Summary of the research program

Project Title	**A Study on the Efficacy of Dual-Carrier-Frequency Transcranial Alternating Current Stimulation Modulating the Hippocampus for the Treatment of Chronic Non-Specific Low Back Pain**
Study Objectives	**Primary Objective:**To evaluate the effects of dual-carrier-frequency transcranial alternating current stimulation targeted at the hippocampus on pain intensity and associated emotional symptoms including anxiety and depression in patients with chronic non-specific low back pain. **Exploratory Objective:**To explore the functional changes in pain-related neural networks and emotion regulation networks following dual-carrier-frequency tACS targeted at the hippocampus, with a focus on functional connectivity changes between the hippocampus and brain regions including the prefrontal cortex and primary sensory cortices, and to analyze the relationships between changes in brain network metrics and improvements in pain, anxiety, depression, and other emotional symptoms.
Research Hypotheses	Dual-carrier-frequency transcranial alternating current stimulation targeting the hippocampus significantly reduces pain intensity and anxiety symptoms in patients with chronic non-specific low back pain. This effect may be mediated by the modulation of functional connectivity between the hippocampus and pain- as well as emotion-related brain regions.
Study Design	Single-center, randomized, double-blind, controlled clinical trial
Study Participants	**Inclusion Criteria:** 1. Age between 18 and 80 years; 2. Meets the diagnostic criteria for non-specific low back pain as specified in the *Chinese Clinical Guidelines for the Diagnosis and Treatment of Non-specific Low Back Pain (2022)* [1]; 3. Diagnosed with chronic non-specific low back pain within the past 3

	months, or pain located between T12 and the gluteal fold for at least half of the days within the past 6 months; 4. VAS score ≥ 3 (Visual Analogue Scale, 0–10); 5. Mechanical pain induced by posture, activity, and movement (e.g., flexion and functional movement tasks); 6. Voluntarily agrees to participate in this study and has signed the informed consent form. **Exclusion Criteria:** 1. Clear etiology of specific low back pain, such as vertebral fracture, severe spinal cord/nerve root compression symptoms (progressive neurological deficit), spinal infection, tumor, ankylosing spondylitis, etc.; 2. Prior major lumbar surgery or implants (e.g., internal fixation, spinal cord stimulation devices, etc.), or recent (within 3 months) invasive analgesic treatments such as nerve blocks or radiofrequency ablation; 3. Contraindications to MEG or MRI, including the presence of ferromagnetic metal implants in the body (e.g., cardiac pacemakers, hearing aids, carotid artery stents, etc.), claustrophobia that precludes MEG examination, or failed MEG acquisition; 4. History of severe cardiovascular disease, epilepsy, or other conditions that contraindicate electrical stimulation; 5. Pregnancy or lactation; 6. Cognitive impairment, psychiatric disorders, or other conditions that prevent understanding or compliance with the study procedures; 7. Rheumatic diseases, connective tissue diseases, or diabetes.
Diagnostic Criteria	1. Pain predominantly located in the lumbodorsal region (from below the costal margin to above the gluteal fold), which may be accompanied by discomfort in the buttocks. 2. Symptoms are persistent or recurrent for ≥ 3 months. 3. After evaluation based on medical history, physical examination, and

	auxiliary examinations when necessary, the pain cannot be attributed to a definite specific pathological cause (i.e., not due to tumor, infection, fracture, inflammatory spondyloarthropathy, etc.).
Sample Size and Calculation Basis	The study design is primarily informed by the randomized controlled trial published by Blanco-Giménez et al. (2024) in *BMC Musculoskeletal Disorders*, which investigated the effects of exercise combined with manual therapy or kinesiotaping on sEMG and pain perception in patients with chronic low back pain. Sample size estimation was performed using G*Power® software (Franz Faul, Universität Kiel, Kiel, Germany, version 3.1.9.7). Repeated-measures analysis of variance (ANOVA) was planned for data analysis. Accordingly, sample size calculation was based on the primary outcome measure, the Visual Analogue Scale (VAS). Referring to the results of previous studies, the Cohen's d effect size was set at 0.44, which was converted to an effect size of $f = 0.22$ for repeated-measures ANOVA (conversion formula: $f = d/2$), with a statistical power of 0.90, a significance level of $\alpha = 0.05$, four groups, five repeated measurements, a repeated-measures correlation coefficient of 0.5, and a non-sphericity correction coefficient of $\varepsilon = 1$. The calculation yielded a total required sample size of 48 participants. Additionally, considering a 20% dropout rate during the follow-up period, three additional participants were added per group (15 per group), resulting in a total enrollment of 60 participants. Reference: Blanco-Giménez P, Vicente-Mampel J, Gargallo P, Maroto-Izquierdo S, Martín-Ruíz J, Jaenada-Carrilero E, Barrios C. Effect of exercise and manual therapy or kinesiotaping on sEMG and pain perception in chronic low back pain: a randomized trial. *BMC Musculoskelet Disord*. 2024;25:583. doi:10.1186/s12891-024-07667-9.
Intervention	**Intervention Type:**Dual-carrier-frequency transcranial alternating current stimulation

	Intervention Intensity and Frequency: Right hippocampus; 10 Hz; 2 mA; 20 minutes per session; 5 sessions per week; continuous intervention for 2 weeks; total of 10 sessions
Outcome Variables	**Primary Endpoint:** Visual Analogue Scale (VAS) score assessed at the end of Week 2 of intervention. **Secondary Endpoints:** 1. McGill Pain Questionnaire (MPQ) 2. Hamilton Depression Scale (HAMD) 3. Hamilton Anxiety Scale (HAMA) 4. Pittsburgh Sleep Quality Index (PSQI) 5. Central Sensitization Inventory (CSI) 6. Oswestry Disability Index (ODI) 7. Functional near-infrared spectroscopy (fNIRS) 8. Functional magnetic resonance imaging (fMRI) 9. Electroencephalography (EEG) 10. Magnetoencephalography (MEG) 11. Surface electromyography (EMG) 12. Sensors 13. Ultrasound **Assessment Time Points:** Outcome measures will be evaluated at five time points: before treatment (T0), immediately after the first stimulation session (T1), at mid-treatment (T2), at the end of treatment (T3), and at 2 weeks post-treatment follow-up (T4). The primary outcome is the VAS score at T3 (end of Week 2 of intervention). Secondary subjective measures (MPQ, HAMA, HAMD, ODI, CSI) will be assessed at all five time points. Due to equipment availability and operational limitations, objective measures including MEG and fNIRS will be collected at four time points (T0, T1, T3, and T4),

