

# Effects of different PTSD modeling methods on fear and depressive behavior in rats: a protocol for systematic review and network meta analysis

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**Protocol**

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# Abstract

**Background:** Posttraumatic stress disorder (PTSD) is a debilitating mental disorder that occurs after exposure to traumatic events. This disorder can result in a interference to personal and family functioning, causing great threat to people's life and public health. Animal models remain the effective tools to the pathophysiological study of PTSD, There are many model paradigms of PTSD, and the purpose of this study is to evaluate the effect of different model paradigms of PTSD on depression-like and fear-like behavior using systematic review and network meta-analysis (NMA).

**Methods:** The authors will retrieve a total of seven electronic databases by April 2020. After a series of screening, the two researchers will use Aggregate Data Drug Information System (ADDIS) and Stata software to analyze the data extracted from randomized controlled animal studies in PTSD rats . Ultimately, the evidence grade of the results will be evaluated.

**Discussion:** The results of this study will provide references for evaluating the influence of different rats models of PTSD on depression-like and fear-like behavior, and provide decision-making references for clinical practice.

**Systematic review registration:** PROSPERO CRD42020182389.

## 1. Background

Posttraumatic stress disorder (PTSD) is a trauma-related disorder that occurs when symptoms develop after exposure to aversive details of, a potentially one or more traumatic events, such as death, war, sexual assault or serious injury [1]. PTSD affects many individuals in the general population, studies have shown that 60.7% of men and 51.2% of women may be exposed to traumatic events that lead to the development of PTSD [2, 3]. Previous studies have revealed that an estimated lifetime prevalence of PTSD of 3.9% in the general population and 5.6% among persons who have previously experienced traumatic event [4, 5], in addition, up to half of those diagnosed with PTSD tend to show persistent symptoms and an unremitting chronic course for years and decades. Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) classifies the symptoms of PTSD within three symptom clusters, re-experiencing of the trauma, persistent avoidance, and hyperarousal or physiologic reactivity [1]. Individuals with PTSD is often comorbid with depression, anxiety disorders, and substance abuse, meanwhile, those people are at risk for reduced quality of life, suicide, reduced productivity, domestic violence and damaged relationships, which resulting in a severe burden on public health [6–8]. The mechanisms leading to PTSD have not yet been fully understood, current research suggests that the pathogenesis of PTSD is multifactorial, including the activation of the hypothalamic–pituitary–adrenal (HPA) axis, regulation of neuroendocrine and immune systems, or even genetic factors, furthermore, the morphological changes in subcortical structures may also associated with PTSD symptoms [9, 10].

Despite the high complexity of human psychopathology, animal models of mental illness remain the best tool, which will help to understand the neural basis of human pathology and provide a preclinical

assessment for new treatments [11]. Animal models provide an important approach for the pathophysiological study of PTSD, because the acquisition of PTSD in human is incidental and the nature of the trauma is highly variable, furthermore, inducing PTSD in healthy volunteers is not ethically viable. However, animal models can circumvent these shortcomings in human research, First of all, researchers can manipulate all aspects of the stressor including the type, timing, and intensity in animal models; Secondly, animal studies allow for pre-exposure and immediate post-exposure interventions and manipulations; Finally, animal model studies enable the assessment of concomitant biomolecular changes in dissected brain areas and pharmacological experimentations with potential therapeutic effects [12, 13]. An ideal animal models of PTSD should meet the following criteria of validity including[14, 15]: (1) Face validity—the model reproduces symptoms associated with the human syndrome such as re-experiencing traumatic memories, hypervigilance and emotional numbness, and these symptoms can assess the animal's damage or recovery; (2) Construct validity—usually judged by stress response, neurological changes and comorbidity, for instance, Elevated Plus Maze (EPM) test as a measure of anxiety; (3) Predictive validity—the animal's core symptoms may respond to the treatment of the drug (Such as selective serotonin reuptake inhibitors). In the above criteria of validity, PTSD symptoms is a critical aspect, Depression-like behavior and fear-like behavior are the two most common symptoms cluster in animal models of PTSD, which parallel aspects of traumatic stress-induced behaviors in people, it can be detected using a variety of classical behavioral experiments, including EPM, open field test and startle response test, therefore, the animal level of depression and fear through behavioral testing can reflect the effectiveness of the animal model to some extent. There is no single generally accepted model of PTSD, although several stress paradigms of PTSD have been built. The present model varies according to the induction of traumatic stressors, including physical stressors: footshock, underwater trauma (UWT), restraint stress, single prolonged stress(SPS); Social stressors: housing instability, early life stress; Psychological stressors: Predator scent stress(PSS). [16–18].

