International Platform of Registered Systematic Review and Meta-analysis Protocols

INPLASY

Comparative efficacy of different acupuncture therapies for animal models of post-stroke depression: A systematic review and network meta-analysis protocol

INPLASY202660033
doi: 10.37766/inplasy2026.6.0033
Received: 6 June 2026
Published: 6 June 2026

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ADMINISTRATIVE INFORMATION

Support - No financial support.

Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202660033

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 6 June 2026 and was last updated on 6 June 2026.

INTRODUCTION

Review question / Objective This systematic review and network meta-analysis (NMA) aims to evaluate the comparative efficacy of different acupuncture therapies in post-stroke depression (PSD) animal models, identify their response profiles under distinct pathophysiological conditions, and dissect their underlying neurobiological mechanisms.

1. Primary Objective (Efficacy Comparison Across Pathophysiological Dimensions):

To compare the relative efficacy of various acupuncture modalities (e.g., manual acupuncture, electroacupuncture, warm needle acupuncture) across distinct pathophysiological dimensions. Studies will be stratified based on modeling protocols: pure stroke models (e.g., MCAO) representing the "primary focal neural injury and localized neuroinflammation" dimension, and stroke-stress composite models (e.g., MCAO+CUMS) representing the "superimposed chronic environmental stress and neuroendocrine dysregulation" dimension. This helps clarify how different acupuncture modalities perform when targeting distinct pathological components of PSD.

2. Secondary Objective (Pathway-Specific Responsiveness):

To quantitatively compare the regulatory effects of distinct acupuncture interventions on five core neurobiological pathways: neurotransmitters (5-HT, DA, NE), neurotrophins (BDNF, NGF), neuroinflammation (IL-1β, IL-6, TNF-α), HPA axis (CRH, ACTH, CORT), and oxidative stress (SOD, MDA, GSH-Px).

3. Translational Medicine Objective (Mechanism-Driven, Precision Acupuncture Framework):

To construct a preclinical-to-clinical translational framework linking specific pathological features with optimal acupuncture selections. Although clinically dividing PSD patients into rigid subtypes is challenging due to overlap, clinicians regularly encounter distinct predominant phenotypes—such as cognitive/neurological deficits ("lesion-dominant" pathology) versus severe anxiety, insomnia, and elevated cortisol ("stress-dominant" pathology). Mapping preclinical pathway sensitivities will provide a mechanism-driven,

biomarker-guided decision-making blueprint for clinical personalized acupuncture, reducing therapeutic trial-and-error.

Rationale Post-stroke depression (PSD) is a highly prevalent neuropsychiatric complication, affecting approximately 20% to 65% of stroke survivors. PSD seriously hinders neurological recovery, reduces quality of life, and increases long-term morbidity. Clinically, PSD exhibits high heterogeneity, primarily manifesting as two distinct subtypes: "organic/lesion-induced PSD" (triggered directly by neural circuit damage and localized neuroinflammation) and "reactive/psychosocial stress PSD" (triggered by severe disability and post-stroke existential distress). Acupuncture has emerged as a promising non-pharmacological therapy for PSD, with clinical evidence confirming its symptom-alleviating effects. However, the precise biological mechanisms underlying different acupuncture modalities remain partially understood, and clinical prescription selection still largely relies on empirical trial-and-error.

Preclinical evidence demonstrates that acupuncture alleviates depressive-like behaviors in PSD animal models, modulating diverse pathways including neurotransmitters, neurotrophic factors, neuroinflammation, the HPA axis, and oxidative stress. Nevertheless, existing animal studies mostly focus on a single acupuncture modality or isolated pathways, lacking a systematic comparison of their relative mechanistic profiles. Crucially, it remains unanswered whether different acupuncture therapies exhibit selective sensitivity toward specific mechanistic modules or distinct animal models. For instance, whether electroacupuncture (characterized by constant physical electrical stimulation) is more sensitive to localized neuroinflammation and neuroplasticity pathways in pure ischemic models (such as MCAO), and whether manual acupuncture (characterized by gentle tactile stimulation) is more selective for systemic endocrine dysregulation (such as HPA axis hyperactivity) in ischemic-stress composite models (such as MCAO+CUMS), remains a critical evidence gap.