	whereas fMRI will be performed at two time points only (T0 and T3).
Follow-up Plan	None
Sample Collection	☑ No □ Yes
Non-Medical Purpose Examinations	☑ No □ Yes (e.g., unnecessary CT scans, contrast imaging, laboratory tests, etc.)
Statistical Methods	All blinded data will first undergo normality and homogeneity of variance testing. Baseline comparability among the four groups will be assessed using ANOVA, Kruskal-Wallis H test, or chi-square test as appropriate. For the primary outcome and scale-based secondary outcomes, a 4 × 5 two-way repeated-measures ANOVA will be performed; for objective device-derived measures, a 4 × 4 repeated-measures ANOVA will be used. When the sphericity assumption is violated, the Greenhouse-Geisser correction will be applied. Post-hoc comparisons will be conducted using Tukey's method, and effect sizes will be reported as partial eta squared (η^2p). Correlations between change values (Δ = post-value − baseline value) of subjective and objective measures will be analyzed using Pearson or Spearman correlation, with Bonferroni correction for multiple comparisons. A two-sided p-value < 0.05 will be considered statistically significant.
Self-Assessment of Research Risk Level	□ High Risk □ Moderate Risk ☑ Low Risk **Brief Justification:** The interventions in this study pose minimal safety risks. Magnetoencephalography (MEG) involves a weak magnetic field environment and is a non-invasive, radiation-free procedure that carries virtually no additional risk.

Confidentiality Statement:

The information contained in this research protocol is provided solely for review by the investigators of this project, the Clinical Research Management Committee, and relevant regulatory agencies. No information may be disclosed to any third party

unrelated to this study without prior approval from the Principal Investigator.

1. Rationale

Low back pain (LBP) is a globally prevalent condition [2] and one of the leading causes of disability and functional limitations [3]. According to data from the Global Burden of Disease (GBD) study, approximately 619 million people worldwide were affected by LBP in 2020, and this number is projected to increase to 843 million by 2050, with the burden of disease continuing to rise [4]. LBP is generally defined as pain or discomfort localized to the area between the lower margin of the 12th rib and the gluteal fold, with or without radiating pain to the lower extremities [5]. Notably, approximately 85% of patients with LBP lack a clear pathoanatomical etiology [6], and conventional imaging examinations often fail to provide definitive etiological evidence. When LBP persists for more than 3 months, it is defined as chronic low back pain. Among these, chronic non-specific low back pain (CNSLBP) accounts for approximately 90%–95% of LBP cases and is one of the most common types of chronic pain encountered in clinical practice [7].

Chronic non-specific low back pain (CNSLBP) often lacks a clear structural etiology. Its development and maintenance are associated not only with peripheral tissue factors but also with central sensitization and long-term neuroplastic changes [5,8]. As a key structure of the limbic system, the hippocampus plays a critical role in contextual memory formation [9], contextual association, and emotional processing [10]. Recent studies suggest that the hippocampus may be involved in the development and maintenance of chronic pain. On the one hand, the hippocampus integrates and associates pain experiences with contextual cues such as actions and environmental contexts, thereby forming pain-related memory traces [11,12]. Berger et al. [11] further demonstrated that the hippocampus not only mediates memory encoding during this process but also that morphological characteristics of specific regions in the left posterior hippocampus directly determine the degree of pain memory bias—chronic pain patients consistently overestimate prior pain intensity by 16%–18%, and this bias is stable and modulated by loss-aversion personality traits. These mechanisms collectively enhance individuals' threat expectancy and avoidance behaviors toward

pain, promoting pain persistence and reactivation [13]. On the other hand, the chronic stress state, sleep disturbances, and negative emotions (such as anxiety and depression [14]) accompanying chronic pain may further affect hippocampal network function and neuroplasticity [15]. Empirical evidence from Vachon-Presseau et al. [16] provides a specific pathway for this link: smaller hippocampal volume elevates basal cortisol levels, which in turn enhances parahippocampal responses to painful stimuli, thereby exacerbating clinical pain experience. This suggests that hippocampal volume may constitute an individual vulnerability factor to stress, interacting with emotional and sleep factors to form a mutually reinforcing pathological process. The present study identifies the hippocampus as a key brain region of interest and aims to systematically analyze hippocampal structural characteristics, functional connectivity patterns, and their relationships with pain intensity, functional disability, and emotional indicators, in order to explore the potential mechanisms underlying the formation and maintenance of CNSLBP symptom phenotypes and to provide a theoretical basis for chronic pain intervention strategies targeting central neural mechanisms.

Dual-carrier-frequency transcranial alternating current stimulation is an important form of transcranial alternating current stimulation technology that employs two pairs of transcranial alternating currents at different frequencies to generate a low-frequency interference effect within the brain [17]. Thus, while maintaining non-invasiveness and favorable safety, this technique demonstrates potential for modulating deep brain regions [18]. Its associated adverse effects are limited to mild local skin sensations (e.g., tingling, itching), with no reports of serious adverse events or epileptic activity, confirming its good safety and tolerability in human applications [19]. This technology has been preliminarily validated in healthy subjects and patient populations such as those with Parkinson's disease: studies targeting the striatum have observed frequency-dependent modulatory effects on motor learning [20-23], while multiple studies targeting the hippocampus have consistently found that theta-band (5–10 Hz) envelope stimulation effectively modulates hippocampal activity during memory encoding. Violante et al. [22], using simultaneous functional magnetic resonance imaging, confirmed that it reduces task-evoked hippocampal signals and enhances episodic

memory accuracy. Beanato et al. [24] found that intermittent theta-burst stimulation accelerates spatial information retrieval and improves spatial navigation efficiency. Wang et al. [25], based on memory reconsolidation theory, applied 10 Hz stimulation targeting the hippocampus following drug cue exposure and significantly reduced craving scores in individuals with addiction. To date, clinical studies have demonstrated that this technique induces activation effects in the striatum, hippocampus, and other deep brain regions in healthy subjects and patients with conditions such as Parkinson's disease [20-23]. A recent study found decreased hippocampal function in chronic pain patients [26]. However, therapeutic applications targeting the hippocampus in chronic pain patients remain to be investigated, and its clinical efficacy and optimal stimulation parameters await systematic establishment through standardized research [27].