So far, there has been no NMA of the differences between different PTSD animal models. We will carry out direct or indirect comparative analysis by using the method of NMA in the relevant randomized controlled trials (RCTs). Hopefully the best animal model paradigm of PTSD can be obtained, so as to provide references for the further study of PTSD mechanisms and clinical practice.

## **2. Objectives**

Currently, SPS and its improved method, restraint and electric shock, PSS and UWT are the most common PTSD modeling paradigms, but their differences and advantages have not been quantitatively evaluated. The purpose of this NMA is to evaluate the evidence for the effectiveness of different PTSD modeling paradigms in creating models of fear and depression by comparing the above commonly used PTSD modeling paradigms.

## **3. Methods**

### **3.1. Protocol and registration**

This protocol following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) guidelines [19]. To improve transparency and completeness, a completed PRISMA-P 2015 checklist is provided [see Additional file 1]. This study has been registered in International Prospective Register of Systematic Reviews (PROSPERO) in the registration, registration number: CRD42020182389, (<https://www.crd.york.ac.uk/prospero/>).

## **3.2 Ethics**

This research does not require ethical approval.

## **3.3. Eligibility criteria**

The participant (P), intervention (I), comparator (C), outcome (O), and study design (S) are the 5 main factors determining the inclusion and exclusion criteria of this research.

### **3.3.1. Type of participant**

Adult Sprague-Dawley (SD) rats (SPF grade) without sex or weight restriction.

### **3.3.2. Type of interventions and comparators**

Different PTSD modeling methods are used as intervention factors. The methods are built based on SPS and its improved method, restraint and electric shock, PSS, and UWT. The control group is a blank control group.

### **3.3.3. Type of outcomes**

The behavior test is carried out by open field test, EPM and foot shock experiment. The outcome indexes include: horizontal span number and vertical number measured in open field test; open arm residence time and open arm entry times measured in EPM; freezing time measured by conditioned fear reaction test.

### **3.3.4. Study design**

Studies must be RCTs that include different interventions and satisfy the selection principle of P, I, C and O. If the above four criteria are met at the same time, the study is included. Case reports, cross-over studies, studies without individual controls, preliminary studies, systematic evaluations, incorrect data, duplication, and unclear literature will be excluded.

## **3.4. Literature retrieval strategy**

Computer retrieval of published RCTs of PTSD rats study is conducted in PubMed, the Cochrane Library (issue 4, 2020), EMBASE, China National Knowledge Infrastructure (CNKI), China Biological Medicine (CBM), Chinese Scientific Journals Database, and wan-fang databases. The time limit of document retrieval is from the establishment of each database to April 30, 2020. The language is limited to Chinese and English. In addition, inclusive literature from the field and references from previous evaluations will

be manually retrieved to find other potentially relevant articles. Chinese search terms mainly include: "post-traumatic stress disorder", "rat model"; English search words include "post-traumatic stress disorder", "PTSD", "rats", "animal models", etc. Taking PubMed as an example, the initial retrieval strategy is shown [see Additional file 2] and will be adjusted according to the specific database.