Therefore, this systematic review and network meta-analysis is warranted. By integrating preclinical evidence from the perspective of "therapy-mechanism" mapping, this study will compare the relative efficacy of different acupuncture modalities across diverse PSD models and identify their selective pathway responsiveness. This is expected to provide systematic preclinical evidence for subsequent mechanistic validation studies and the optimization of acupuncture protocols, while offering helpful insights and a theoretical foundation for exploring future mechanism-guided, individualized clinical acupuncture strategies.

Condition being studied Post-stroke depression (PSD) is one of the most common neuropsychiatric complications following a stroke, affecting approximately 20% to 65% of survivors. It is characterized primarily by persistent depressed mood, anhedonia, and loss of interest, which significantly hinder neurological recovery, deteriorate quality of life, and increase the risk of poor long-term outcomes. Pathophysiologically, PSD is multifactorial and closely associated with neurochemical and structural alterations, including neurotransmitter dysregulation, decreased neurotrophic factors, neuroinflammatory activation, HPA axis hyperactivity, and oxidative stress. Current clinical interventions primarily rely on pharmacotherapy (antidepressants), psychotherapy, and rehabilitative care. While these treatments are effective for some patients, they are often limited by delayed onset of therapeutic effects, high inter-individual variability in response, adverse side effects, and low long-term compliance. Acupuncture has been widely investigated and utilized in both preclinical studies and clinical practice for PSD. However, the comparative efficacy of distinct acupuncture modalities and their selective mechanism-regulating profiles still lack a systematic, evidence-based evaluation.

METHODS

Search strategy We will systematically search seven electronic databases, including PubMed, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database, and Chinese Biomedical Literature Database (CBM/SinoMed), from database inception to June 2026. The search will be limited to studies published in Chinese or English. A combination of subject terms and free-text terms will be used. The main search terms will include post-stroke depression, poststroke depression, PSD, stroke, cerebral infarction, cerebral ischemia, cerebral hemorrhage, middle cerebral artery occlusion, MCAO, depression, depressive-like behavior, anhedonia, acupuncture, electroacupuncture, manual acupuncture, scalp acupuncture, warm needle acupuncture, moxibustion, acupoint, animal, rat, mouse, rodent, and animal model. Reference lists of included studies and relevant reviews will also be checked to identify additional eligible studies.

Participant or population 1. Inclusion Criteria:

Animal models of post-stroke depression (PSD) established on either ischemic stroke (e.g., middle cerebral artery occlusion [MCAO], photothrombosis) or hemorrhagic stroke (e.g., intracerebral hemorrhage [ICH] induced by autologous blood or collagenase injection). Eligible subjects are limited to rodents, including rats (e.g., Sprague-Dawley, Wistar) and mice (e.g., C57BL/6, ICR), with no restrictions on strain, age, weight, or sex. Methods of modeling depression are unrestricted and include stroke followed by chronic unpredictable mild stress (CUMS), social isolation, chronic social defeat stress (CSDS), or other recognized, validated PSD modeling protocols.
2. Exclusion Criteria:
Non-rodent animal models (e.g., primates, dogs, rabbits); Pure stroke models without any depressive-like behavior assessment, or pure depression models without stroke surgical/pathological modeling; Animals with severe genetic or acquired comorbidities prior to modeling (e.g., spontaneously hypertensive rats [SHR], diabetic rodent models); Human clinical trials, in vitro studies, or ex vivo tissue/cell experiments.

Intervention 1. Inclusion Criteria:
Eligible interventions will include acupuncture-related therapies administered as monotherapies. These eligible modalities will be mapped as independent nodes in the network meta-analysis, including: Manual Acupuncture (MA) (conventional manual needle insertion with mechanical manipulation, such as twisting, lifting, thrusting); Electroacupuncture (EA) (electrical current stimulation applied through inserted acupuncture needles); Warm Needle Acupuncture (WNA) (warming of the needles using burning moxa attached to the needle handle); Scalp Acupuncture (SA) (acupuncture applied to specific scalp zones); and Moxibustion (Mox) (burning of moxa on or near the skin over acupoints). There are no restrictions on acupoint selection (e.g., Baihui, Yintang), needle materials, retention time, stimulation parameters, frequency, or duration of treatment.
2. Exclusion Criteria:
Non-invasive physical stimulations without needle penetration (except classic moxibustion), such as transcutaneous electrical acupoint stimulation (TEAS), laser acupuncture, or acupressure; Interventions involving drug or chemical delivery, such as acupoint injection (hydro-acupuncture), herbal patching at acupoints, or acupoint catgut embedding, to avoid confounding pharmacological mechanisms; Acupuncture combined with other active therapies (e.g., chemical antidepressants, herbal medicine, hyperbaric oxygen therapy) to isolate the pure therapeutic effects of distinct acupuncture modalities.