2. Study Objectives

2.1 Primary Objective:

To evaluate the effects of dual-carrier-frequency transcranial alternating current stimulation targeted at the hippocampus on pain intensity and associated emotional symptoms including anxiety and depression in patients with chronic non-specific low back pain.

2.2 Secondary and Exploratory Objectives:

To explore the functional changes in pain-related neural networks and emotion regulation networks following dual-carrier-frequency transcranial alternating current stimulation targeted at the hippocampus, with a focus on functional connectivity changes between the hippocampus and brain regions including the prefrontal cortex and primary sensory cortices, and to analyze the relationships between changes in brain network metrics and improvements in pain, anxiety, depression, and other emotional symptoms.

3. Study Plan

3.1 Study Design

This study is designed as a prospective, randomized, double-blind, sham-controlled

clinical trial aimed at evaluating the efficacy and safety of dual-carrier-frequency transcranial alternating current stimulation in patients with chronic non-specific low back pain.

3.2 Selection of Study Participants

3.2.1 Inclusion Criteria:

(1) Age between 18 and 80 years;

(2) Meets the diagnostic criteria for non-specific low back pain as specified in the *Chinese Clinical Guidelines for the Diagnosis and Treatment of Non-specific Low Back Pain (2022)*[1];

(3) Diagnosed with chronic non-specific low back pain within the past 3 months, or pain located between T12 and the gluteal fold for at least half of the days within the past 6 months;

(4) VAS score ≥ 3 (Visual Analogue Scale, 0–10);

(5) Mechanical pain induced by posture, activity, and movement (e.g., flexion and functional movement tasks);

(6) Voluntarily agrees to participate in this study and has signed the informed consent form.

3.2.2 Exclusion Criteria:

(1) Clear etiology of specific low back pain, such as vertebral fracture, severe spinal cord/nerve root compression symptoms (progressive neurological deficit), spinal infection, tumor, ankylosing spondylitis, etc.;

(2) Prior major lumbar surgery or implants (e.g., internal fixation, spinal cord stimulation devices, etc.), or recent (within 3 months) invasive analgesic treatments such as nerve blocks or radiofrequency ablation;

(3) Implanted electronic medical devices such as cardiac pacemakers or defibrillators;

(4) History of severe cardiovascular disease, epilepsy, or other conditions that contraindicate electrical stimulation;

(5) Pregnancy or lactation;

(6) Cognitive impairment, psychiatric disorders, or other conditions that prevent understanding or compliance with the study procedures;

(7) Rheumatic diseases, connective tissue diseases, or diabetes;

(8) Significant cognitive deficits or communication impairments that prevent cooperation with this study.

3.3 Criteria for Participant Withdrawal from the Trial

(1) If the patient's condition continues to deteriorate during the trial and potential risk events may occur, the study physician may determine that clinical research should be discontinued, and the case will be withdrawn from the clinical study;

(2) The participant develops certain complications, comorbidities, or special physiological changes during the study that may make continued participation inappropriate;

(3) Participants who experience serious adverse events;

(4) The participant is unwilling to continue participation in the clinical research and requests termination from the attending physician; in such cases, the case may be withdrawn from the clinical study.

3.4 Early Termination of the Trial / Closure of the Study Site

If symptoms such as dizziness or headache occur repeatedly during the study and persist without relief, the trial will be terminated early. If serious complications or other severe events occur, the study site will be closed.

3.5 Treatment

3.5.1 Treatments Received by Study Participants

This study includes four groups: Group 1 (dual-carrier-frequency transcranial alternating current stimulation group), Group 2 (dual-carrier-frequency transcranial alternating current stimulation with iTBS group), Group 3 (HF-tES superficial stimulation control group), and Group 4 (sham stimulation control group). Participants in all four groups will receive standardized routine pain rehabilitation therapy in addition to the corresponding experimental interventions.

3.5.2 Method of Participant Allocation

A computer-generated random number table will be used for group allocation. Participants will be randomly assigned at a 1:1:1:1 ratio to Group 1 (dual-carrier-frequency transcranial alternating current stimulation group), Group 2 (dual-carrier-frequency transcranial alternating current stimulation with iTBS group), Group 3 (HF-tES superficial stimulation group), and Group 4 (sham stimulation group). The random allocation sequence will be generated and sealed by personnel not involved in the implementation of the study. Participants, investigators, and outcome assessors will remain blinded to group allocation.

3.5.3 Dose Selection in the Trial

Group 1 (Dual-carrier-frequency transcranial alternating current stimulation group): Participants will receive transcranial temporal interference electrical stimulation. The stimulation delivers multi-channel high-frequency sinusoidal currents through scalp electrodes, with carrier frequencies of 2 kHz and 2.010 kHz interfering with each other to generate a 10 Hz low-frequency envelope modulation effect. The primary stimulation target is the right hippocampus. Stimulation parameters are set at 2 mA baseline-to-peak current per channel (gradually increased from 0.5 mA to 2 mA in 400 μA steps), with electrode placement optimized using standard or individualized head models to focus the envelope electric field. Stimulation will be administered 5 sessions per week for 2 consecutive weeks, totaling 10 sessions.

Participants will receive standardized routine pain rehabilitation therapy immediately after each stimulation session.

