by noise interference filtering, spectrum and power calculation, time-domain and frequency-domain data storage, contrast curve drawing and display and frequency value calculation. Shown in Figure 2.

First, the smoothing filtering method was used to filter the original EEG data. Second, the selected stable segments were analyzed using a Hanning window and fast Fourier transform (FFT) to compute the spectra in the frequency domain. Next, a grand average was calculated for each subject, reflecting the spectral power in the alpha band ( $\mu V^2/Hz$ ) at the regions of interest (left parietal P3, right parietal P4, left occipital O1, and right occipital O2).

Another part is that extracting the dominant frequency change of the regions of interest. A program was written to calculate the corresponding instantaneous frequency value according to the time-domain fluctuating waveform by peak-to-peak measuring. Finally, the frequency of the alpha band in the occipital region was computed as the mean value.

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### Supplementary Figure 2. Alpha frequency calculation.

#### Clinical assessment

Each patient with GAD underwent clinical assessment based on the HAM-A, HAM-D<sub>17</sub> and Clinical Global Impression-Severity (CGI-S) scales.

HAM-A consists of 14 questions that can be used to evaluate the anxiety level of patients from two aspects: psychological symptoms and somatic symptoms.

The 17-item HAM-D is suitable for evaluating a depressed mood in GAD patients, but not major depressive disorder.

CGI-S is rated on the following seven-point scale: 1=normal, not at all ill; 2=borderline mentally ill; 3=mildly ill; 4=moderately ill; 5=markedly ill; 6=severely ill; 7=among the most extremely ill patients, as a disease severity measure at baseline.

#### Primary outcome

The primary clinical outcomes were HAM-A scores at baseline, post-treatment and the one-month follow-up.

#### Secondary outcome

A 50% reduction in HAM-A score compared to that at baseline was considered a response, and a score less than 7 was considered remission. The changes in HAM-D<sub>17</sub>

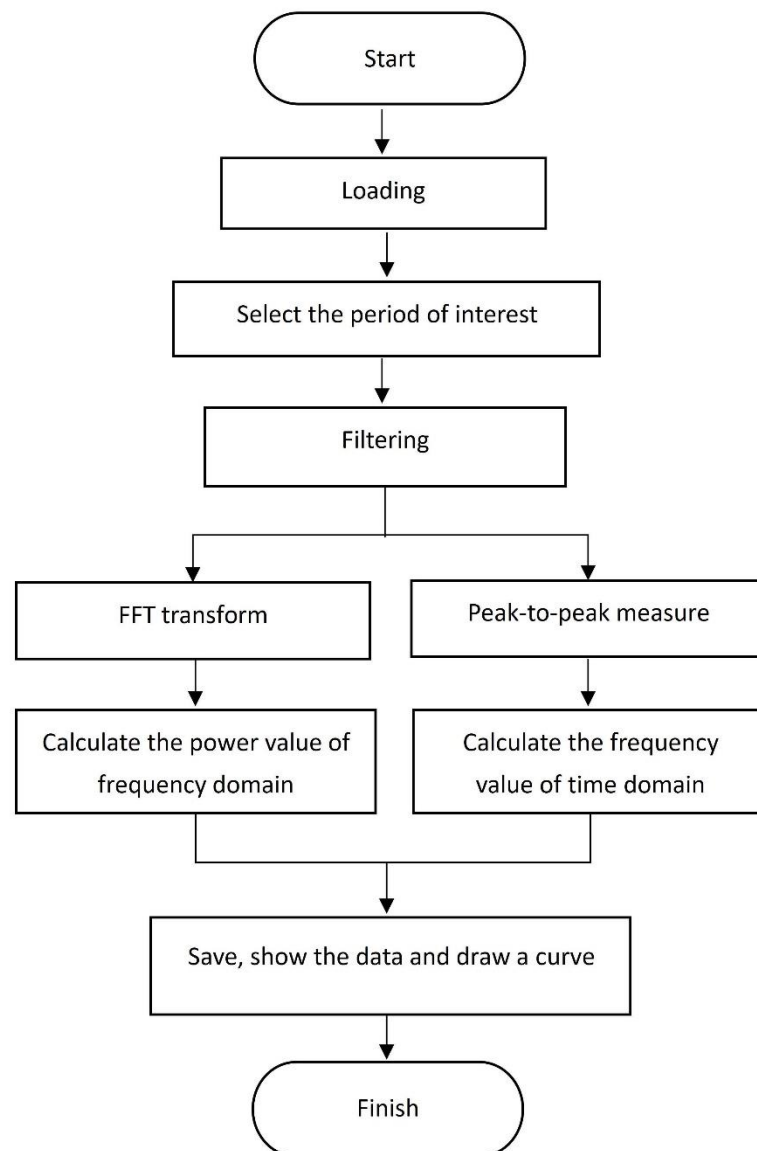
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scores and alpha oscillations, including frequency and power, were measured at post-treatment and one-month follow-up.

## **Ethical standards**

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013. All procedures involving human participants/patients were approved by The Second Affiliated Hospital of Dalian Medical University Ethics Committee (No. 108, 2019).

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Supplementary Figure 3. EEG signal processing.

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