

A Comparative Project on the Efficacy of Temporal Interference Stimulation (TIS), a Non-invasive Deep Brain Stimulation Technique, at Different Targets for Parkinson's Disease

Study Title: A Comparative Project on the Efficacy of Temporal Interference Stimulation (TIS), a Non-invasive Deep Brain Stimulation Technique, at Different Targets for Parkinson's Disease

Protocol Number: Version 2.2

Principal Investigator: Mei Bin

Department: Department of Neurology

Study Duration: June 2026 – December 2027

Zhongnan Hospital of Wuhan University

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1. Study Synopsis

1.1 Abstract

Study Title: A Comparative Project on the Efficacy of Temporal Interference Stimulation (TIS), a Non-invasive Deep Brain Stimulation Technique, at Different Targets for Parkinson's Disease

Study Summary: To explore the therapeutic effects of Temporal Interference Stimulation (TIS), a non-invasive deep brain stimulation technique, in patients with Parkinson's disease (PD), and to further investigate the potential mechanisms underlying TIS treatment for PD. TIS offers the advantage of non-invasive stimulation. This study will use TIS to explore different stimulation targets in PD patients, and may also provide reference for target selection in subsequent DBS surgery. The study will provide preliminary exploratory evidence for the application of non-invasive deep brain stimulation in the treatment of Parkinson's disease.

Study Objective: Based on exploring the improvement effects of TIS on motor and non-motor symptoms in PD patients, this study aims to systematically compare the differences in symptom improvement, safety, and compliance among PD patients receiving TIS intervention at different targets through a randomized, double-blind, target-exploratory design.

Study Population: A total of 16 subjects are planned to be enrolled.

Study Site/Location: Zhongnan Hospital of Wuhan University / Department of Neurology

Study Intervention: TIS stimulation targets are the subthalamic nucleus (STN) or the globus pallidus (GP), with a current intensity of 2 mA, base frequency of 2000 Hz, frequency difference of 130 Hz, and stimulation duration of 20 minutes.

Study Duration: June 1, 2026 – December 31, 2027

Subject Participation Duration: Each subject will be followed for 3 months from enrollment to completion of all follow-up assessments.

2. Study Background

2.1 Significance of the Study

Parkinson's disease (PD) is a neurodegenerative disease for which, to date, no curative therapy

exists. Moreover, as the disease progresses, the effects of existing symptomatic treatments become increasingly limited. At the same time, the incidence of PD is increasing year by year. Given this trend, there is an urgent need to develop new therapeutic strategies to improve the future quality of life of PD patients, and non-invasive neuromodulation techniques represent one such approach. Transcranial alternating current stimulation (tACS) is a commonly used non-invasive neuromodulation method that influences brain neural activity by placing electrodes on the scalp and applying alternating current stimulation. However, this method is significantly attenuated by tissues such as the scalp and skull, making it difficult to reach deep brain regions. The introduction of Temporal Interference Stimulation (TIS) has made non-invasive deep brain stimulation possible. This method delivers two pairs of high-frequency stimulation signals simultaneously to stimulation electrodes placed on the scalp, creating a strong overlapping electric field in specific deep brain regions, with the electric field oscillating at the difference frequency of the high-frequency signals. The resulting modulated signal can overcome the limitations of traditional transcranial electrical stimulation, such as insufficient depth of penetration and electric field dispersion. The effectiveness of TIS modulation has been validated in rat model studies. In the field of Parkinson's disease research, TIS not only has potential therapeutic effects but also provides an important tool for exploring and validating key disease-modulating targets (such as the subthalamic nucleus and globus pallidus). By modulating neural activity in different brain regions, it is expected to further clarify the core mechanisms underlying PD pathology and lay the foundation for Deep Brain Stimulation (DBS) therapy. Furthermore, with its advantages of being non-invasive, safe, and highly reproducible, TIS holds promise as a novel intervention for PD, offering new possibilities for expanding clinical treatment strategies.

2.2 Study Background

PD is a common progressive neurodegenerative disease associated with severe disability and negative impacts on quality of life [1]. Although the etiology of PD remains unclear, pathologically it involves the loss or dysfunction of dopaminergic neurons in the substantia nigra pars compacta [2]. Typical clinical manifestations include difficulties in coordinated movement, such as asymmetric resting tremor, rigidity, and bradykinesia. These symptoms and their response to levodopa form the basis for the clinical diagnosis of PD. Postural instability and gait abnormalities occur in more advanced stages of the disease. Although PD is incurable, numerous pharmacological treatments are available to manage motor and non-motor symptoms.

PD is characterized by progressive motor impairment. Over the past three decades, Deep Brain Stimulation (DBS) has been used as a neurosurgical procedure to treat debilitating motor symptoms in PD patients. Although DBS of the globus pallidus (GPi) has been shown to be effective, its widespread clinical application is limited, in part due to its invasiveness and treatment-related adverse effects [3-5]. Moreover, because therapeutic efficacy varies considerably among individuals, the choice of stimulation target has a critical impact on surgical outcomes. Accurately determining the most suitable DBS target for individual patients prior to surgery remains a major clinical challenge.