Group 2 (Dual-carrier-frequency transcranial alternating current stimulation with iTBS group): Based on the parameters used in Group 1, the stimulation pattern is modified by adding intermittent theta-burst stimulation (iTBS) superimposed on the carrier: a 2-second pulse train is delivered every 10 seconds. Each session lasts 20 minutes, administered 5 times per week for 2 consecutive weeks, totaling 10 sessions. Participants will receive standardized routine pain rehabilitation therapy immediately after each stimulation session.

Group 3 (tES superficial stimulation control group): Participants will receive high-frequency transcranial electrical stimulation (tES). Stimulation electrodes are primarily placed over the primary somatosensory cortex (S1 region), using alternating current parameters to produce neuromodulatory effects primarily characterized by superficial cortical activation and sensorimotor network modulation. Stimulation intensity is set within a safe threshold tolerated by participants. Each session lasts 20 minutes, administered 5 times per week for 2 consecutive weeks, totaling 10 sessions. Participants will receive standardized routine pain rehabilitation therapy immediately after each stimulation session.

Group 4 (Sham stimulation control group): Participants will receive sham transcranial temporal interference stimulation that is identical to the active treatment groups in terms of electrode placement and device setup. A 5-second ramp-up and ramp-down is applied at the start and end of stimulation to simulate the sensory experience of active treatment, but the current output during the treatment period is 0 mA, with the same carrier frequencies applied without any frequency difference, thereby avoiding the generation of effective envelope modulation. The duration of each session, stimulation frequency, and intervention period are consistent with those of the active treatment groups. This sham stimulation protocol ensures effective blinding of participants while producing no neuromodulatory effects.

All participants will receive standardized routine pain rehabilitation therapy, including shock wave therapy, magnetic therapy, shortwave therapy, and high-energy laser therapy. The content, intensity, and duration of rehabilitation training will be identical across all four groups.

Rationale for the 10 Hz Parameter Selection:The 10 Hz difference frequency was selected as the key modulation frequency in this study primarily because it aligns well with the intrinsic rhythmic activity of the hippocampus [28]. The hippocampus plays a crucial role in emotional processing and pain-related memory formation, and its neural oscillations exhibit frequency selectivity to exogenous rhythmic inputs. Previous studies have suggested that 10 Hz stimulation can effectively modulate hippocampal neural activity and sustain after-effects following stimulation, potentially inducing neuroplastic changes that persistently influence hippocampal function. Therefore, this study selects 10 Hz as the key parameter for hippocampal-targeted stimulation, with the aim of modulating pain experience and associated emotional and anxiety responses through the regulation of hippocampal neural activity, thereby offering a novel neuromodulatory approach for the treatment of chronic pain and its comorbid emotional disorders.

3.5.4 Blinding

Participants, outcome assessors, and treatment providers will all be blinded to group allocation. Data management will be performed by a clinical coordinator, and the final dataset will be provided to a statistician for statistical analysis.

3.5.5 Measures to Ensure Treatment Adherence

Patient education: Provide clear information about the treatment protocol, explaining the purpose, procedures, and expected effects of the intervention to help patients understand its importance.

Simplification of treatment protocol: Streamline treatment procedures as much as possible (e.g., using user-friendly devices) to reduce patient burden.

Incentive mechanisms: Consider implementing incentive measures, such as

providing small rewards to encourage patients to adhere to the treatment regimen.

Support systems: Establish support networks involving family members, friends, or treatment team members to provide emotional and practical support, encouraging patients to follow the treatment protocol.

Use of technological tools: Utilize mobile applications or other digital tools to remind patients to attend sessions or adhere to the intervention schedule.

3.6 Study Outcomes

3.6.1 Primary Outcome Variable

Visual Analogue Scale (VAS) score at the end of the second week of intervention.

3.6.2 Secondary Outcome Variables

(1) McGill Pain Questionnaire (MPQ)

(2) Hamilton Depression Scale (HAMD)

(3) Hamilton Anxiety Scale (HAMA)

(4) Pittsburgh Sleep Quality Index (PSQI)

(5) Central Sensitization Inventory (CSI)

(6) Oswestry Disability Index (ODI)

(7) Functional near-infrared spectroscopy (fNIRS)

(8) Functional magnetic resonance imaging (fMRI)

(9) Electroencephalography (EEG)

(10) Magnetoencephalography (MEG)

(11) Surface electromyography (EMG)

(12) Sensors

(13) Ultrasound

3.6.3 Assessment Time Points

Outcome Measure	T0 (Baseline)	T1 (Post-first stimulation)	T2 (Mid-treatment)	T3 (End of treatment)	T4 (2-week follow-up)
VAS (Primary)	✓	✓	✓	✓	✓
MPQ,HAMA, HAMD,ODI,CSI	✓	✓	✓	✓	✓
MEG,fNIRS	✓	✓	None	✓	✓
fMRI	✓	None	None	✓	None

Note: T0 = before treatment; T1 = immediately after first stimulation; T2 = mid-treatment; T3 = end of Week 2 of intervention; T4 = 2 weeks post-treatment follow-up.

4. Statistical Methods and Sample Size Determination

4.1 Statistical and Analysis Plan

(I) Data Distribution Testing and Baseline Comparisons

All continuous data will first be assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. For demographic characteristics (e.g., age, disease duration) and baseline outcome measures among the four groups, one-way ANOVA will be used for data that are normally distributed with equal variances; otherwise, the Kruskal-Wallis H test will be applied. Categorical variables (e.g., sex ratio) will be compared across groups using the chi-square test.

(II) Outcome Measures Analysis

The primary outcome is the VAS score at the end of the second week of intervention. Given the four-group, multiple-time-point repeated-measures design, the primary efficacy analysis will be performed using a linear mixed-effects model. The model will include group, time, and group × time interaction as fixed effects, with

participants as random effects. If baseline VAS, age, sex, disease duration, or other variables exhibit significant imbalance across groups or are considered clinically important, they may be included as covariates for adjustment. The primary focus will be on the group × time interaction effect and the differences in VAS change between each active treatment group and the sham control group at the end of intervention. Results will be presented as least-squares mean differences with 95% confidence intervals and p-values.

