PROTOCOL TITLE:

Efficacy and mechanistic of repeated transcranial magnetic stimulation in children with autism spectrum disorder: an open-label clinical trial

NCT NUMBER: *05472870*

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STUDY SUMMARY

Study Title	Efficacy and mechanisms of repeated transcranial magnetic stimulation in children with autism spectrum disorder: an open-label clinical trial
Primary Objective	Preliminarily explore whether accelerated continuous theta-burst stimulation (a-cTBS) over left primary motor cortex (M1) can improve clinical symptoms of children with autism spectrum disorder (ASD) in China.
Secondary Objective	Explore the appropriate intervention model, effective adaptation population and underlying neurological mechanisms of repeated transcranial magnetic stimulation (rTMS) for ASD children.
Sample Size	≥30 individuals
Trial Design	This study is a single-arm open-label clinical trial, designed to enroll at least 30 children with ASD aged over 4 years old. The children seen in our outpatient clinics, who meet the DSM-5 ASD diagnostic criteria with ADOS and strict inclusion/exclusion criteria, can be enrolled for the intervention after informed consent. During the trial, participants will receive accelerated continuous theta-burst stimulation (a-cTBS)on the left primary motor cortex (M1) for 5 consecutive days and complete clinical assessments within 2 weeks before the cTBS intervention (pre-cTBS), repeated within 3 days after the completion of the cTBS course (post-cTBS) and 1 month following the last cTBS session (one month follow-up), respectively.
Outcome measures	The primary outcome measure is the parent-reported Social Responsiveness Scale (SRS) score at pre-intervention, immediately after the 5 days of intervention and 4 weeks after the 5 days of intervention. Secondary outcome measures are Childhood Autism Rating Scale (CARS) ; Repetitive Behaviors Scale-Revised (RBS-R); Conners parent symptom questionnaire (PSQ), Behavior Rating Inventory of Executive Function(BRIEF), Peabody Picture Vocabulary Test (PPVT); Chinese Communication Development Inventory (CCDI) ; Clinical Efficacy Rating Scale (CGI) scores. Exploratory indicators are changes in resting-state Electroencephalogram (EEG) characteristics before and after the intervention, and Multilingual Assessment Instrument for Narratives (MAIN) scores.
Safety Evaluation	During the intervention and follow-up periods, a careful physical examination will be conducted by clinician when participants come to the hospital. Meanwhile, A semi-structured

	interview will be administered following each treatment session and follow-up by the rTMS technician, special attention will be paid to TMS common discomforts, such as spasms, convulsions, scalp discomfort, local pain, headache, dizziness, tinnitus, nausea,etc. If any adverse events or physical discomfort were reported, detailed descriptions were recorded and graded according to their severity.
Inclusion criteria	A. Children aged ≥4 years old B. Meet the diagnostic criteria for ASD of the DSM-5 C. ASD Diagnosis confirmed by the Autism Diagnostic Observation Schedule (ADOS) or Autism Diagnostic Interview, Revised (ADI-R) D. Provide written informed consents
Exclusion criteria	A. With metal implants in the body B. History of epilepsy or other neurological disease C. Require surgical treatment due to structural abnormalities indicated by brain MRI D. With genetic or chromosomal abnormalities E. With psychiatric/mental disorder (e.g., very early-onset schizophrenia) other than ASD F. Suffer from serious heart disease and/or severe hearing impairment G. Intracranial hypertension H. Participating in other clinical trials I. Participants who received other interventions within 4 weeks prior to enrollment.
Intervention	Participants will receive cTBS over the left M1for 5 consecutive days. The detailed parameters as follows: 80% of RMT, 60 cycles of 10 bursts of three pulses at 50 Hz were delivered in 2-second trains (5 Hz) with no intertrain interval (i.e. triplet standard cTBS, 1800 pulses, 120s). Stimulation sessions were delivered hourly, 10 sessions were applied per day (18,000 pulses/day).
Research time	Expect to recruit at least 30 ASD children from May 2022 to December 2023. A total of approximately 5 weeks per subject will spend to complete a baseline assessment, a 5-day intervention, and a 4-week follow-up after the intervention.