Temporal Interference Stimulation (TIS), a non-invasive deep brain stimulation technique, offers a non-invasive approach to modulating neuronal activity in deep brain nuclei for the potential treatment of motor symptoms in PD [6]. TIS is a non-invasive method targeting deep brain structures and has shown promise in improving motor symptoms of PD in previous

proof-of-concept studies. The principle of TIS involves placing two pairs of transcranial stimulation electrodes on the scalp, each delivering high-frequency currents with slightly different frequencies. The human cerebral cortex does not respond to high-frequency currents. However, when these two currents interfere in deep brain regions (i.e., the target area), they generate a low-frequency electrical stimulation (the difference between the two high frequencies) that affects deep brain areas. TI stimulation has demonstrated the potential to modulate DBS-related brain regions, such as the subthalamic nucleus and globus pallidus, in animal models and preliminary clinical studies. Thus, it achieves stimulation of deep brain nuclei while minimizing activation of superficial cortical areas. The breakthrough of TIS also lies in its flexibility in targeting: the stimulation target can be adjusted by changing the current ratio between electrode pairs without altering electrode positions. This makes TIS highly suitable for future clinical applications in modulating deep nuclei and developing personalized diagnostic and therapeutic plans for PD patients.

An exploratory study conducted by our collaborative team showed that unilateral stimulation of the subthalamic nucleus (STN) using TIS in PD patients alleviated motor symptoms, as demonstrated by pre- versus post-treatment comparisons [7]. In a recent exploratory study of TIS stimulation of the right globus pallidus interna (GPI), TIS was confirmed to be feasible and safe for alleviating motor symptoms, particularly bradykinesia and tremor, in patients with mild PD; compared to the right side, motor symptoms (especially bradykinesia) on the left side showed more significant improvement. PD patients with more severe bradykinesia and tremor prior to stimulation achieved greater improvement after TIS treatment [6]. Current findings suggest that TIS has the potential to improve motor symptoms in PD patients. However, in the two studies mentioned above, some patients still did not respond to TIS stimulation. To further investigate the effects of TIS stimulation on PD patients, this study plans to enroll 16 PD patients and use TIS to stimulate the GPI and STN to compare the efficacy of TIS stimulation at different targets for treating motor symptoms in PD. This study aims to explore the effects of TIS on motor symptoms, gait, and postural control in PD patients, as well as the effects of TIS stimulation on brain activation and related brain networks. It will also provide validation of the effectiveness of TIS as a new physical therapy for treating motor symptoms in PD in clinical settings.

References

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3. Expected Outcomes of the Study

- 1) TIS improves motor symptoms, gait, and postural control in PD patients;
- 2) TIS treatment activates relevant brain regions and strengthens connectivity in related brain networks in PD patients;
- 3) TIS will elucidate the physiological basis and stimulation-response patterns of different stimulation targets for symptom improvement in PD, advancing the development of non-invasive neuromodulation therapy for PD from experience-based selection to data-driven, mechanism-informed optimization.

2.4 Risk/Benefit Assessment

2.4.1 Known Potential Risks

The TIS treatment process may be associated with the following adverse events, including: headache, fatigue, and itching at the stimulation site. As a new technology, its long-term effects have not yet been established.

2.4.2 Known Potential Benefits

TIS is a non-invasive brain neuromodulation technique that, compared to invasive treatments, improves subject safety. It also enables stimulation of deep brain nuclei and is more likely to benefit subjects than superficial stimulation techniques, with potential effects including improvement of motor symptoms, gait, and postural control in PD.

2.4.3 Potential Risk/Benefit Assessment

TIS is a non-invasive brain neuromodulation technique that can provide additional therapeutic options for PD patients without interfering with conventional treatment. The breakthrough of TI technology also lies in the flexibility of its stimulation targets: the stimulation target can be adjusted by changing the current ratio between electrode pairs without altering electrode positions. This makes it highly suitable for future clinical applications involving multi-target (simultaneous/sequential) modulation and personalized diagnostic and treatment planning for PD patients. Overall, it is therefore considered that the potential benefits of conducting this study outweigh the potential risks.