Secondary outcomes based on scales include MPQ, HAMA, HAMD, PSQI, CSI, and ODI. These will also be analyzed using linear mixed-effects models with the same model structure as the primary outcome analysis. For measures that do not meet the assumptions for continuous variable modeling or are markedly skewed, appropriate data transformations, generalized linear mixed models, or non-parametric methods will be used for sensitivity analyses.

For objective brain functional measures (fMRI, fNIRS, MEG, etc.), predefined key brain regions, network connections, or physiological parameters will be prioritized for analysis. When objective measures are collected at T0, T1, T3, and T4, linear mixed-effects models including group, time, and group × time interaction will be applied. For analyses involving multiple brain regions, multiple functional connections, or multiple frequency bands, the false discovery rate (FDR) method or Bonferroni method will be used for multiple comparison correction as appropriate. Exploratory whole-brain or multi-channel analyses that are not predefined will be reported as exploratory results.

When the overall model indicates a statistically significant group main effect, time main effect, or group × time interaction, pre-specified between-group comparisons will be performed. The primary comparison will be between Group 1 and the sham control group for VAS improvement at the end of treatment. Comparisons between Group 2 vs. sham and Group 3 vs. sham will be considered secondary comparisons. Comparisons between Group 1 vs. Group 2 and Group 1 vs. Group 3 will be considered exploratory comparisons. Multiple comparisons will be adjusted using the Holm-Bonferroni method or Tukey's method, and adjusted p-values will be reported.

The relationship between objective measures and subjective symptom improvement will be examined as exploratory analyses. Change values from baseline to end of treatment and from baseline to end of follow-up will be calculated for each participant, such as ΔVAS, ΔHAMA, ΔHAMD, and changes in functional connectivity. Pearson correlation analysis will be used if change values are normally distributed; otherwise, Spearman's rank correlation analysis will be applied.

Safety analysis will include the incidence of adverse events, serious adverse events, and adverse events related to the intervention. Between-group comparisons of adverse event rates will be performed using the chi-square test or Fisher's exact test. All adverse events will be summarized descriptively by time of onset, severity, management measures, outcome, and relationship to the intervention.

4.2 Sample Size Determination

The study design is primarily informed by the randomized controlled trial published by Blanco-Giménez et al. (2024) in *BMC Musculoskeletal Disorders*, which investigated the effects of exercise combined with manual therapy or kinesiotaping on sEMG and pain perception in patients with chronic low back pain [29]. Sample size estimation was performed using G*Power® software (Franz Faul, Universität Kiel, Kiel, Germany, version 3.1.9.7). Repeated-measures analysis of variance (ANOVA) was planned for data analysis. Accordingly, sample size calculation was based on the primary outcome measure, the Visual Analogue Scale (VAS). Referring to the results of previous studies, the Cohen's d effect size was set at 0.44, which was converted to an effect size of f = 0.22 for repeated-measures ANOVA (conversion formula: f = d/2), with a statistical power of 0.90, a significance level of $\alpha = 0.05$, four groups, five repeated measurements, a repeated-measures correlation coefficient of 0.5, and a non-sphericity correction coefficient of $\varepsilon = 1$. The calculation yielded a total required sample size of 48 participants. Additionally, considering a 20% dropout rate during the follow-up period, three additional participants were added per group (15 per group), resulting in a total enrollment of 60 participants.

The selected effect size falls within the small effect size range (0.20–0.59), and

this setting is supported by findings from both previous and subsequent studies. In the event of dropout, non-adherence, or missing outcome data, intention-to-treat analysis will be applied in this study.

5. Adverse Events

5.1 Adverse Event Monitoring

During the clinical trial, investigators will assess the occurrence of adverse events through close observation, physical examinations, and necessary laboratory and imaging evaluations. In addition, participants are obliged to report any physical discomfort experienced during the trial to the research center, and the physician will evaluate and manage these events accordingly.

5.2 Definitions of Adverse Events

5.2.1 Adverse Events

An adverse event is defined as any untoward or uncomfortable symptom, sign, or physical injury that occurs during the trial, regardless of whether a causal relationship with the clinical trial is established. The incidence rate of adverse events is calculated as: Adverse event rate (%) = (Number of adverse events in the group / Total number of participants in the group) × 100%.

Potential adverse events related to the study that may occur during the trial include dizziness, headache, nausea, etc. Any accidental injury or acute illness occurring during the study will be recorded as an adverse event. The severity, time of onset, duration, and management measures will be documented on the observation form, and the relationship to the clinical intervention will be evaluated based on comprehensive consideration.

5.2.2 Serious Adverse Events

None

5.2.3 Unexpected Adverse Events

None

5.2.4 Relationship Between Adverse Events and Trial Drug/Device

None

5.3 Reporting of Serious Adverse Events / Pregnancy

In the event of complications or adverse events (severe cases should be reported within 24 hours), the lead site and the sponsor must be notified, and a Serious Complication/Adverse Event Report Form must be completed. Investigators are responsible for follow-up observation and documentation of the outcome of all complications/adverse events. Participants who withdraw from the trial due to complications or adverse events will be followed until the event is fully resolved.

5.4 Risk Management Plan for Clinical Research

This study may involve risks such as participant discomfort, failure to complete the protocol as planned, missing or inconsistent data, and participant withdrawal. The research team will provide clear instructions on precautions in advance, implement key step verification and reminders, and ensure timely recording and quality checking of data. In the event of discomfort or abnormality, the related procedures will be immediately discontinued and properly managed, with complete documentation of the process and outcomes. If necessary, the principal investigator and the ethics committee will be notified, and corrective measures will be implemented.

6. References

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Informed Consent Form

Study Title: Efficacy of Dual-Carrier-Frequency Transcranial Alternating Current Stimulation Targeting the Hippocampus for the Treatment of Chronic Non-Specific Low Back Pain

Principal Investigator: Wang Yonghui

Study Duration: July 3, 2026 – September 24, 2029

Planned Number of Participants:60 participants

Name:_____

Sex:□ Male □ Female

Phone Number:_____

Mailing Address:_____

You are invited to participate in a clinical research study. This informed consent form provides you with information to help you decide whether to participate in this study. Please read it carefully. If you have any questions, please feel free to ask the study doctor in charge of this research.