ABBREVIATIONS/DEFINITIONS

ASD	Autism spectrum disorder
ADOS	Autism Diagnostic Observation Schedule
ADI-R	Autism Diagnostic Interview, Revised (ADI-R)
AE	Adverse Events
a-cTBS	Accelerated continuous theta-burst stimulation
BRIEF	Behavior Rating Inventory of Executive Function
CCDI	Chinese Communication Development Inventory
CGI	Clinical Efficacy Rating Scale
cTBS	Continuous theta-burst stimulation
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
EEG	Electroencephalogram
M1	Primary motor cortex
MAIN	Multilingual Assessment Instrument for Narratives
PSQ	Conners parent symptom questionnaire
PPVT	Peabody Picture Vocabulary Test
RBS-R	Repetitive Behaviors Scale-Revised
rTMS	Repeated transcranial magnetic stimulation
SRS	Social Responsiveness Scale
SAE	Serious Adverse Events
TMS	Transcranial magnetic stimulation

Catalogue

Efficacy and mechanistic of repeated transcranial magnetic stimulation in children with autism spectrum disorder: an open-label clinical trial

1.0 Background

Autism spectrum disorder (ASD), also known as childhood autism, was first reported and named by American psychiatrist Kanner in 1943. It is a neurodevelopmental condition with a specific combination of impairments in social communication and repetitive behaviors, highly restricted interests and/or sensory behavior, beginning early in life. In recent years, the global incidence of autism in children has been rising sharply, affecting approximately 1/100 children around the world and more in some countries and districts [1]. Moreover, a significant proportion of ASD children have a combination of different degrees of intellectual impairment, who need more attention and care, bringing heavy burden to families and society [2].

Currently, it is believed that individualized precise treatment based on etiology is the most recommended way to treat neuropsychiatric diseases including ASD[3]. However, ASD is thought to be the result of a combination of many factors acting on the nervous system, including the individual's genetic background, adverse environment, so there is a significant heterogeneity on the behavioral symptoms and severity phenotype of children with ASD, which further increases the difficulty of diagnosis and treatment. So far, there are few highly evidence-based and effective treatment for ASD worldwide[4], especially for core social communication symptom, and children’s treatment is still based on symptomatic behavioral intervention, such as behavioral analysis therapy (ABA), Denver early intervention mode, etc. Many families cannot afford or do not have access to professional intervention, and only small-to-medium effects of improvement have been achieved despite some children received timely high-intensity interventions. Therefore, it is imperative to find new treatments that are safe, tolerable, fast acting, durable, and effective.

Repetitive transcranial magnetic stimulation (rTMS), a non-invasive neuromodulation technology, has been proposed as potential therapeutic options for the modification of the pathological neuroplasticity involved in neuropsychiatric disorders including ASD[5], and the use of rTMS in patients is relatively well tolerated and safe [6,7]. Consistant Theta-burst stimulation (cTBS) is a more efficient form of rTMS that produces stronger brain plasticity changes through lower overall stimulation intensity and shorter stimulation duration[8]. To date, There are many rTMS studies on dorsolateral prefrontal cortex (DLPFC), which found that the intervention group improved in social interaction, cognitive and executive function, repetitive and stereotyped behavior to varying degrees, and had good clinical benefits in ASD [9-13]. We noticed the primary motor cortex (M1) might be a potential stimulation target for two reasons: 1) atypical neuroplasticity was observed in M1 in autistic individuals using TMS[14,15]; 2)M1 is highly involved in the processing of

action execution[16] and emotion evaluation[17-18]. However, there has been no clinical trial targeting M1 examined the efficacy of improving ASD symptoms.