Comparator Eligible comparator groups will serve as critical reference nodes linking acupuncture interventions in the network layout, including:
Model Control (Untreated PSD): Animal models that have undergone full stroke and depression induction protocols but receive no therapeutic interventions. To minimize operational bias, animals in this group must be subjected to identical mock-restraining or immobilization procedures for the same duration and frequency as the acupuncture groups.
Sham/Placebo Acupuncture: Controls designed to isolate physiological non-specific effects of needles, including: superficial needle insertion at non-acupoints; "Minimal acupuncture" (shallow insertion at acupoints with no manual manipulation/Deqi sensation); inactive electroacupuncture (needles inserted with electrodes attached but keeping the power stimulator turned off or sending 0 mA current); mock moxibustion (using heat-insulated pads or non-burning moxa to mimic the visual/tactile procedure without heat transfer).
Active Pharmacological Control: Rodents treated with standard clinical first-line chemical antidepressants, specifically Selective Serotonin Reuptake Inhibitors (SSRIs, e.g., Fluoxetine/Prozac) to establish a chemical treatment baseline.
Normal Control (Blank Control): Intact, healthy rodents of the same strain, age, and sex raised under identical husbandry conditions without any stroke or depression modeling procedures, serving as the biological baseline.

Study designs to be included Randomized controlled laboratory animal studies (experiments) with parallel-group designs evaluating the efficacy of acupuncture therapies for animal models of post-stroke depression (PSD). Eligible publications are limited to peer-reviewed studies published in English and Chinese.Exclusion Criteria: Non-randomized animal experiments or studies without parallel control; Self-controlled studies or cross-over design experiments; Review articles, systematic reviews, meta-analyses, conference abstracts, patents, letters, or case reports; In vitro experiments, ex vivo tissue studies, or human cli.

Eligibility criteria 1. Inclusion Criteria:
Rodent models of post-stroke depression (PSD) established via validated ischemic or hemorrhagic stroke methods, regardless of strain, age, weight, or sex. Eligible invasive acupuncture therapies (MA, EA, WNA, SA, Mox) administered as monotherapies. Non-acupuncture controls,

including normal control, untreated/model control, sham acupuncture, and active pharmacological control (SSRIs). Randomized controlled laboratory animal trials with parallel-group designs. Full-text peer-reviewed articles published in English or Chinese.
2. Exclusion Criteria:
Non-randomized, non-parallel, self-controlled, or cross-over animal experiments. Reviews, systematic reviews, meta-analyses, conference abstracts, editor comments, case reports, or patents. In vitro cell experiments, ex vivo tissue/organ studies, or human clinical trials. Acupuncture combined with other active therapies (e.g., antidepressants, herbal medicine) to avoid confounding therapeutic effects. Studies with duplicated data (e.g., a master's/doctoral thesis and a journal article originating from the same experiment). In such cases, the version with the most complete and comprehensive dataset will be included, and others will be excluded to prevent double-counting. Studies with incomplete data, logical errors, or data presented only in graphs that cannot be extracted accurately using digitizing software (e.g., Engauge Digitizer) even after attempting to contact the primary authors.

Information sources Information sources include China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database (CQVIP), Chinese Biomedical Literature Database (CBM/SinoMed), PubMed, Embase, and Web of Science. The search period will be from the inception of each database to June 2026. Reference lists of included studies and relevant reviews will also be checked to identify potentially missed studies. For missing or incomplete data, we will attempt to contact the primary authors of the original studies.