(I) Study Content and Study Purpose:

This study aims to evaluate the efficacy of dual-carrier-frequency transcranial alternating current stimulation targeted at the hippocampus in improving pain intensity and associated emotional symptoms (including anxiety and depression) in patients with chronic non-specific low back pain (CNSLBP), and to explore changes in pain-related neural networks and emotion regulation networks following stimulation, as well as to analyze the relationships between changes in brain network metrics and clinical symptom improvement.

Study Objectives:

To evaluate the improvement effects of dual-carrier-frequency transcranial alternating current stimulation targeted at the hippocampus on pain intensity (VAS) and associated emotional symptoms (including anxiety and depression) in participants with CNSLBP; to explore the effects of this stimulation regimen on functional connectivity changes in pain-related neural networks and emotion

regulation networks, with a particular focus on functional connectivity changes between the hippocampus and brain regions including the prefrontal cortex and primary sensory cortices; and to analyze the associations between changes in brain network metrics and improvements in pain and emotional symptoms, thereby providing evidence for elucidating the role of the hippocampus in the pathological mechanisms of CNSLBP and for targeted neuromodulation strategies.

(II) Study Design:

This study is a single-center, double-blind, randomized controlled trial. If you agree to participate, the researcher will first conduct a screening assessment to confirm whether you meet the inclusion/exclusion criteria. Please provide truthful information regarding your basic personal details, medical history, and recent medication use, and cooperate with the completion of the informed consent process. After enrollment, you will undergo clinical scale assessments and objective device-based examinations at five time points: before treatment (T0), immediately after the first stimulation (T1), at mid-treatment (T2), at the end of treatment (T3), and at 2 weeks of follow-up (T4). Scale-based assessments (including questionnaires on pain, mood, sleep, and functional disability) will be conducted at all five time points. Objective examinations including magnetoencephalography (MEG) and functional near-infrared spectroscopy (fNIRS) will be collected at four time points (T0, T1, T3, T4), while functional magnetic resonance imaging (fMRI) will be performed at two time points only (T0 and T3). Detailed study procedures are described in Section 5.

The MEG examination must be completed within a magnetic shielding device. It is a non-contact, non-invasive procedure that does not require contrast agent injection. The total experimental paradigm duration is approximately 10 minutes (including eyes-open/eyes-closed resting-state and spatial memory tasks). Where available, cranial MRI may be performed for coregistration with MEG data to improve the accuracy of brain region localization. Before the examination, please carefully read the relevant precautions, remove all metal objects and items that may affect the examination, and wear clothing without metal accessories. This study does not involve blood sample collection. All data will be de-identified using coded

identifiers, and your personal information will be strictly protected.

(III) **Institutional Qualifications:**

Our institution is a Class A Tertiary hospital capable of conducting standardized, high-quality clinical research in pain and related fields.

(IV) **Participant Inclusion and Exclusion Criteria**

This study plans to enroll 60 eligible participants with chronic non-specific low back pain.

Inclusion Criteria (All of the following must be met):

1. Age between 18 and 80 years, regardless of sex;
2. Meets the diagnostic criteria specified in the *Chinese Clinical Guidelines for the Diagnosis and Treatment of Non-specific Low Back Pain (2022)*;
3. Diagnosed with chronic non-specific low back pain within the past 3 months, or pain located between T12 and the gluteal fold for at least half of the days within the past 6 months;
4. Visual Analogue Scale (VAS) score ≥ 3 (0–10 scale);
5. Mechanical pain induced by posture, activity, and movement (e.g., flexion, functional tasks);
6. Voluntarily agrees to participate and has signed the informed consent form.

Exclusion Criteria (Presence of any one criterion precludes participation):

1. Presence of a clear specific etiology for low back pain (e.g., vertebral fracture, severe spinal cord/nerve root compression, spinal infection, tumor, ankylosing spondylitis, etc.);
2. Prior major lumbar surgery or implants (e.g., internal fixation, spinal cord stimulation devices, etc.), or receipt of invasive analgesic treatments such as nerve blocks or radiofrequency ablation within the past 3 months;
3. Presence of ferromagnetic metal implants in the body (e.g., cardiac pacemakers, hearing aids, carotid artery stents, etc.), or claustrophobia that precludes MEG or MRI examination;
4. Severe cardiovascular disease, epilepsy, or other conditions that contraindicate electrical stimulation;

5. Pregnancy or lactation;
6. Cognitive impairment, psychiatric disorders, or other conditions that prevent understanding or cooperation with the study procedures;
7. Rheumatic diseases, connective tissue diseases, or diabetes.

(V) **Study Procedures**

Phase	Duration	Content
Screening Period	1–3 days before enrollment	Medical history collection, physical examination, assessment of inclusion/exclusion criteria, signing of informed consent form
Treatment Period	2 weeks (10 sessions total)	5 sessions per week, 20 minutes per session, total of 10 sessions + routine rehabilitation therapy
Follow-up Period	2 weeks	Return visit at 2 weeks after treatment completion (T4)

A computer-generated random number table will be used to allocate you in a 1:1:1:1 ratio to Group 1 (dual-carrier-frequency transcranial alternating current stimulation group), Group 2 (dual-carrier-frequency transcranial alternating current stimulation with iTBS group), Group 3 (tES superficial stimulation group), or Group 4 (sham stimulation group). Neither you nor the study evaluators will be aware of your group assignment.

(VI) **Examinations and Assessments Required in This Study**

This study does not involve blood sample collection. All examinations are non-invasive and non-intrusive.