Given the scarcity of studies on rTMS stimulation over M1 and the lack of trials examining its efficacy, especially in social communication impairment, we design this single-arm open-label trial to investigate the efficacy and safety of an accelerated and high-dose continuous Theta-burst stimulation (a-cTBS) targeting left M1 for autistic core deficits in ASD children. Before drafting this protocol, our group has included 20 high-functioning ASD children aged 6-10 years old to receive cTBS on M1 in 10 consecutive days [NCT05238298], the results suggested that more than half of the children had improvement in social and language function, meanwhile, it was safe and well tolerated. The above research experience provide a guarantee for the implementation of this trial.

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2. 0 Objectives

To whether accelerated continuous theta-burst stimulation (a-cTBS) over left primary motor cortex (M1) can improve clinical symptoms of children with autism spectrum disorder (ASD) in China, and to investigate the appropriate intervention model, effective adaptation population and underlying neurological mechanisms of repeated transcranial magnetic stimulation (rTMS) for ASD children..

3. 0 Study design

This study is a single-arm open-label clinical trial, planned to include at least 30 ASD children aged over 4 years old.

The children seen in our outpatient clinics, who meet the DSM-5 ASD diagnostic criteria with ADOS and strict inclusion/exclusion criteria, can be enrolled for the intervention after informed consent.

During the trial, participants will receive accelerated continuous theta-burst stimulation (a-cTBS)on the left primary motor cortex (M1) for 5 consecutive days and complete clinical assessments from the baseline to 1 month following the last cTBS session (Pre-intervention, Post-intervention and one month follow-up, respectively).

The detailed a-cTBS parameters as follows: 80% of RMT, 60 cycles of 10 bursts of three pulses at 50 Hz were delivered in 2-second trains (5 Hz) with no intertrain interval (i.e. triplet standard cTBS, 1800 pulses, 120s). Stimulation sessions were delivered hourly, 10 sessions were applied per day (18,000 pulses/day).