Main outcome(s) Depressive-like behavioral assessments in PSD animal models, representing distinct psychiatric clinical end-points, including: Sucrose Preference Test (SPT): To evaluate anhedonia (the core clinical symptom of depression). The primary extracted quantitative parameter will be the sucrose preference percentage (%). Forced Swimming Test (FST): To evaluate behavioral despair. The primary extracted quantitative parameter will be the duration of immobility (seconds).
Open Field Test (OFT): To evaluate autonomous exploratory activity and anxiety-like states. Extracted quantitative parameters will include the total distance traveled (meters or centimeters) and/or time spent in the central area (seconds). Other validated depressive-like behavior tests, such as the Tail Suspension Test (TST) or Novelty Suppressed Feeding (NSF) test, will be included as main outcomes if available in the primary studies.

Additional outcome(s) Quantitative expression of representative neurobiological biomarkers across five core mechanistic pathways (measured in central brain regions such as the hippocampus, prefrontal cortex, and striatum, or peripheral circulation such as serum/plasma): Neurotransmitter Pathway: Serotonin (5-HT), dopamine (DA), and norepinephrine (NE). Neurotrophic Pathway: Brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Neuroinflammatory Pathway: Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 β (IL-1β). HPA Axis Pathway: Corticosterone (CORT), adrenocorticotropic hormone (ACTH), and corticotropin-releasing hormone (CRH). Oxidative Stress Pathway: Superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px).

Data management Literature screening and data management will strictly follow the PRISMA 2020 guidelines. All retrieved references will be imported into EndNote X9 reference management software for de-duplication and systematic archiving.
Two reviewers will independently screen the literature in two sequential phases: a preliminary screening of titles and abstracts to exclude obviously irrelevant studies, followed by a full-text evaluation against the pre-defined inclusion and exclusion criteria. Discrepancies at any stage will be resolved through consensus discussion, and if unresolved, will be arbitrated by a third senior reviewer.
A standardized data extraction spreadsheet will be developed prior to extraction. Two reviewers will independently extract data using a pre-piloted Excel spreadsheet. The extracted details will consist of:
Study Metadata: Primary author, publication year, country of origin, and language. Animal Characteristics: Species, strain, age, weight, sex, and health status prior to modeling. Modeling Details: Stroke surgical/pathological protocols (e.g., ischemic MCAO vs. hemorrhagic ICH), depression modeling stressors (e.g., CUMS, social isolation, CSDS), and duration of modeling.
Acupuncture Parameters: Specific modality (MA, EA, WNA, SA, Mox), selected acupoints (e.g., GV20, GV29), needle materials, retention time, electrical stimulator parameters (frequency, waveform, current intensity in mA), moxibustion temperature/duration, and overall treatment duration/frequency.

Control Interventions: Type of control (Normal, Model mock-restraint, Sham acupuncture specifics, Active pharmacological agent dosages).
Quantitative Outcomes: Sample size (n), mean, and standard deviation (SD) for behavioral tests (SPT, FST, OFT) and neurobiological mechanism biomarkers.
For incomplete data, we will proactively attempt to contact the primary authors to request the original datasets. If the data are presented only in graphical format, digital graph extraction software (WebPlotDigitizer or Engauge Digitizer) will be utilized by both reviewers independently to convert graphs into precise numerical coordinates, and the average values will be used.

Quality assessment / Risk of bias analysis The risk of bias for the included studies will be independently assessed by two reviewers using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool for animal studies (Hooijmans et al., 2014). The assessment covers 10 domains: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers/investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. Each domain will be categorized as "low risk," "high risk," or "unclear risk" of bias. Discrepancies between the two reviewers will be resolved through discussion; if consensus cannot be reached, a third senior reviewer will make the final decision. Visualizations of the risk of bias assessment will be generated using Review Manager (RevMan 5.4).