You will be assessed at the following time points:

Assessment Time Point	Subjective Assessment Content	Objective Device Examinations
Before treatment (T0)	VAS,MPQ,HAMA, HAMD,PSQI,CSI,ODI	MEG, fMRI, fNIRS, EEG, EMG, Sensors, Ultrasound

Immediately after first treatment (T1)	Same scales as above	MEG, fNIRS, EEG, EMG, Sensors, Ultrasound
Mid-treatment (T2)	Same scales as above	None
End of treatment (T3)	Same scales as above	MEG, fNIRS, fMRI, EEG, EMG, Sensors, Ultrasound
2-week follow-up (T4)	Same scales as above	MEG, fNIRS, EEG, EMG, Sensors, Ultrasound

Scale Descriptions:

- **VAS:**Visual Analogue Scale for pain (primary outcome)
- **MPQ:**McGill Pain Questionnaire
- **HAMA:**Hamilton Anxiety Scale
- **HAMD:** Hamilton Depression Scale
- **PSQI:**Pittsburgh Sleep Quality Index
- **CSI:**Central Sensitization Inventory
- **ODI:**Oswestry Disability Index

Objective Examination Descriptions:

- **Magnetoencephalography (MEG):**Performed in a magnetically shielded room; non-invasive, radiation-free, approximately 10 minutes.
- **Functional Magnetic Resonance Imaging (fMRI):**Performed in an MRI scanner; approximately 10–20 minutes; radiation-free.
- **Functional Near-Infrared Spectroscopy(fNIRS),**

Electroencephalography (EEG), Surface Electromyography (EMG), Sensors, and Ultrasound: All are non-invasive, painless, and radiation-free.

(VII) **Data Management and Statistical Analysis**

All data in this study will be managed using coded de-identification, and the analysts will remain blinded to participant identities. All continuous data will first be assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. For demographic characteristics (e.g., age, disease duration) and baseline outcome measures among the four groups, one-way analysis of variance

(ANOVA) will be used for data that are normally distributed with equal variances; otherwise, the Kruskal-Wallis H test will be applied. Categorical variables (e.g., sex ratio) will be compared across groups using the chi-square test.

The primary outcome measure and scale-based secondary outcome measures will be analyzed using two-way repeated-measures ANOVA (4 groups × 5 time points). For objective device-based measures, which are collected at only four time points, a 4 groups × 4 time points analysis will be used. When the sphericity assumption is violated, the Greenhouse-Geisser correction will be applied. Post-hoc multiple comparisons will be performed using Tukey's method, and effect sizes will be reported as partial eta squared (η^2p).

To explore the relationship between changes in brain network functional connectivity and clinical symptom improvement, change values (Δ = post-treatment value − baseline value) from baseline to end of treatment and from baseline to end of follow-up will be calculated for both objective and subjective measures in each participant. Pearson correlation (for normally distributed data) or Spearman correlation (for skewed data) will be used based on data distribution, with Bonferroni correction applied for multiple comparisons. A two-sided p-value < 0.05 will be considered statistically significant for all analyses.

(VIII) **Risks and Discomforts:**

While all examinations and non-invasive stimulation procedures are considered to have a favorable safety profile, the following risks may arise:

Stimulation-related adverse effects: Some participants may experience localized skin tingling, itching, mild burning sensation, redness, or transient headache, fatigue, drowsiness, nausea, nervousness, or visual disturbances. These reactions are generally mild and tend to resolve without intervention. Should symptoms persist or worsen, stimulation will be stopped immediately and appropriate care will be provided.

MEG/MRI examinations: In rare instances, unforeseeable discomfort may occur due to individual idiosyncrasies. Participants with claustrophobia may find the examination environment challenging; please inform the research team in advance.

Unknown risks: There may be adverse effects not described in this informed consent form. If you experience any unusual symptoms, please report them to the research staff without delay.

Contingency plan: In the event of a serious adverse event, the intervention will be terminated promptly, emergency medical treatment will be administered, and the Ethics Committee will be notified.

(IX) **Costs of Participation:**

This study will not charge you any additional fees. The specific details are as follows:

1. All examinations and treatment procedures involved in this study—including dual-carrier-frequency transcranial alternating current stimulation, all scale-based assessments, and MEG, fMRI, fNIRS, EEG, EMG, sensors, and ultrasound—will be provided free of charge.
2. During the intervention period of this study, if you receive other routine diagnostic or therapeutic services (e.g., medications or examinations for other comorbid conditions, or other rehabilitation therapies), the associated costs will be borne by you in accordance with the hospital's standard billing practices and are not covered by this study.

(X) **Your Rights and Responsibilities:**

You have the following rights:

1. **Privacy protection:** If you decide to participate in this study, your participation and all personal information collected during the study will be kept confidential. Your information will be identified by a study number rather than by your name. Any information that could identify you will not be disclosed to individuals other than the research team members unless we obtain your permission. All research team members are required to maintain the confidentiality of your identity. Your records will be kept in a locked file cabinet and will be accessible only to the research staff. The results of this study may be published in scientific journals, but your address, name, or other personal identifying information will not appear.
2. **Voluntary participation and withdrawal:** You may choose not to participate

in this study, or you may withdraw from the study at any time by notifying the study doctor. If you withdraw, your data will not be included in the study results, and your medical care and rights will not be affected in any way.

You have the following responsibilities:

1. Provide truthful information regarding your personal background, medical history, and diagnosis/treatment history to facilitate the conduct of the study.
2. Cooperate with the study doctor and complete all relevant examinations during the clinical research process.
3. Carefully read and clearly understand the precautions for the examinations, as well as the potential risks, unexpected events, and unforeseeable circumstances that may arise.
4. Agree that, during the examination, the physician may adjust the examination protocol as deemed necessary based on your specific condition. In the event of any risks or emergencies, you authorize the medical staff to provide appropriate care in accordance with routine medical practice.

(XI) **Benefits and Compensation:**

By participating in this study, you may experience direct therapeutic benefits, including reduced pain intensity, alleviation of anxiety and depressive symptoms, and improved daily functioning. At the same time, your participation will help us accumulate valuable scientific data, contributing to the development of better treatment options for more chronic pain patients in the future. Please note, however, that all free examinations (MEG, fMRI, etc.) and device-based assessments provided in this study are research support measures and are not considered therapeutic benefits; cost-related details are described in Section 9.