3. Principal Investigator Information

3.1 Principal Investigator Name, Qualifications, and Contact Information

Name	GCP Training	Role in Study (e.g., PI, Sub-I, CRC)
Mei Bin	Yes	PI
Pan Yang	Yes	Sub-I

3.2 Key Study Personnel

No.	Name	Gender	Age	Title	Specialty	GCP Training	Role in Study
1	Mei Bin	Male	51	Chief Physician	Neurology	Yes	PI
2	Pan Yang	Female	39	Chief Physician	Neurology	Yes	Sub-I
3	Xia Yingfeng	Male	36	Attending Physician	Neurology	Yes	Investigator
4	Nie Chuang	Female	28	Resident Physician	Neurology	Yes	Investigator
5	Tu Xin	Female	29	Research Assistant	Neurology	Yes	Data Manager

4. Study Objectives

To investigate the effects of TIS on motor symptoms, gait, and postural control in PD patients, and to explore its impact on brain activation and related brain networks. This study aims to provide more targeted and predictive evidence for target selection in subsequent TIS treatment for PD patients, enhance TIS efficacy, and promote the clinical translation of this technology in the field of Parkinson's disease. Through its non-invasive advantages, the study will evaluate the effects of stimulating different targets for the treatment of Parkinson's disease.

5. Study Design

5.1 Overall Design

This study plans to enroll 16 PD subjects, who will be randomly assigned to two groups (Group A and Group B). Subjects in Group A will receive TIS stimulation targeting the subthalamic nucleus for 7 consecutive days; subjects in Group B will receive TIS stimulation targeting the globus pallidus for 7 consecutive days. Prior to stimulation, all subjects will undergo MDS-UPDRS, MoCA, Timed Up and Go (TUG) test assessments, as well as MRI scanning, 64-channel EEG, and serum sample collection. Immediately after the first treatment and at 1 month, MDS-UPDRS assessments, functional MRI scanning, 64-channel EEG, and serum sample collection will be repeated.

To minimize subjective bias and psychological effects, and to enhance the objectivity and credibility of the study, this experiment employs a randomized, controlled, double-blind design. The randomization process will be performed by an independent statistician using computer-generated random sequences (SPSS, R, or Excel) to ensure objectivity and unpredictability of group assignment. Each participant's group number will be automatically assigned by the system, with no intervention by other researchers. To ensure double-blinding, the stimulation protocol will be administered by an operator through encrypted settings. The operator will be aware of group assignments but will not participate in any other data recording, collection, or analysis activities. Subjects will also be unable to perceive which type of stimulation they are receiving. All study evaluators, including the principal investigator, clinicians, and data analysts, will remain blinded throughout the study period.

In the event of study completion or the need for unblinding due to a serious adverse event, an

independent unblinding administrator will handle the case individually based on the coding, without affecting the blind status of other study personnel, thereby ensuring scientific rigor and ethical compliance. During data processing, data analysts will not be aware of group assignments and will perform data classification and processing based only on subject numbers.

5.2 Definition of Study Endpoints

5.2.1 Primary Efficacy Endpoints

Primary efficacy endpoints:

(1) Change in MDS-UPDRS Part III scores from baseline to immediately after the first treatment and at 1 month;

(2) Changes in brain activation and functional connectivity, assessed by comparing fMRI and 64-channel EEG data at baseline, post-treatment, and at 1-month follow-up, to determine the effects of TIS on brain activation and network functional connectivity at different targets.

Secondary Efficacy Endpoints:

(1) Change in MDS-UPDRS Part II scores from baseline to immediately after the first treatment and at 1 month;

(2) Montreal Cognitive Assessment (MoCA) scores between the two groups at baseline;

(3) Changes in Timed Up and Go (TUG) test performance from baseline to post-treatment and at 1-month follow-up.

5.3 Sample Size Determination

This study plans to enroll 16 subjects.

6. Study Population

6.1 Inclusion Criteria

(1) Adult males or females aged ≥ 40 years;

(2) Diagnosis of idiopathic Parkinson's disease according to the UK Brain Bank criteria, with age of onset ≥ 40 years;

(3) Previous or current treatment with dopamine replacement therapy (e.g., levodopa) with good response;

(4) Hoehn and Yahr (H&Y) stage 1.5 – 2.5;

(5) Able to walk independently for at least 5 minutes without assistive devices;

(6) No severe freezing of gait (FOG);

(7) Disease duration ≥ 2 years since diagnosis, clinically stable, and able to comply with study assessments and interventions;

(8) Stable medication regimen for at least 4 weeks prior to the study;

(9) Signed informed consent form; subject or legal guardian is able to understand and willing to participate in this study.

6.2 Exclusion Criteria

(1) Presence of other neurological disorders that may affect the study (e.g., ...);

(2) Mild cognitive impairment or above (MoCA ≤ 23);

(3) Orthopedic or other medical conditions that may affect gait or balance;

(4) Contraindications to MRI, such as claustrophobia;

(5) History of antipsychotic, antidepressant, or other medications that may affect dopamine

levels;

- (6) History of other severe psychiatric disorders;
- (7) History of epilepsy, traumatic brain injury, or implanted metallic devices in the brain or heart (e.g., stimulators, pacemakers) or other contraindications;
- (8) History of electroconvulsive therapy (ECT);
- (9) Currently participating in other gait- or balance-related intervention training;
- (10) Physician-diagnosed cardiovascular risk factors for exercise.

