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Efficacy and Mechanisms of Non-Pharmacological Interventions for Post-Stroke Cognitive Impairment in Animal Models: A Systematic Review and Meta-Analysis

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Peng, WQ; Zhang, YX;Tang, XR; Liu, JH.

Corresponding author:
Xiaorong Tang

tangxr@gzucm.edu.cn

Author Affiliation:
Guangzhou University of Chinese Medicine, Guangzhou, China.

ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202590009

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 4 September 2025 and was last updated on 4 September 2025.

INTRODUCTION

Review question / Objective P – Population
Laboratory animals (e.g., rats, mice) with experimentally induced post-stroke cognitive impairment (resulting from cerebral ischemia or intracerebral hemorrhage). Animal models that received sham surgery only will be excluded.
I – Intervention
Any non-pharmacological intervention. Examples include: exercise training (voluntary wheel running, treadmill running), environmental enrichment, acupuncture (body acupuncture, electroacupuncture, scalp acupuncture), physical therapy (transcranial magnetic stimulation, transcranial direct current stimulation), music therapy, cognitive training, etc. Interventions may be initiated at any time point after stroke induction.
C – Comparison
The control group should consist of stroke model animals receiving standard housing conditions and/or a placebo/sham intervention (e.g., sham acupuncture, non-acupoint stimulation, locked running wheel).
O – Outcomes
Primary outcome: Performance in behavioral tests of cognitive function (e.g., escape latency or time s.

Rationale Post-stroke cognitive impairment (PSCI) is a common and devastating sequelae of stroke, severely affecting patients' quality of life and functional independence. However, there is a lack of universally effective pharmacological treatments for PSCI. Non-pharmacological interventions (e.g., exercise, environmental enrichment, acupuncture) represent an attractive alternative or adjunctive strategy due to their high safety profile and potential for multi-targeted therapy.

Animal models serve as a crucial platform for investigating potential interventions for PSCI and their underlying mechanisms. While a substantial number of preclinical studies have explored the effects of various non-pharmacological interventions on animal models of PSCI, the results are heterogeneous, and individual studies are often limited by small sample sizes and insufficient statistical power.
Therefore, a rigorous systematic assessment and quantitative synthesis of the existing evidence through a systematic review and meta-analysis is imp.

Condition being studied Post-stroke cognitive impairment in animal models.

METHODS

Search strategy We will search the following electronic databases from their inception to September 2025:
PubMed
Embase (via Ovid)
Web of Science Core Collection
Cochrane Library (primarily CENTRAL)
China National Knowledge Infrastructure (CNKI)
Wanfang Data
Chongqing VIP Database (CQVIP)
China Biology Medicine
The search strategy will utilize a combination of controlled vocabulary (e.g., MeSH in PubMed, Emtree in Embase) and free-text terms related to:
Population (P): stroke, animal models
Intervention (I): non-pharmacological interventions
Outcome (O): cognitive impairment
Study design (S): animal studies, controlled trials
Boolean operators (“OR” within concepts, “AND” between concepts) and field tags (e.g., [tiab], [mesh]) will be employed. No language restrictions will be applied. The reference lists of included articles will be hand-searched for additional eligible studies.
Sample Search Strategy for PubMed:
#1 "Stroke"[Mesh] OR stroke[tiab] OR "Brain Ischemia"[Mesh] OR brain ischemia[tiab] OR cerebral ischemia[tiab] O.

Participant or population Laboratory animals (e.g., rats, mice) with experimentally induced post-stroke cognitive impairment (resulting from cerebral ischemia or intracerebral hemorrhage). Animal models that received sham surgery only will be excluded.

Intervention Any non-pharmacological intervention. Examples include: exercise training (voluntary wheel running, treadmill running), environmental enrichment, acupuncture (body acupuncture, electroacupuncture, scalp acupuncture), physical therapy (transcranial magnetic stimulation, transcranial direct current stimulation), music therapy, cognitive training, etc. Interventions may be initiated at any time point after stroke induction.

Comparator The control group should consist of stroke model animals receiving standard housing conditions and/or a placebo/sham intervention (e.g., sham acupuncture, non-acupoint stimulation, locked running wheel).

Study designs to be included Randomized controlled animal trials (RCTs) will be included.Definition: Studies must report the use of a random method (e.g., random number table, computer-generated randomization) to allocate animals to experimental or control groups.Excluded study types: Non-randomized studies, reviews, meta-analyses, case reports, conference abstracts, comments, and study protocols will be excluded.