### 3.5. Literature selection and data extraction

The study selection program will follow the Prisma guidelines, KS and FJX will independently screen literatures according to inclusion and exclusion criteria: (a) The retrieved literatures will be imported into Endnote X9 software for rechecking, and duplicate references are removed; (b) By reading the title and preliminary screening the abstract, exclude the literature that obviously does not meet the inclusion criteria; (c) Download and read the full text for re-screening; (d) After the final inclusion, the pre-designed data extraction table is used for data extraction, and the results will be cross-checked; (e) If there is any disagreement, the third researcher ALH will be asked to assist in the judgment. The main content of data extraction includes: basic information of literature (title, journal, author, publication date), basic situation of the research object, and the extraction of the outcome indicators are continuous variable, and expressed as a mean and standard deviation, respectively. At the same time, the key factors of bias risk assessment are extracted.

## 3.6. Quality assessment/methodological quality of included studies

The methodological quality of systematic review reflects the risk of bias or validity in its process and results. Methodological quality will be assessed based on the SYRCLE bias risk assessment tool. Two trained researchers KS and ALH will independently evaluate the risk of bias of the included studies. In case of dispute, submit to corresponding author HZ for arbitration.

SYRCLE's bias risk assessment tool will be used to assess the risk of RCTs being included in NMA for 10 items, including [20]: (a) How to generate the assignment sequence of experimental animals? Is the application correct? (b) Is the baseline of each group comparable? Or are the potential confounders adjusted? (c) Whether to hide the experimental animal grouping scheme? (d) In the course of the experiment, are the experimental animals (cages) placed randomly? (e) During the experiment, in order to avoid the breeders and researchers of experimental animals from knowing what kind of intervention the experimental animals receive, should they be blinded? (f) In the process of evaluating experimental results, are experimental animals randomly selected? (g) In the evaluation of experimental results, whether to blind the results evaluators? (h) Does the analysis of the final measurement index include all the experimental animals? If the data from experimental animals were not included in the analysis, are there possible reasons why reporting missing data would not affect the authenticity of the measurements? (i) Are possible conditions and relevant information of alternative reporting measures reported? (j) Are other possible causes of bias reported? The 10 items were judged in the form of "yes", "no" and "unclear". "Yes" means low bias and low risk; "No" represents high bias and high risk; the degree

to risk which "not clear" represents is uncertain. Finally, the evaluation results of all included original literature are presented in the form of text, table or graph.

## **3.7. Data synthesis and statistical methods**

### **3.7.1. Network meta-analysis**

This study uses ADDIS 1.16.8 for NMA [21]. ADDIS software uses markov chain - monte carlo (MCMC) algorithm to for priori evaluation and processing the extracted data based on bayesian framework, so as to provide support for further research and decision. Preset model parameters: 4 chains are used for simulation analysis, with an initial value of 2.5, a step size of 10, 20,000 annealing times, and 50,000 simulation iterations. Firstly, the network evidence plot is generated according to different outcome indicators (horizontal span number, vertical number, open-arm residence time, open-arm entry number, and freezing time), and MD is used as the effect quantity and 95% CI is used for statistical analysis. According to the results of the NMA, rank probability plot of various modeling methods is generated and sorted by dominance, with Rank1 being the optimal sort.

### **3.7.2. Statistical model selection**

Node-split model is used to verify the consistency of the corresponding data. If there is no statistical difference ( $P > 0.05$ ) between direct comparison and indirect comparison, the consistency model is used, whereas the inconsistency model is used for analysis. If the consistency model is adopted, then the stability of the results is verified by the inconsistency model: when the inconsistency factors including 0, at the same time inconsistency standard deviation including 1 says the result of consistency model is more stable and reliable. At the same time, various analysis models are iterated with preset parameters, and the convergence of iteration effect is judged by potential scale reduced factor (PSRF). When the PSRF value is close to or equal to 1 ( $1 \leq \text{PSRF} \leq 1.05$ ), the convergence is complete, the model has good stability, and the conclusion of analysis is reliable. If the PSRF value is not in this range, the iteration continues manually until the PSRF value reaches the range standard.

### **3.7.3. Heterogeneity test**

Before the combination of effect size, the heterogeneity of the included literature is tested using Stata. When inter-study heterogeneity exists, the random effect model is used. For comparison of each pair, heterogeneity is assessed by the statistic  $I^2$  value. When  $I^2 > 50\%$ , it indicates that there is heterogeneity between studies, and the source of heterogeneity should be further searched. When  $I^2 < 50\%$ , inter-study heterogeneity is considered to be small or there is no obvious heterogeneity.