6.3 Subject Recruitment

(1) Recruitment Channels

Medical institutions: Eligible patients will be referred through professionals in the Department of Neurology, Zhongnan Hospital of Wuhan University, and other institutions.

Online promotion: Recruitment information will be published via the Department of Neurology's WeChat public account and related online forums.

(2) Recruitment Process

Subject screening: Potential participants will be initially assessed via telephone or online questionnaire to confirm whether they meet the inclusion criteria.

Appointment for interview: Participants who pass the initial screening will be scheduled for an in-person interview to provide detailed information about the study purpose, procedures, potential risks, and benefits.

Informed consent: After confirming their understanding of the study, participants will be asked to sign the informed consent form.

Assessment and enrollment: Willing participants will undergo professional assessments, including motor symptom evaluation (MDS-UPDRS Part III) and cognitive function assessment (MoCA), to confirm final enrollment.

(3) Participant Benefits

> Health assessment: All participants will receive comprehensive health assessments and relevant health recommendations.

> Treatment protocol: Participants will receive TIS intervention during the study and may potentially benefit from effective treatment.

> Compensation: Participants will receive financial compensation to cover time and transportation costs.

(4) Ethical and Privacy Protection

> All participant information will be strictly confidential and used solely for the purposes of this study.

> The study will comply with the review and approval of the Ethics Committee to ensure participants' rights and safety.

6.4 Method of Subject Allocation

A random number generator software will be used to generate a random sequence of numbers 1 and 2 corresponding to the order of visit, which will be used to assign subjects to groups. Number 1 is designated as the experimental group and number 2 as the control group. Subjects will be assigned to groups according to the random number corresponding to their order of visit. Each subject's number and group assignment will be recorded and kept by a single study team.

member who is not involved in the experimental procedures.

7. Study Intervention

7.1 Administration of Study Intervention

7.1.1 Description of Study Intervention

The medical device used in this study is as follows:

Product Name	Non-invasive Brain Neurostimulator NervioX
Manufacturer	Suzhou Dome Medical Technology Co., Ltd.
Model/Specification	TES NEURO-800
Structural Composition	The TES NEURO-800 product consists of a stimulator main unit, electrode adapters, algorithm and control software (optional accessories), stimulation electrode leads (optional accessories), electrode caps (optional accessories), and animal electrodes (optional accessories). The NVX-36T amplifier, as part of the computer system, is used for training systems, clinical and scientific research. Each channel has a DC cascade input and a separate 24-bit ADC for EEG sampling up to 10,000 times per second. An internal high-resolution Direct Digital Synthesis (DDS) current stimulator is used to generate DC or AC current through any EEG electrode or set of electrodes. Application software is used to set up experiments and record EDF+, BDF+, and stimulation data. Software libraries are provided for user application design.

The investigational device has completed safety compliance and electromagnetic compatibility testing at qualified testing institutions.

7.1.2 Dosage and Administration

This study plans to enroll 16 PD subjects, who will be randomly assigned to Groups A and B. Stimulation targets are the globus pallidus and subthalamic nucleus:

Group A: Stimulation target: subthalamic nucleus (frequency difference = 130 Hz), stimulation duration: 20 minutes, with 30-second ramp-up at the beginning and 30-second ramp-down at the end. All four stimulation impedances must be maintained at $\leq 10 \text{ K } \Omega$. Duration: 7 consecutive days.

Group B: Stimulation target: globus pallidus (frequency difference = 130 Hz), stimulation duration: 20 minutes, with 30-second ramp-up at the beginning and 30-second ramp-down at the end. All four stimulation impedances must be maintained at $\leq 10 \text{ K } \Omega$. Duration: 7 consecutive days.

The frequency difference is fixed for all subjects. The stimulation current intensity varies considerably among individuals due to differences in tolerance and will be adjusted according to each subject's specific condition and clinical needs. Other than the target difference, there are no other differences in treatment intervention between the two groups.

7.2 Study Schedule

Study Procedure	Screening	Treatment Period I	Treatment Period II	Follow-up
Visit	V0	V1	V2	V3
	Day -7 to 0	Day 1	Day 2–7	1 Month
Time Window	—		+2 days	+2 days
Informed Consent	X			
Demographics	X			
Medical History	X			
Inclusion/Exclusion Criteria	X			
Randomization		X		
MDS-UPDRS	X	X		X
Timed Up and Go (TUG)	X	X		X
64-channel EEG	X	X		X
MRI Scan	X	X		X
Serum Samples	X	X		X
TIS Intervention		X	X	
Adverse Events		X	X	

7.3 Study Procedures

1) Visit 0 – Screening Period (Day -7 to 0):

- Sign informed consent;
- Demographics (date of birth, sex, etc.);
- Record medical history and concomitant medications;
- Assess inclusion/exclusion criteria;
- Perform MDS-UPDRS, Timed Up and Go (TUG) test, and 64-channel EEG;
- MRI scanning (T1 structural imaging, diffusion tensor imaging, and resting-state fMRI);
- Serum sample collection.