Attached: Study design Flow

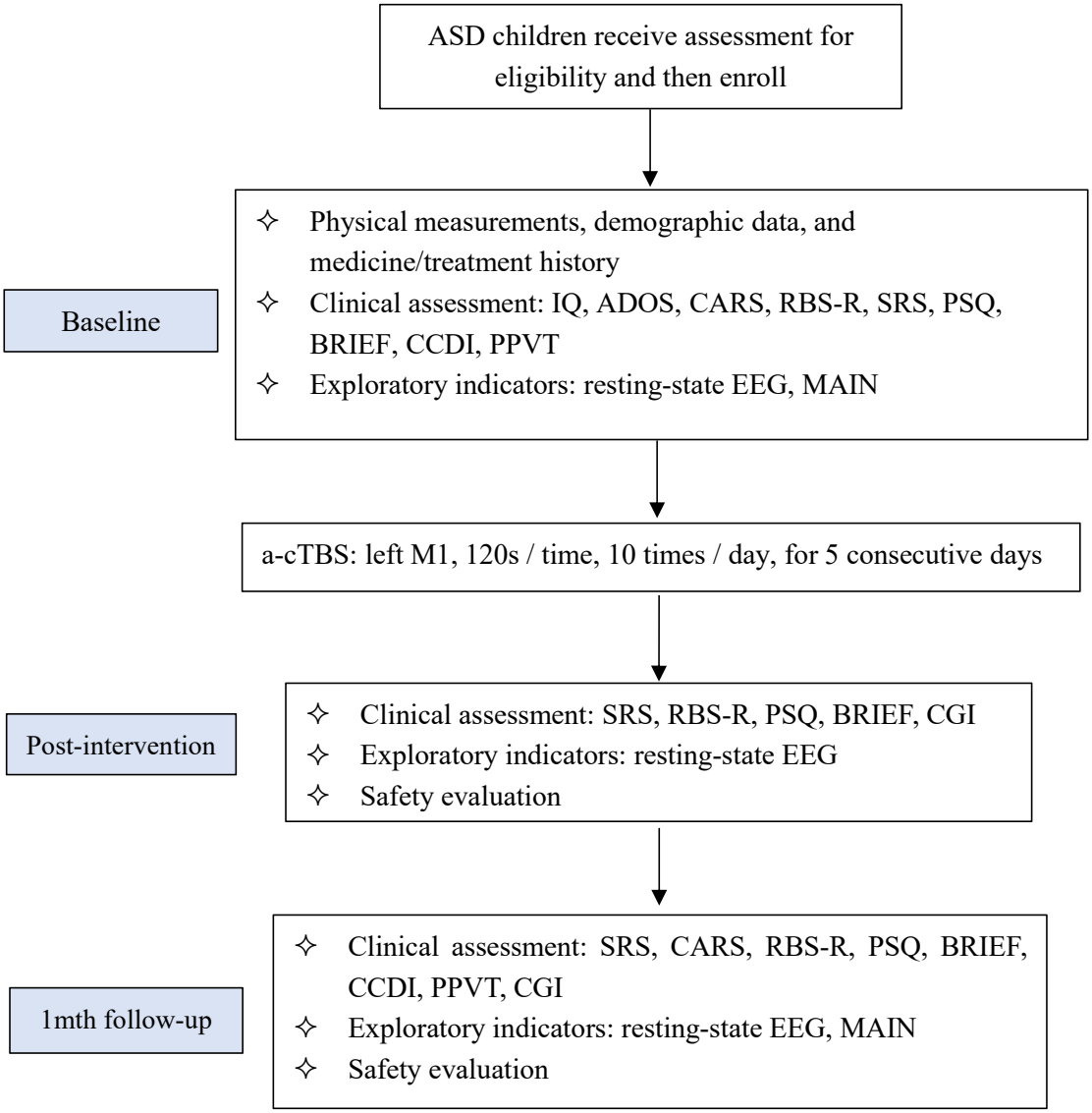

Figure 1. Study flow

4.0 Study subjects

4.1 Indications

Children with ASD.

4.2 Sample size and calculation method

This study will recruit at least 30 ASD children aged over 4 years old at one trial center.

Sample size calculation method:

This study is a single-arm, open-label clinical trail and most of previous rTMS studies included 10-20 ASD patient and found their symptoms had improved after rTMS intervention. For example, Wang, et al. reported the improvement of language and social symptoms in 13 children aged 3-12 years with ASD after 15 days (15 rTMS sessions in total) [Wang, et al., Frontiers in Psychiatry 2019], Fahnestock, et al. found a reduction of the disease severity among 16 children with ASD (mean age: 9 years old) after one-month rTMS intervention (20 sessions in total) [Fahnestock, et al., Drug Development Research 2021].

A minimum total sample size was estimated using G*Power, being powered (80% power and two-sided 5% significance) to detect a moderate (0.5)-to-large (0.8) standardized effect of treatment. The estimated effect size was guided by existing study results on social behavior deficits (Ni HC, et al.,Autism, 2021, Barahona-Correa et al., Front Integr Neurosci, 2018). Considering the 10 % dropout rate and the fact that this study would include both low-function and high-function ASD children (further stratified analysis is required), the final sample size of this study is estimated to be at least 30.

4.3 Inclusion criteria

A. Children aged ⩾4 years old

B. Meet the diagnostic criteria for ASD of the DSM-5

C. ASD Diagnosis confirmed by the Autism Diagnostic Observation Schedule (ADOS) or Autism Diagnostic Interview, Revised (ADI-R)

D. Provide written informed consents

4.4 Exclusion criteria

A. With metal implants in the body

B. History of epilepsy or other neurological disease

C. Require surgical treatment due to structural abnormalities indicated by brain MRI

D. With genetic or chromosomal abnormalities

E. With psychiatric/mental disorder (e.g., very early-onset schizophrenia) other

than ASD

F. Suffer from serious heart disease and/or severe hearing impairment

G. Intracranial hypertension

H. Participating in other clinical trials

I. Participants who received other interventions within 4 weeks prior to enrollment.

4.5 Exit criteria

A. The child had obvious side effects during the intervention

B. Self-withdrawal (patient withdrawal of consent, etc.)

C. Lost to visit

D. Other reasons for the subject's inability to complete the full process of this trial (e.g., severe somatic disease were found after enrollment)

4.6 Case rejection criteria

A. Those participants found not to meet the trail criteria after enrollment

B. Poor compliance, do not cooperate with the protocol to complete the 5-day intervention course, etc.

5.0 Intervention

5.1 Intervention program

❶ Machine: Pulsed magnetic Stimulation Device (M-100 Ultimate, Shenzhen Yingchi Technology Co.,Ltd, Shenzhen, China)

❷Intervention protocol: target left M1, and details of parameter are shown below.