Eligibility criteria Inclusion Criteria:
Types of Studies: Randomized controlled animal trials (RCTs). Studies will be included if they are described as "randomized" or use a method of random allocation (e.g., random number table).
Types of Subjects: rats or mice models of ischemic or hemorrhagic stroke induced by any established method (e.g., MCAO suture occlusion, photothrombosis, endothelin-1-induced, autologous thromboembolism) that report the presence of cognitive impairment (confirmed by behavioral tests).
Types of Interventions: Any non-pharmacological intervention aimed at improving cognitive function. Examples include: exercise training, environmental enrichment, acupuncture/electroacupuncture, physical therapy (e.g., rTMS, tDCS), music therapy, cognitive training, etc. Interventions can be single or combined.
Types of Comparators: Stroke model control groups receiving standard housing conditions, sham intervention (e.g., sham acupuncture, non-acupoint stimulation, locked running wheel), or placebo intervention.
Types.

Information sources To identify all potentially relevant studies, the following sources will be searched from their inception to September 2025:
Electronic Databases:
PubMed
Embase (via Ovid)
Web of Science Core Collection

Cochrane Library (Cochrane Central Register of Controlled Trials, CENTRAL)
China National Knowledge Infrastructure (CNKI)
Wanfang Data
Chongqing VIP Database (CQVIP)
China Biology Medicine
Trial Registries:
Animal Study Registry
Grey Literature and Supplementary Search Strategies:
The reference lists of all included studies will be hand-searched.
The reference lists of relevant systematic reviews will be examined.
We will attempt to contact experts in the field to inquire about unpublished data.
No language restrictions will be applied.

Main outcome(s) Cognitive function scores : escape latency,Time spent in the target quadrant in MWM, discrimination index in NOR.

Additional outcome(s) Potential mechanism indicators (e.g., expression of synaptic plasticity-related proteins (PSD-95, Synapsin-1); levels of neuroinflammatory cytokines (IL-1β, TNF-α); markers of neurogenesis (DCX, BrdU/NeuN double labeling); apoptosis-related proteins (Bcl-2, Bax)).Secondary: Neuroplasticity markers (BDNF, synaptophysin), infarct volume reduction.

Data management Data Extraction Process: Two reviewers will independently extract data using a pre-designed, piloted standardized data extraction form. Any discrepancies will be resolved through discussion or by consultation with a third reviewer.
Data to be Extracted: The extraction form will include the following items:
Study identification: first author, publication year, country, funding source, declaration of conflicts of interest.
Study characteristics: animal species, strain, age, weight, stroke model type, modeling method.
Intervention and comparator details: type of intervention, specific parameters (e.g., intensity, frequency, duration), time of initiation post-stroke, course of treatment, description of the control condition.
Outcome data: sample size, mean, standard deviation (SD), standard error of the mean (SEM), or 95% confidence intervals for all outcome measures in each group. If necessary, numerical data will be extracted from figures using software (e.g., WebPlotDigitizer).
Data for risk of bias assessment.

Quality assessment / Risk of bias analysis The methodological quality and risk of bias of included studies will be independently assessed by two reviewers using the SYRCLE's risk of bias tool (SYstematic Review Centre for Laboratory animal Experimentation). Any discrepancies will be resolved through discussion or by consulting a third reviewer.
This tool evaluates the following 10 key domains:
Sequence generation: Was the allocation sequence adequately generated and applied?
Baseline characteristics: Were the groups similar at baseline or appropriately adjusted for confounders?
Allocation concealment: Was the allocation sequence adequately concealed prior to and during assignment?
Random housing: Were animals randomly housed during the experiment?
Blinding of investigators: Were the caregivers and/or investigators blinded from knowledge of which intervention an animal received?
Random outcome assessment: Were animals selected at random for outcome assessment?
Blinding of outcome assessor: Was the outcome assessor blinded?
Incomplete outcome data: Were.