2) Treatment Period (7 days + 2 days)

TIS intervention is administered once daily for 7 consecutive days, 20 minutes per session.

Visit 1 – Treatment Period I (First Treatment)

- Randomization: subjects are assigned to groups using a computer-generated random sequence (SPSS, R, or Excel) generated by an independent statistician; TIS intervention mode is determined based on randomization results;
- Based on randomization results, administer 20-minute TIS intervention;
- Immediately after the first treatment, repeat MDS-UPDRS assessment, functional MRI scanning, 64-channel EEG, and serum sample collection;
- Record adverse events and concomitant medications for the disease.

Visit 2 – Treatment Period II (Days 2 – 7 + 2 days)

- Based on randomization results, administer 20-minute TIS intervention;
- Record adverse events and concomitant medications for the disease.

3) Visit 3 – Follow-up Period (1 month + 2 days)

- Perform MDS-UPDRS, Timed Up and Go (TUG) test, and 64-channel EEG;
- MRI scanning (T1 structural imaging, diffusion tensor imaging, and resting-state fMRI);
- Serum sample collection;
- Record medical history and concomitant medications.

8. Study Endpoint Evaluation

8.1 Primary Endpoint Evaluation

(1) Changes in motor symptoms in PD patients: assessed by comparing changes in the total MDS-UPDRS score and Part III score before and after treatment.

(2) Changes in brain activation and functional connectivity: assessed by comparing pre-and post-treatment fMRI to determine the effects of TIS on brain activation and functional connectivity at different targets in PD patients.

8.2 Secondary Endpoint Evaluation

Changes in motor symptoms in PD patients: assessed by comparing MDS-UPDRS Part III scores and 64-channel EEG data immediately before and after the first treatment.

8.3 Safety and Other Evaluations

Safety analyses will be based on the Safety Analysis Set (SS). Safety endpoints in this study include known anticipated adverse events associated with TIS technology and the occurrence of other adverse events and serious adverse events during the study. The number and incidence of adverse events/serious adverse events will be calculated and presented with detailed listings.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence in a subject during the course of the clinical study, whether or not considered related to the study intervention.

8.4.2 Definition of Serious Adverse Event (SAE)

A serious adverse event (SAE) is any untoward medical occurrence during the clinical study that meets any of the following criteria:

- 1) Death;
- 2) Life-threatening disease or injury;
- 3) Permanent impairment of body structure or body function;
- 4) Requires hospitalization or prolongation of existing hospitalization;
- 5) Requires medical intervention to prevent permanent impairment of body structure or body function;
- 6) Results in fetal distress, fetal death, or congenital abnormality/birth defect.

8.4.3 Classification of Adverse Events

8.4.3.1 Severity

Mild: Tolerable by the subject, does not affect the study intervention, requires no special treatment, and has no impact on the subject's health.

Moderate: Intolerable to the subject, requires interruption of the study intervention or special treatment, and has a direct impact on the subject's health.

Severe: Life-threatening, fatal, or disabling; requires immediate discontinuation of the study

intervention or emergency treatment.

8.4.3.2 Relationship to Study Intervention

The investigator should assess the relationship between adverse events and TIS treatment according to the following classification criteria:

Related to treatment: (1) There is a reasonable temporal relationship; (2) The adverse event is a known risk of the intervention or can be explained by the mechanism of the intervention; (3) The injury diminishes or resolves upon discontinuation of treatment; (4) The injury reappears upon re-administration of treatment; (5) Cannot be explained by other factors. If all five criteria are met, it is judged as "definitely related"; if two criteria are met, it is judged as "possibly related."

Not related to treatment: (1) There is no reasonable temporal relationship; (2) The adverse event is not of a type that could be caused by the intervention; (3) The adverse event can be explained by concomitant medication/device use, disease progression, or other treatments. If all three criteria are met, it is judged as "definitely not related"; if one criterion is met, it is judged as "probably not related."

8.4.3.3 Expected Adverse Reactions and Suspected Unexpected Serious Adverse Reactions

The examination methods used in this study (MRI scans) comply with international standards and are widely used in clinical research. They may produce minor side effects in subjects, such as fever and increased heart rate, which are mostly not serious and are clinically acceptable. If psychological discomfort or other reactions occur during the study, subjects may contact the study physician at any time for timely management. In addition, follow-up surveys may involve personal privacy issues, which may cause anxiety or psychological discomfort. If necessary, researchers will refer subjects to a psychologist or clinician for counseling.

In addition, TIS is a non-invasive brain neurostimulator that may have motor symptom-improving effects. The TIS treatment process may be associated with adverse events including headache, fatigue, and itching at the stimulation site. If any of these symptoms occur, subjects may promptly report to the study staff for rapid management. If a subject experiences rapid deterioration of motor function during the study, that subject will be treated as a dropout and will receive appropriate diagnostic and therapeutic interventions based on their confirmed condition.