### **3.7.4. Sensitivity analysis**

If necessary, the sensitivity analysis will be used to assess the effect of each study on the random effects model. The sensitivity of the general combined effect of all outcome indicators is analyzed by the exclusion method. That is, each study is excluded, and the remaining studies will be re-analyzed to

identify the stability of the results. If there is no qualitative change in the combined effect showed in the results, the results are stable.

### **3.7.5. Subgroup analysis**

If necessary, we will conduct a subgroup analysis of rat age, body weight and time of different modeling methods.

### **3.7.6. Small sample effect/publication bias**

If 10 or more studies are included in the NMA, a comparison-adjusted funnel plot is developed using Stata to evaluate the presence of small sample effects or publication bias in the intervention network. Descriptive analysis will be carried out through the symmetry of funnel plot. If the plot is asymmetric and there is no inverted funnel shape, it indicates that there may be publication bias. This may be related to the difficulty in the publication of the literature with negative results and the low quality of the inclusion methods.

### **3.7.7. Dealing with missing data**

If the required data is lost or incomplete, we will contact the corresponding author of the original document or the relevant email address of the first author. If there is no response, the record is excluded.

## **4. Discussions**

PTSD is excessive physical and mental stress caused by a major traumatic event, characterized by tardiness and persistence, or even lifelong onset. Humans are being severely tested by the COVID-19, and there is also the possibility of PTSD. Animal modeling is a widely used way of studying PTSD without the need for actual victims, and any findings in the model can provide scientists with predictions and some valuable ideas [22].

Over the past few decades, a variety of PTSD models have been established, including SPS, restraint and foot shock, PSS, UWT, etc. Although the stressors of the above paradigm are different, including single stressors, compound stressors, psychological stressors and social stressors. Each paradigm can only exhibit part of symptoms of PTSD, and still cannot fully reveal the full spectrum of symptoms. For example, Ardi et al [23] confirmed the consistency between the rats with PTSD phobia and clinical patients by using UWT model. However, Zoladz et al [24] suggested that PSS model may be more consistent with the symptoms of long-term traumatic memory and chronic PTSD. Since the usefulness of animal models of PTSD depends on their ability to reproduce the human syndrome, it is still worth exploring whether different rats models of PTSD exhibit differences in depression-like and fear-like behavior.

Therefore, it is one of the emphases for the current PTSD mechanism research to compare the advantages of various rats model paradigms and to explore the most effective modeling methods that are most consistent with the physiological and pathological changes of human PTSD. Through the

systematic review and NMA method, this study analyzes and compares various commonly used PTSD rats model paradigms, hoping to provide an advantageous scheme for the selection of modeling methods in animal experiments of PTSD and provide clues for clinical research, so as to further explore the evidence of its effectiveness in humans.

## Abbreviations

PTSD: Post-traumatic stress disorder, DSM = Diagnostic and Statistical Manual of Mental Disorders, SPS = single prolonged stress, PSS = Predator scent stress, UWT = Underwater trauma, SD = Sprague-Dawley, EPM = Elevated Plus Maze, NMA = Network Meta analysis, PROSPERO = International Prospective Register of Systematic Reviews, RCTs = randomized controlled trials, CBM = China Biological Medicine, CNKI = China National Knowledge Infrastructure, ADDIS = Aggregate Data Drug Information System, PSRF = potential scale reduced factor

## Declarations

## Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

KS and FJX made the same contribution to the research and design, and wrote the original draft of the protocol. The search strategy has been developed by KS and FJX. KS and FJX will conduct literature retrieval and collation. KS and ALH will evaluate the risk of bias in the literature. Data analysis and article

writing will be done by KS, FJX. HZ, as the corresponding author, will be responsible for overseeing every process of the audit review to control the quality of the study. All authors read and approved the final manuscript.

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Not applicable.

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