Strategy of data synthesis Data extraction will be performed independently by two reviewers using a pre-piloted form. Discrepancies will be resolved by consensus. Extracted data will include study characteristics, participant demographics, intervention details, comparator, outcomes, and risk of bias data.
We will first present a narrative synthesis summarizing the findings of all included studies. If studies are sufficiently homogeneous in terms of participants, interventions, and outcomes, we will perform meta-analysis using RevMan software. For dichotomous outcomes, we will calculate risk ratios (RR) with 95% confidence intervals (CIs). For continuous outcomes, we will calculate the standardized mean difference (SMD) with 95% CIs. A random-effects model will be used due to anticipated clinical heterogeneity.
Heterogeneity will be assessed using the I^2 statistic and Chi^2 test. An I^2 value greater than 50% will be considered as substantial heterogeneity. If substantial heterogeneity is present, we will explore potential sources through pre-specified subgroup analyses (e.g., by age group, intervention dosage, study design). Sensitivity analyses will be conducted by excluding studies with a high risk of bias. If more than 10 studies are included, publication bias will be assessed using funnel plots and Egger's test.
If meta-analysis is not appropriate due to significant heterogeneity, we will report the results narratively."

Subgroup analysis To explore the potential sources of heterogeneity and to investigate the specific conditions under which non-pharmacological interventions are most effective, we pre-specify the following subgroup analyses. These analyses will be conducted if a sufficient number of studies (e.g., at least 2 studies per subgroup) are available for each outcome：1. By Characteristics of the Animal Model:
Species and strain: e.g., Rats (Sprague-Dawley, Wistar) vs. Mice (C57BL/6, BALB/c).
Type of stroke model:
Ischemia model: Middle cerebral artery occlusion (MCAO) vs. other models (e.g., photothrombosis).
Ischemia vs. Hemorrhage: Models of ischemic stroke vs. models of hemorrhagic stroke.
2. By Characteristics of the Intervention:
Type of non-pharmacological intervention: This is a key subgroup analysis given our research focus. We will compare:
Physical Exercise (e.g., treadmill running, voluntary wheel running) vs Control。
Cognitive Training (e.g., Morris water maze training, other learning paradigms) vs Control.
Neuromodulati.

Sensitivity analysis To assess the robustness of our findings, we will perform the following sensitivity analyses for our primary outcomes:
By risk of bias: Repeating the analysis excluding studies with an overall 'high risk of bias'.
By statistical model: Comparing results from the random-effects model with those from a fixed-effect model.
By study design: Excluding non-randomized controlled studies (if any are included) to ensure findings are driven by rigorous designs.
By outcome metrics: For cognitive function, comparing results when different behavioral tests (e.g., Morris Water Maze vs. Barnes Maze) are analyzed separately, if data permits.
By missing data: Assessing the impact of imputed data (e.g., for missing standard deviations) by comparing results with and without imputation.
The results of the sensitivity analyses will be considered to alter the robustness of the evidence if the direction of effect, statistical significance, or effect size (e.g., SMD changing by >0.5) is meaningfully changed.

Language restriction No, we will not impose any language restrictions.

Country(ies) involved China.

Other relevant information "We will explicitly note whether included studies reported approval from an institutional animal ethics committee and complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. While this will not be an exclusion criterion, it will be considered in the discussion of the findings' reliability."
"As cognitive outcomes are often measured at multiple time points post-intervention, we will pre-specify a primary time point of interest (e.g., immediately at the end of the intervention) for our main analysis.
"In addition to efficacy outcomes, we will systematically extract and narratively synthesize data on potential biological mechanisms investigated in the included studies (e.g., synaptic plasticity markers like BDNF, synapsin-I; neurogenesis; angiogenesis; neuroinflammation; apoptosis). This will provide a mechanistic context for the behavioral findings."
"Our review team includes native Chinese speakers, allowing for the inclusion and accurate data extraction from studies published in Chinese. For studies in other languages, we will use translation software and, if necessary, seek assistance from colleagues or professional services."

Keywords Post-Stroke Cognitive Impairment; Non-Pharmacological Interventions; Animal Models; Systematic Review; Meta-Analysis; Cognitive Function; Neuroplasticity.

Dissemination plans Our primary plan is to publish the full findings in a peer-reviewed journal focused on stroke, neuroscience, or translational medicine. We will also present key results at relevant academic conferences. To ensure rapid dissemination, we may share a preprint via bioRxiv/medRxiv. We are committed to open science and will share the extracted data on a public repository . Finally, we will update this INPLASY record with the publication DOI.

Contributions of each author

Author 1 - Wenqing Peng - Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization.
Email: pengwenqinggnep@outlook.com
Author 2 - Yixin Zhang - Investigation.
Email: 2023051082@stu.gzucm.edu.cn
Author 3 - Xiaorong Tang - Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition.
Email: tangxr@gzucm.edu.cn