	Intensity	Frequency in the cluster	Number of pulses in the cluster	Inter-cluster frequency	Number of inter-cluster pulses	stimulation time	Inter-cluster interval	Repli-cation	Total pulses number	total time
cTBS	80%MT	50HZ	3	5HZ	200	40s	0	3	1800	120s

❸Treatment period: 10 times a day, 1 hour interval, a total of 5 days

5.2 Combined treatment

The combined treatment and (or) medication during the study should be detailed in the case report form, including the name, dose, route of administration, usage, start and end time of treatment and (or) medication.

6.0 Follow-up plan

6.1 Follow-up time arrangement

❶Total of three follow-up time.

- Follow-up visit 1 (V0): Baseline
- Follow-up visit 2 (V1): immediately after the 5-day intervention
- Follow-up visit 3 (V2): 4 weeks after the 5-day intervention

❷Unscheduled follow-up (USV): No limit on number of times

6.2 Follow-up content: Process schedule

Follow-up time / Follow-up content	V0	V1	V 2
	-2w	5d	+4w
Informed consent	×		
Inclusion/exclusion criteria	×		
Demographic data	×		
History taking	×		
check-up	×		
IQ	×		
Autism Diagnostic Observation Schedule (ADOS)/ Autism Diagnostic Interview, Revised (ADI-R)	×		
Childhood Autism Rating Scale (CARS)	×		×
Repetitive Behaviors Scale – Revised (RBS-R)	×	×	×
Social Responsiveness Scale (SRS)	×	×	×
Behavior Rating Inventory of Executive Function(BRIEF)	×	×	×
Conners parent symptom questionnaire (PSQ)	×	×	×
Peabody Picture Vocabulary Test (PPVT)	×		×
Chinese Communication Development Inventory (CCDI)	×		×
Multilingual Assessment Instrument for Narratives (MAIN)	×		×
Clinical Efficacy Rating Scale (CGI)		×	×
Resting-state EEG	×	×	×
Adverse event		×	

7.0 Outcome measures

7.1 Primary outcome measures:

The Social Responsiveness Scale (SRS) provides the multi-dimensional measure of social interaction allowing the rating of social impairment in ASD for children aged 4 – 18 years. It can generate a total raw score and five theoretical subscales scores (labeled social awareness, social cognition, social communication, social motivation,

and autistic mannerisms), and higher scores indicates increased social impairment. We used SRS to monitor the response to the intervention in social domain as it's able to measure subtle changes in the severity of symptoms over time. Parents completed SRS before their child receiving the intervention, immediately after the 5-day intervention and 4 weeks after the 5-day intervention.

7.2 Secondary outcome measures:

At baseline, Post-intervention, 4-week follow-up:

1) Repetitive Behaviors Scale-Revised (RBS-R) is a questionnaire that is used to measure the breadth of repetitive behavior in children, adolescents, and adults with Autism Spectrum disorders. The RBS-R provides a quantitative, continuous measure of the full spectrum of repetitive behaviors. The RBS-R consists of six subscales including: Stereotyped Behavior, Self-injurious Behavior, Compulsive Behavior, Routine Behavior, Sameness Behavior, and Restricted Behavior, that have no overlap of item content. The higher scores indicating severer symptoms.

2) Behavior Rating Inventory of Executive Function-Parent (BRIEF-P). School Version contains eight clinical domains that can generate behavioral regulation and meta-cognition indices and an overall executive composite score; Preschool Version comprises five subscales that can yields inhibitory self-control, flexibility and emergent meta-cognition indices and an overall executive composite score), which provided an ecologically sensitive measure of executive function (EF) performance in everyday (school/home) environments.