9. Statistical Analysis

9.1 General Methods

A statistical analysis plan will be developed by a biostatistician based on the study protocol and finalized before database lock.

The statistical analysis will be performed using SPSS version 28.0 or higher. All statistical tests will be two-sided, and a P-value < 0.05 will be considered statistically significant (unless otherwise specified).

Descriptive statistics for quantitative variables will include mean, standard deviation, maximum, minimum, median, and 25th and 75th percentiles; categorical variables will be described by frequency and percentage.

For between-group comparisons, analyses will be performed according to variable type. For quantitative data, between-group comparisons will use independent-samples t-test (if variance is homogeneous and data are normally distributed) or Wilcoxon rank-sum test. Categorical data will be analyzed using chi-square test or Fisher's exact test (if chi-square test is not applicable).

Ordinal data will be analyzed using Wilcoxon rank-sum test or CMH test.

10. Privacy and Confidentiality

All personal information involved in this study is confidential. Subjects' raw data will be stored encrypted at the research center, and questionnaire and cognitive function task data will be kept in locked file cabinets. All information provided by subjects will be used solely by the project team for scientific research purposes. This information may include names, addresses, phone numbers, medical history, and information obtained during study visits. All subject data will be identified by study numbers rather than subjects' real names. Information that could identify subjects will not be disclosed to individuals outside the research team unless the subject provides personal permission. All research team members are required to maintain the confidentiality of subjects' identities. To ensure the study is conducted in accordance with regulations, government regulatory authorities or members of the Ethics Committee may, when necessary, access subjects' personal data at the research site for audit purposes. When the results of this study are published, no personal information of any subject will be disclosed.

The results of this study may be registered on public websites such as the Chinese Clinical Trial Registry. The research results are jointly owned by the participating research institutions.

11. Conflict of Interest Statement

There are no conflicts of interest related to this study.

Protocol Signature Page

I have carefully read this study protocol and acknowledge that it covers all necessary content required for the conduct of the study. I will conduct the study in accordance with the protocol.

I will provide a copy of this protocol and all relevant materials to all personnel assisting with this study. I will discuss these materials with them to ensure that they fully understand the trial-related information.

Patient Informed Consent Form

Protocol Title: A Comparative Project on the Efficacy of Temporal Interference Stimulation (TIS), a Non- invasive Deep Brain Stimulation Technique, at Different Targets for Parkinson's Disease

Informed Consent Form Version: 2.1

Version Date: July 11, 2025

Research Institution: Zhongnan Hospital of Wuhan University

Principal Investigator: Mei Bin

You are invited to participate in a clinical study. This Informed Consent Form provides you with information to help you decide whether to participate in this study. Please read it carefully, and if you have any questions, please ask the investigator responsible for this study.

What is the background and purpose of the study?

Background: Parkinson's disease (PD) is a common progressive neurodegenerative disease associated with severe disability and negative effects on quality of life. Although the cause of PD remains unknown, pathologically it involves the loss or dysfunction of dopaminergic neurons in the substantia nigra pars compacta. Typical clinical manifestations include difficulty in coordinated movement, such as asymmetric resting tremor, rigidity, and bradykinesia. These symptoms and their response to levodopa form the basis for the clinical diagnosis of PD. Postural instability and gait abnormalities occur in more advanced stages. Although PD is incurable, many medications are available to control motor and non-motor symptoms. PD is characterized by progressive motor impairment. Over the past three decades, deep brain stimulation (DBS) has been used as a neurosurgical procedure to treat debilitating motor symptoms in PD patients. Although DBS of the globus pallidus (GPi) has been shown to be effective, its widespread clinical use is limited partly by its invasiveness and treatment-related adverse effects.

Based on non-invasive deep brain stimulation technology – Temporal Interference (TI) stimulation – a non-invasive technique is now available to modulate neuronal activity in deep brain nuclei, potentially treating motor symptoms in PD. Temporal Interference stimulation is a non-invasive method targeting deep brain structures and has shown promise in improving PD motor symptoms in previous proof-of-concept studies. The principle of TI is to place two pairs of transcranial stimulation electrodes on the scalp, each delivering high-frequency currents with slightly different frequencies. The human cerebral cortex does not respond to high-frequency currents. However, when these two currents interfere in deep brain regions (i.e., the target area), they produce a low-frequency electrical stimulation (the difference between the two high frequencies), similar to transcranial direct current stimulation (tDCS), which affects deep brain areas. This achieves stimulation of deep brain nuclei while minimizing activation of superficial cortical areas. The breakthrough of TI also lies in the flexibility of its stimulation targets: the target position can be adjusted by changing the current ratio between electrode pairs without altering electrode positions. This makes TI highly suitable for future clinical applications in modulating deep nuclei and developing personalized diagnostic and treatment plans for PD patients.