In the event that any study-related injury or adverse effect—such as skin tingling, itching, mild burning sensation, redness, or transient headache, fatigue, drowsiness, nausea, nervousness, or visual disturbances—is determined by the investigator to be associated with this study, the research team will provide you with active diagnosis and treatment and will cover the associated medical expenses arising from such adverse effects. Signing this informed consent form will not impair any of your legal

rights.
If you have any further questions regarding this study, please contact the study physician or designated research staff using the contact information provided below: • **Study Physician:** Xiangxin Xing **Phone:**86-18560086949 • **Designated Research Staff:**Xiaofei Zhang **Phone:**86-15563139097 • **Research Ethics Committee of Qilu Hospital of Shandong University** **Phone:** 0531-82165409
I have accurately explained this informed consent document to the study participant. The participant has read and understood the information provided, and I confirm that he/she was afforded the opportunity to ask questions. I hereby confirm that the participant has provided consent voluntarily. **Investigator Signature:**_____ **Date:**_____ Year _____ Month _____ Day
I have read and understood all the information provided above. The Principal Investigator/Investigator has fully addressed all my questions concerning the study plan. Having fully comprehended the details of this research and after due consideration, I hereby voluntarily consent to participate as a participant in this clinical study. **Participant Signature:**_____ **Date:**_____ Year _____ Month _____ Day

Appendix：

Technical Term	Scientific Explanation
Primary/Secondary Somatosensory Cortex	Located in the postcentral gyrus and parietal operculum, respectively, these are the primary cortical areas responsible for receiving and processing somatosensory information (including touch, pain, and temperature), involved in the sensory discrimination and localization of pain.

Prefrontal Cortex	Located in the anterior part of the frontal lobe, including subregions such as the dorsolateral prefrontal cortex and orbitofrontal cortex. It is involved in higher-order cognitive control, attentional allocation, and emotional regulation. In pain modulation, the prefrontal cortex exerts top-down regulation of pain perception through descending inhibitory pathways.
Brain Network / Neural Network	In the brain, different brain regions form anatomical connections via white matter fiber tracts and exhibit functional connectivity patterns of synchronized activity during specific tasks. These interacting sets of brain regions constitute brain networks. Pain perception and emotional regulation both rely on the coordinated activity of multiple brain networks (e.g., default mode network, salience network, central executive network).
Functional Connectivity	Refers to the statistical dependence (e.g., correlation or coherence) of neural activity signals between different brain regions in the temporal domain, reflecting the degree of synchronization in information transfer between them. Changes in functional connectivity strength can reveal interregional coordinated regulatory relationships.
Magnetoencephalography (MEG)	A non-invasive, radiation-free neurophysiological imaging technique that uses superconducting quantum interference devices within a magnetically shielded room to detect the weak magnetic field signals generated by neuronal excitation. It offers millisecond-level temporal resolution and enables real-time tracking of the temporal dynamics of brain functional activity.

Functional Near-Infrared Spectroscopy (fNIRS)	A non-invasive optical brain imaging technique that uses near-infrared light to penetrate the skull and monitor relative changes in oxyhemoglobin and deoxyhemoglobin concentrations in cortical tissue, indirectly reflecting regional cerebral blood oxygen metabolism and thereby inferring brain region activation status.
Functional Magnetic Resonance Imaging (fMRI)	A magnetic resonance imaging technique based on blood oxygen level-dependent (BOLD) contrast, which detects magnetic susceptibility changes caused by alterations in the ratio of oxygenated to deoxygenated hemoglobin in local cerebral blood flow. It non-invasively reflects the metabolic demands associated with neuronal activity and is used for localizing functional brain areas and assessing functional connectivity.
Inclusion Criteria / Exclusion Criteria	Inclusion criteria are the eligibility conditions that all participants must meet for enrollment; exclusion criteria are conditions that, if any one is present, preclude participation. Together, they form the screening framework for study participants, used to control heterogeneity and ensure internal validity and participant safety.
Sham Stimulation	In electrical stimulation studies, participants in the sham group undergo the same operational procedures and electrode placement as those receiving active stimulation, but the stimulation device delivers no effective current (or only a brief initial current to simulate sensation). This serves as a placebo control to correct for psychological expectations and non-specific operational effects.

Visual Analogue Scale (VAS)	A commonly used one-dimensional self-rating tool for pain intensity. On a continuous 10 cm line, the endpoints are labeled "no pain" and "worst possible pain." Participants mark the point on the line that corresponds to their subjective pain experience, and the distance (in mm or cm) from the zero point is measured as the quantitative value of pain intensity.
McGill Pain Questionnaire (MPQ)	A multidimensional pain assessment tool that requires participants to select words that best describe their pain from multiple descriptive groups, covering sensory, affective, and evaluative dimensions. It quantifies the quality, intensity, and experience of pain.
Pittsburgh Sleep Quality Index (PSQI)	A self-rated questionnaire for assessing sleep quality over the past month, covering seven dimensions: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. A total score ≥ 5 indicates poor sleep quality.
Hamilton Anxiety Scale / Hamilton Depression Scale (HAMA/HAMD)	These are well-validated observer-rated scales for assessing the severity of anxiety and depressive symptoms, respectively. Each scale consists of multiple items, scored by the evaluator based on the participant's clinical presentation and interview results. Higher scores indicate greater severity of emotional disturbance.
Central Sensitization Inventory (CSI)	A self-report questionnaire for assessing the degree of central nervous system hypersensitization, covering dimensions such as amplified pain responses, widespread pain, and non-painful sensory abnormalities. Higher scores indicate more pronounced central sensitization.

Oswestry Disability Index (ODI)	A self-rated scale for assessing low back pain-related functional disability, comprising 10 items covering activities of daily living (such as walking, lifting, sitting, standing, etc.) and social/travel capacity. The total score reflects the severity of functional limitations due to low back pain.
De-identification Management	Refers to the process of separating personally identifiable information (such as name, ID number, contact details) from research data and replacing them with a unique study code, so that data cannot be traced back to specific individuals during analysis, thereby protecting participant privacy.