3) Conners Parent Rating Scale (CPRS-48), which provides a qualitative and quantitative picture of children's emotions and behavior, based on five subscales assessing: Conduct Problems, Learning Problems, Psychosomatic, Impulsive-Hyperactive and Anxiety. And CRPS-48 allows for another factor: the Hyperactivity Index (HI), which includes the 10 items that are considered to be the most sensitive to treatment effects.

At baseline and 4-week follow-up:

4) Childhood Autism Rating Scale (CARS). CARS is a behaviour-rating scale used to assess the presence and severity of the symptoms of autism spectrum disorder(ASD), with scores ranging from 15 to 60 and high scores indicating severe symptoms. Change of the CARS score from baseline to 1 months after treatment to evaluate the effect of rTMS treatment on ASD.

5) Chinese Communicative Development Inventory (CCDI), which is a powerful tool to assess early vocabulary development and the language skills of older children with developmental disorders and the effectiveness of their interventions; raw scores were used here allowing us to analysis change in language level over time.

6) Peabody Picture Vocabulary Test (PPVT), one of the most commonly used assessment tests that measure understanding of single-word. The higher score of PPVT means better single-word comprehension.

At Post-intervention and 4-week follow-up:

7) Clinical Global Impression Scale (CGI, which consists of 3 items that assess global illness severity, overall improvement from the start of treatment, and

therapeutic response(referred to as “CGI-S,” “CGI-I,” and “CGI-E”, respectively), we use CGI-I to rate how much the patient’s illness has improved or worsened relative to a baseline measurement (a seven-point scale: 1 = “very much improved” to 7 = “very much worse”).

7.3 Exploratory outcome measures:

Before receiving the intervention, and 4 weeks after the end of 5-day intervention, children having certain expression ability need to complete the Multilingual Assessment Instrument for Narratives (MAIN) to assess narrative comprehension and production skills.

Resting-state EEG data will be collected before and after the intervention to investigate the effect of the intervention on brain activity.

8.0 Safety evaluation

8.1 Physical Signs

Careful physical examination of participants will be conducted by clinician at each visit, with special attention to the inquiry and checking for spasm, convulsions, scalp discomfort, local pain, headache, dizziness, tinnitus, nausea, etc.

8.2 Adverse events and incidence

A semi-structured interview was administered following each treatment session and follow-up by the rTMS technician. A standardized adverse events assessment form was completed if adverse events or physical discomfort were reported. Using this form, detailed descriptions of the adverse events experienced were recorded, including rating the event as mild (no impairment, no need stopping trial), moderate (some impairment, need stopping trial, no need for intervention), severe (evidence of impairment, need stopping trial and need for intervention).

9. 0 Adverse events and serious adverse events

9.1 Definition of adverse events

Adverse events (Adverse event, AE) are any signs, symptoms or clinical manifestations that are inconsistent with the treatment purpose, even if there is no correlation with the treatment, is to be recorded and followed up for their regression.

9.2 Record of adverse events

Record all adverse events that occurred after signing the informed consent form. The contents of each adverse event record should include the following points:

❶Duration of the adverse event : start time and end time;

❷Severity of adverse events:

Mild-no impairment, no need stopping trial

Moderate-some impairment, need stopping trial, no need for intervention

Severe-evidence of impairment, need stopping trial and need for intervention;

❸Relationship between adverse events and intervention: Investigators assessed the correlation between adverse events and the intervention as: positive / likely related

/ possibly related / likely unrelated / unrelated;

❹ Management of adverse events: The investigator should follow up the adverse events until the symptoms disappear or stabilize; if the adverse event remains at the end of the study, follow up again within one month after the end of the study.

The intervention used in this study is rTMS, in principle, there is no safety risk in the use of rTMS in children if the safety guidelines developed by the international expert panel are strictly adhered to. However, rTMS may bring temporary minor discomfort such as scalp discomfort, localized pain, dizziness, headache, fatigue, tinnitus, anxiety, etc., mostly relieved within 24 hours. Serious side effects of seizures and neurocardiogenic syncope induced during stimulation have also been reported, but subjects who experienced these two adverse reactions had a history of depression and/or syncope.