An exploratory study by our team members showed that TI stimulation of the unilateral subthalamic nucleus (STN) in PD patients alleviated motor symptoms, as observed by pre-versus post-treatment comparisons. In a recent exploratory study of TI stimulation of the right GPi, TI was found to be feasible and safe for alleviating motor symptoms, especially bradykinesia and tremor, in mild PD; compared with the right side, motor symptoms (especially bradykinesia) on the left side improved more significantly. PD patients with more severe bradykinesia and tremor before stimulation experienced greater improvement after TI. Current findings indicate that TI has the potential to improve motor symptoms in PD. However, in both studies, some patients still did not respond to TI stimulation. To further investigate the differences between responders and non-responders to TI stimulation in PD patients, this study plans to enroll 16 PD patients to explore the effects of TI on motor symptoms, gait, and postural control, as well as the

effects of TI stimulation on brain activation and related brain networks. This will provide validation of the effectiveness and a new physical therapy for the clinical application of TI in treating motor symptoms of Parkinson's disease.

Study Purpose: To explore the effects of non - invasive deep brain stimulation on motor symptoms, gait, and postural control in PD patients, and to investigate the impact of TI stimulation on brain activation and related brain networks.

Study Device: The medical device used in this study is the Non- invasive Brain Neurostimulator NervioX, developed by Suzhou Dome Medical Technology Co., Ltd. It consists of a stimulator main unit, electrode adapters, algorithm and control software (optional accessories), stimulation electrode leads (optional accessories), electrode caps (optional accessories), and animal electrodes (optional accessories). The product is currently still in the experimental development phase and has not yet received marketing approval from the National Medical Products Administration (NMPA). However, the product has completed safety and electromagnetic compatibility testing at qualified testing institutions, and the test results are satisfactory.

What will I need to do if I participate in the study?

If you agree to participate in this study, we will assign you a study number and establish a medical file. During the study, we will comprehensively evaluate your personality traits, sleep issues, etc., and professional medical staff will conduct clinical scale assessments and related examinations for you.

【Clinical Scales】

You will need to complete the following scales: MDS Unified Parkinson's Disease Rating Scale (MDS- UPDRS) and Montreal Cognitive Assessment (MoCA).

【Motor Function Assessment】

Motor function assessment: A physician will evaluate your motor function using the Timed Up and Go (TUG) test.

【MRI Scanning and Blood Collection】

A physician will perform MRI scanning and blood sample collection for you. This examination has no radiation; you only need to lie quietly and complete specific cognitive tasks while trying to keep your body still. MRI data will be collected using the MRI scanner at the Imaging Center, including T1 structural imaging (scan duration about 6 minutes), diffusion tensor imaging (scan duration about 15 minutes), and resting- state functional MRI (total scan duration about 9 minutes). The purpose is to assess whether there are visible structural changes in your brain, as well as invisible changes in structure and functional status.

MRI scanning must be performed at the lead institution, Zhongnan Hospital of Wuhan University.

【Procedures and Duration】

1. After signing the informed consent, the physician will ask you for basic information such as personal details, medical history, and PD medication use. At the same time, you will undergo MDS- UPDRS and MoCA assessments, and the TUG test. In addition, you will undergo MRI scanning, 64- channel EEG, and blood collection at Zhongnan Hospital of Wuhan University. Your PD medication use will be recorded. The study requires that your antidepressant medication regimen remain unchanged within 30 days prior to signing the informed consent and throughout the entire study period. If the above assessments indicate that you are suitable for participation in this study, you will formally enter the study and receive treatment.

2. During the treatment phase, the physician will randomly assign you to a group using a randomization system. You may be assigned to Group A to receive TIS stimulation targeting the subthalamic nucleus, once daily for 7 days, or to Group B to receive TIS stimulation targeting the globus pallidus, once daily for 7 days. You have an equal chance of being assigned to either group, and the grouping result is not subject to human interference. According to your group assignment, you will receive TIS stimulation targeting either the right subthalamic nucleus or the globus pallidus, with a current intensity of 2 mA, base frequency of 2000 Hz, frequency difference of 130 Hz, and stimulation duration of 20 minutes. Before stimulation, you will undergo MDS-UPDRS, MoCA, and TUG assessments, as well as MRI scanning and blood collection. Immediately after the first treatment and at 3 months, MDS-UPDRS assessment, functional MRI scanning, 64-channel EEG, and blood collection will be repeated. During this period, your PD medication use and any physical discomfort will be recorded.

As a study participant, you are required to provide truthful information about your medical history and current health status; inform the study physician of any discomfort you experience during the study; not change your PD medication or its dosage/administration on your own during the study; inform the study physician if you are currently participating in any other studies; and if you or your spouse become pregnant during the study, please inform your study physician promptly. The study physician will also answer any questions you may have during the clinical trial.

