Meta-Analysis

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
50(2) 1–26

DOI: 10.1177/03000605221075816
journals.sagepub.com/home/imr
SAGE

Validity of chronic restraint stress for modeling anhedonic-like behavior in rodents: a systematic review and meta-analysis

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Abstract

Background: Chronic restraint stress (CRS) is widely used to recapitulate depression phenotypes in rodents but is frequently criticized for a perceived lack of efficacy. The aim of this study was to evaluate anhedonic-like behavior in the CRS model in rodents by performing a meta-analysis of studies that included sucrose preference tests.

Methods: This meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. We comprehensively searched for eligible studies published before June 2021 in the PubMed, Embase, Medline, and Web of Science databases. We chose sucrose preference ratio as the indicative measure of anhedonia because it is a core symptom of depression in humans.

Results: Our pooled analysis included 34 articles with 57 studies and seven rodent species/strains and demonstrated decreased sucrose preference in the stress group compared with controls. The duration of CRS differentially affected the validity of anhedonic-like behavior in the models. Rats exhibited greater susceptibility to restraint stress than mice, demonstrating inter-species variability.

Conclusions: Our meta-analysis of studies that used the CRS paradigm to evaluate anhedonic-like behavior in rodents was focused on a core symptom of depression (anhedonia) as the main endpoint of the model and identified species-dependent susceptibility to restraint stress.

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Keywords
Depression, chronic restraint stress, sucrose preference, anhedonia, validity, meta-analysis

Date received: 3 August 2021; accepted: 5 January 2022

Introduction

Depression is currently among the top five leading causes of the global disease burden, affecting 20% of the world's population.[1,2] According to the World Health Organization, over 300 million people suffer from major depressive disorder (MDD) worldwide.[3] Depression is a mood disorder characterized by a depressed mood, social isolation, anhedonia, and feelings of worthlessness that negatively influence overall quality of life, sometimes even causing patients to endanger their lives through recurrent suicidal thoughts.[4,5] Depression represents a chronic and recurrent psychiatric condition with varying symptoms among patients. Patients with chronic diseases have a higher risk of depression, which in turn reduces recovery from chronic diseases and treatment compliance. Depression not only imposes a large healthcare and economic challenge on society but also presents considerable social impacts. MDD is now the main risk factor for suicide-related deaths and the second leading cause of disability worldwide.[5] Unfortunately, 30% to 50% of patients suffering from depression do not respond to current antidepressant treatments.[6] Stress, or psychological stress, is a reaction mode. When the human body is stimulated by external adverse factors it will trigger stress reactions (anxiety, depression, fear, and other adverse emotions). Chronic stress, also called long-term stress, means that the stress process and event that cause stress will last longer.[7] It has been recognized that physiological responses to chronic stress are potent modulators of immune, endocrine, and metabolic pathways.[8] Chronic stress is a significant risk factor for the development of depression, which leads to synaptic changes and depressive-like behaviors in rodents. Currently, chronic stress models are the most widely used animal models of depression.[9]

It is difficult to determine what the underlying mechanisms of MDD might be in human studies. In contrast, animal studies allow the experimental induction of depression-relevant behaviors, which permits deeper investigations into molecular pathways. Thus, modeling depression in animals is vital for uncovering mechanisms underlying the human condition. Great progress has been made over the past 50 years in elucidating the pathophysiology of depression, much of which is attributable to the implementation of numerous animal models of depression.[2,10,11] Most of the current knowledge about the mechanisms underlying depression has come from animal models, although no animal model can be entirely congruent with the human condition. Chronic psychosocial stressors are risk factors for the development of depression in humans.[12,13] Chronic stressors are detrimental because they disrupt the normal stress response of the brain, eventually contributing to the development of depression.[14–16] Additionally, chronic stressors enhance levels of stress-related hormones by disrupting the hypothalamic-pituitary-adrenal (HPA) axis and suppress the production of new neurons in the hippocampus.[16–18]

Several chronic stress models including chronic social defeat stress (CSDS), chronic restraint stress (CRS), and chronic unpredictable mild stress (CUMS) have been shown to recapitulate depression-like behaviors in rodents, and thus have been used to model depression and investigate its underlying mechanisms. Depression-like behaviors induced by the animal models have been examined including by the sucrose preference test (SPT; indicative of anhedonia) and forced swim and tail suspension test (indicative of despair). Changes in the performance of model animals in these tests can often be reversed by chronic antidepressant treatments.[19] However, it is noteworthy that stress designs in the model contribute to stress susceptibility. Anhedonia is a decreased ability to experience pleasure that is recognized as a core symptom of human depression. SPT is widely applied as a behavioral measure of anhedonia.[20] Experimental animals are given a free choice between drinking water or a weak sucrose solution (1%–2% [weight/volume] sucrose)[8] and exhibit a preference for the latter, reflecting the hedonic state of rodents.