The intervention process of this study is conducted in the hospital, and the subjects will be closely followed and supported in case of adverse reactions; if medical treatment is needed, the pediatrician will provide the treatment plan and follow-up visits. In addition, the study will continue to follow up the adverse events within 4 weeks after the 5-day intervention.

9.3. Serious Adverse Events

Serious adverse events (SAE) are clinical medical events that occur during the course of the study that result in death or life-threatening, require hospitalization or prolonged hospitalization, and cause disability or dysfunctional impairment .

If a serious adverse event occurs during the study, whether related to the intervention, appropriate treatment measures must be taken immediately and reported to the principal investigator and the ethics committee within 24 hours.

10.0 Ethics committee and informed consent form

10.1 Ethics committee

Prior to the start of the study, the protocol and informed consent should be reviewed and approved by the ethics committees of the study centers. During the study, any change of the study protocol to the Ethics Committee shall be filed with the Ethics Committee; in case of serious adverse event, the Ethics Committee shall be notified within 24 hours.

10.2 Informed consent form

Prior to enrollment, the investigator should explain in detail the purpose, procedure, duration, patient benefits and the possible risks of this trial to children's guardians. Informed consent signed in writing by the subject's legal representative should be obtained before enrollment in this study.

11. 0 Data management and statistical analysis

11.1 Data Collection

The data of each subject should be filled in the case report form (CRF) according to the requirements of the study timely, and the investigators should also keep the original data (copy) of participants as much as possible to ensure the accuracy of the CRF data.

11.2 Data Monitoring and Auditing

During the study, the principal investigator and the assisting investigator should monitor the collection and filling of the data regularly, review the data together with the statisticians, and complete the final definition and judgment of the analyzed population.

11.3 Selection of statistical analysis data

Statistical analysis of data from subjects who completed the full 5-day intervention and follow-up assessments (mainly for primary outcome measure, i.e., SRS) .

11.4 Statistical analysis of the population

❶ Analysis of outcome measures: analysis of the population who have completed the corresponding data collection;;

❷ Safety evaluation: included in the analysis as long as he/her had received one session of intervention.

11.5 Statistical analysis method

Data will be preprocessed and/or analyzed using SPSS25.0, SAS9.2, Matlab, and R.

❶ Analysis of the primary outcome measures:

SRS scores at baseline, immediately after the end of 5-day intervention and 4 weeks after the end of 5-day intervention will be analyzed using repeated measures ANOVA.

❷ Analysis of other behavioral outcome measures:

RBS-R, BRIEF, PSQ scores at baseline, immediately after the end of 5-day intervention, and 4 weeks after the end of 5-day intervention will be analyzed using repeated measures ANOVA or Friedman test.

CARS, PPVT, CCDI, MAIN scores at baseline and 4 weeks after the end of 5-day intervention will be analyzed using paired-samples t-tests or Mann-Whitney Wilcoxon test.

❸ EEG analysis

EEG data will be preprocessed and analysed by MATLAB. Changes in the electrophysiologicalactivity characteristics of the brain at baseline, immediately after the end of 5-day intervention and 4 weeks after the end of 5-day intervention will be analyzed and then correlated with clinical behavioral outcome.

❹ Safety evaluation

Summarize the occurrence of adverse events: summarize the number of cases,

instances, and incidence of adverse events and list all adverse events, those related to the study intervention protocol, serious adverse events, and those leading to exit; list the number of cases, instances, and incidence of mild, moderate, and serious adverse events; give a description for adverse events related to the intervention.

❺Analysis of baseline characteristics

The analysis of baseline characteristics included: demographics (age and gender); baseline analysis of each observation used in this study.

The number of cases, mean, standard deviation, median, minimum and maximum values were calculated when the variables were quantitative indicators; the number of cases and composition ratio were calculated when the variables were categorical indicators.

12. 0 Principal Investigator and contact person

12.1 Principal Investigator

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