Strategy of data synthesis Since the outcomes of interest are continuous variables, the pooled effect sizes will be expressed as standardized mean differences (SMDs) with 95% confidence intervals (CIs); if the measurement units and assessment methods are consistent across studies, mean differences (MDs) will be employed instead. Direct comparison evidence will be initially described, and pairwise meta-analysis will be conducted using a random-effects model when study numbers and methodological homogeneity allow.
Given that animal experiments are highly prone to heterogeneity in animal strains, modeling protocols, acupuncture parameters, and assessment techniques, the primary quantitative analysis will employ a random-effects model. Inter-study heterogeneity will be evaluated using the I^2 and tau^2 statistics. Sensitivity analysis will be performed by comparing the results of the random-effects and fixed-effect models to evaluate the robustness of the study conclusions.
When the evidence network is connected and data conditions permit, a frequentist network meta-analysis (NMA) will be conducted to integrate both direct and indirect evidence, comparing the relative effects of different acupuncture therapies. The primary statistical analyses will be completed using R software and the netmeta package (employing key functions such as netmeta()). P-scores (the frequentist analog of the Surface Under the Cumulative Ranking [SUCRA] curve) will be calculated using the netrank() function to rank the relative efficacy of different interventions.
Behavioral outcomes (SPT, FST, OFT) and neurobiological mechanism biomarkers (5-HT, BDNF, TNF-α, IL-6, CORT, SOD, MDA) will be analyzed separately. They will be synthesized according to specific outcomes; different types of behaviors or distinct pathways will not be merged into a single composite outcome. If the number of studies for a specific outcome or node is insufficient, or if the evidence network is disconnected, pairwise meta-analysis or narrative synthesis will be performed.
Local inconsistency in the network layout will be evaluated using the node-splitting method via the netsplit() function. Global inconsistency will be assessed using the design-by-treatment interaction model via the decomp.design() function. If ge 10 studies are included for a specific outcome, potential publication bias or small-study effects will be evaluated using comparison-adjusted funnel plots via the funnel.netmeta() function.

Subgroup analysis If sufficient data are available, subgroup analyses will be conducted to explore potential sources of heterogeneity and to test our translational medical hypotheses. The subgroup analyses will be stratified according to the following biological, intervention-related, and methodological covariates:
Modeling Pathological Features (Model Specificity): Stratified based on the distinct pathophysiological components simulated by different modeling protocols: pure stroke models (e.g., MCAO, photothrombosis) simulating "primary focal neural injury and localized neuroinflammation" pathological features, and stroke-stress composite models (e.g., MCAO+CUMS) simulating "superimposed chronic stress and neuroendocrine dysregulation" pathological features.
Acupuncture Parameters: Specific acupuncture modalities (Manual Acupuncture [MA] vs. Electroacupuncture [EA] vs. Warm Needle Acupuncture [WNA] vs. Scalp Acupuncture [SA] vs. Moxibustion [Mox]); treatment duration (long-term

ge 14 days vs. short-term < 14 days); and electrical stimulation parameters (high frequency ge 50 Hz vs. low frequency < 50 Hz).
Animal Characteristics: Animal species (Rats vs. Mice) and strains (e.g., Sprague-Dawley vs. Wistar; C57BL/6 vs. ICR).
Methodological Quality: Low risk of bias vs. high or unclear risk of bias based on key SYRCLE domains (e.g., sequence generation, blinding of outcome assessors).

Sensitivity analysis Sensitivity analyses will be performed to evaluate the robustness and stability of the pooled effect sizes and to identify potential outlier studies. The following strategies will be employed:
Alternative Statistical Model: Comparing the pooled results obtained from the random-effects model with those from the fixed-effect model. Significant discrepancies between the two models will prompt a critical re-examination of potential clinical and methodological heterogeneity. This will be assessed using the following methods:
Leave-One-Out Approach: Systematically omitting one study at a time and re-calculating the pooled effect size to evaluate whether the overall findings are driven by any single study or outlier.
Exclusion of High-Risk Studies: Excluding studies assessed as having a high risk of bias in key SYRCLE domains (e.g., lack of randomized allocation or blinding) to determine if study quality significantly impacts the stability of the pooled estimates.
For outcomes exhibiting significant heterogeneity (e.g., I2 > 50%) or unstable results during sensitivity checks, further exploratory analyses of study characteristics (e.g., animal strain, modeling protocols, or acupuncture parameters) will be conducted to identify potential sources of heterogeneity and verify the reliability of the evidence.

Language restriction Chinese and English.

Country(ies) involved China.

Other relevant information Non

Keywords post-stroke depression; acupuncture; electroacupuncture; moxibustion; animal model; mechanisms; systematic review; network meta-analysis.

Dissemination plans The results of this study will be submitted to a peer-reviewed academic journal for publication and may be presented at relevant academic conferences.

Contributions of each author
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