The study physician will contact you by phone to remind you of your hospital visit dates. To ensure we can reach you, please inform the physician in advance if your phone number changes. The duration of your participation in this study is expected to be approximately three months. A total of 16 subjects will be enrolled in this study.

Are there risks in the study?

The examination methods used in this study (MRI scanning) and blood collection comply with international standards and are widely used in clinical diagnosis and treatment. They may cause minor side effects such as fever and increased heart rate, but most do not cause serious effects. In addition, the study may involve some of your personal privacy issues, which may cause anxiety or psychological discomfort. If necessary, the researchers will refer you to a psychological counselor or clinician for appropriate consultation. The non-invasive deep brain stimulation (TI) process may be associated with the following adverse reactions, including headache, fatigue, itching at the stimulation site, switching to mania, suicidal ideation, etc. If any of these symptoms occur, you may promptly report to the study physician and receive rapid management.

What are the possible benefits of participating in the study?

This study may alleviate your Parkinson's disease symptoms, but it may also not benefit you. In addition, we hope that the information obtained from your participation in this study may in the future benefit you or others with similar needs.

Will I have expenses or receive compensation for participating in the study?

Expenses: You will receive study-related examinations and assessments free of charge, including MRI scanning, 64-channel EEG, scale assessments, motor function evaluation, and blood sample collection. All costs of non-invasive deep brain stimulation are also covered by the

research funding. The study will not cover your medication costs or examinations outside the study.

Compensation: During your participation in this study, you will receive a transportation allowance of 200 RMB and a nutritional subsidy of 50 RMB. The compensation will be paid in one lump sum when you return to the hospital for follow-up visits.

What if I am harmed as a result of participating in the study?

If you experience complications or other injuries during this trial, the physician will truthfully document the situation and determine whether the adverse event is related to the study procedures. If an adverse event is indeed caused by the study procedures and causes harm to you, you may receive active treatment at your research institution and/or receive appropriate compensation in accordance with relevant Chinese laws and regulations. However, this study will not cover costs unrelated to the study.

Is my information confidential?

If you decide to participate in this study, your participation and all personal information collected during the study will be kept confidential. Information that could identify you will not be disclosed to individuals outside the research team unless you give your permission. All research team members are required to maintain the confidentiality of your identity. The information and data generated by this study will not be sent outside. Your study-related medical records will be kept in a locked filing cabinet at this medical institution and will be accessible only to research personnel. To ensure the study is conducted in accordance with regulations, government regulatory authorities or members of the Ethics Committee may, as necessary, access your personal data at the study site for audit purposes. When the results of this study are published, no personal information about you will be disclosed.

Do I have to participate?

You may voluntarily choose to participate or not participate in this study, or you may notify the investigator at any time to withdraw from the study. Your data will not be included in the study results, and your medical treatment and rights will not be affected in any way. Your participation is entirely voluntary. If you do not participate, it will not affect your medical services at this hospital.

If you need other treatment, or if you do not comply with the study plan, or if a study-related injury occurs, or if there are other reasons that continuing participation might increase the risk of harm to you, the study physician may terminate your continued participation in this study.

Whom should I contact if I need more information?

You may at any time learn about information and progress related to this study. If new safety information related to this study becomes available, we will notify you promptly. If you have questions about this study, or if you experience any discomfort or injury during the study, or if you have questions about your rights as a study participant, you may contact Dr. Pan at 19291751431.

This study has been reviewed by the Medical Ethics Committee of Zhongnan Hospital of Wuhan University. If you have any questions or concerns regarding your rights and health related to

participation in this study, you may contact the Ethics Committee at this institution: telephone 027- 67812787; contact person: Ms. Hu.

Signature Page of Informed Consent

I have read this Informed Consent Form.

I have had the opportunity to ask questions, and all my questions have been answered.

I understand that participation in this study is voluntary.

I may voluntarily choose to participate or not to participate in this study, or I may withdraw at any time by notifying the investigator without discrimination or retaliation, and my medical treatment and rights will not be affected in any way.

If I need other treatment, or if I do not comply with the study plan, or if a study- related injury occurs, or if there are other reasons that continuing participation might increase the risk of harm to me, the study physician may terminate my continued participation in this study.

I will receive a signed copy of this Informed Consent Form.

Name of Subject

Signature of Subject

Date (Year/Month/Day)

Name of Legal Representative (if applicable)

Signature of Legal Representative

Date (Year/Month/Day)

Name of Witness (if applicable)

Signature of Witness

Date (Year/Month/Day)

(Note: A witness signature is required if the subject is illiterate; a legal representative's signature is required if the subject has no capacity or limited capacity to act.)

I have accurately explained this document to the subject, asked him/her to read this Informed Consent Form carefully, and provided careful answers to all questions raised.

Name of Investigator

Signature of Investigator

Date (Year/Month/Day)