The CRS model is a convenient, inexpensive, and stable rodent model of chronic stress because of its relative simplicity and easy workflow; therefore, it is widely used to establish depression rodent models.[2] Previous publications have used many strains of rats and mice to establish the CRS model. Additionally, the restraint duration, intensity, and other conditions have been varied across different studies. Some studies have reported that exposure to CRS induced anhedonia in rodents on the basis of decreased sucrose preference, a core symptom of human depression.[21,22] In contrast, a conflicting study reported that CRS failed to induce anhedonia-like behaviors.[23] Thus, it remains unclear whether CRS can be used as a valid animal model of depression that recapitulates anhedonia-like behavior in different rodent species and strains.

Systematic reviews and meta-analyses, as standard practices in clinical research, have been increasingly performed to validate preclinical studies of disease etiology, diagnosis, and prognosis. In terms of animal experiments, it has been estimated that approximately 50% of published results are not reproducible, which has been described as a "replication crisis".[2] However, few pooled analyses have been conducted within basic life science research to evaluate the reliability of results. The aim of this study was to evaluate the anhedonic-like behavior induced by the CRS model in rodents by performing a meta-analysis of studies that reported SPT results.

Methods and materials

Search strategy

The meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. We comprehensively searched for eligible studies published before June 2021 in the PubMed, Embase, Medline, and Web of Science databases. We searched for the following keywords and corresponding terms in titles and/or abstracts: "chronic restraint stress" OR "chronic psychological stress" AND "animal model".

Eligibility criteria and study selection

All studies enrolled in this meta-analysis satisfied the following criteria: (1) published in English; (2) reported as original research; (3) reported the implementation of CRS protocols in rodents (mice or rats) for at least 1 week; (4) examined depressive-like behaviors including SPT (calculated according to the following formula: % sucrose preference = [sucrose intake/total

fluid intake] × 100); (5) provided SPT outcomes (%) in the text, figures, and/or graphs; and (6) used normal (wild-type) experimental animals that were housed in a suitable environment. Studies were excluded from the meta-analysis if they did not meet all of the above criteria. The selection of included studies was conducted independently by two authors (YM and YX). Discrepancies between the two authors were solved in face-to-face conferences with the third author (XY).

Data extraction

Two authors (YM and YX) independently extracted data from the included studies and any disagreements were settled in face-to-face consultations with the third author (XY). The authors summarized the main characteristics of the studies and collected all information regarding CRS design and SPT protocols. The following information was directly extracted from the selected studies: name of first author, publication year, model animal features (sex, strains), CRS model design (restraint stress duration, period/day), examined depression-like behaviors, measurement of water and food consumption, measurement of body weight, determination of corticosterone and catecholamine, details of SPT (test onset time, training protocols, water and food deprivation period, sucrose concentrations, testing period), and sample sizes (*n*) of the experimental and control groups. For the pooled analysis, SPT outcomes, including mean and standard error (SE), standard deviation (SD), or standard error of mean (SEM), were directly extracted from graphs or figures using Engauge Digitizer software.

Statistical analysis

We evaluated the efficacy and stability of the CRS protocol in modeling depressive-like behavior according on SPT results in model animals. Standardized mean differences (SMDs) with 95% confidence intervals were defined as the indicator of efficacy, and the meta-analysis was performed by pooling mean sucrose preference (%) results, SD/SEM/SE of the mean, and sample size[2] using Stata software version 11.1 (STATA Corporation, College Station, TX, USA). SMD is a measure of effect size that reflects the degree of outcomes in the experimental (stressed) group differing from that of the controls (calculated according to the following formula: $SMD = (M_1–M_2) \div SD$, where $M_1–M_2$ is the difference in the means of the two groups, and SD is the pooled and weighted standard deviation).[2] A fixed-effect model was adopted in the pooled analysis. Results of the meta-analysis are displayed as forest plots.

The Higgins I^2 statistic was used to estimate the heterogeneity among the enrolled studies. This statistic represents the percentage of variation between studies ranging from 0% to 100%. A P value ≤ 0.1 or $I^2 \geq 50\%$ indicates substantial statistical heterogeneity between studies. Publication bias was assessed using a funnel plot (a visual aid for detecting bias). The effect measure (log|SMD|) versus its precision (SE of log|SMD|) was plotted in the funnel plot. In cases of absence of publication bias, the data are expected to be distributed in a funnel-shaped area in the plot.

Results

Literature search and study selection

The flowchart for identifying eligible articles for the meta-analysis is shown in Figure 1. The initial literature search in the PubMed, Embase, Medline, and Web of Science databases yielded a total of 2217 distinct articles. Subsequently, 1761 articles were excluded on the basis of their abstracts, and the full-texts of the

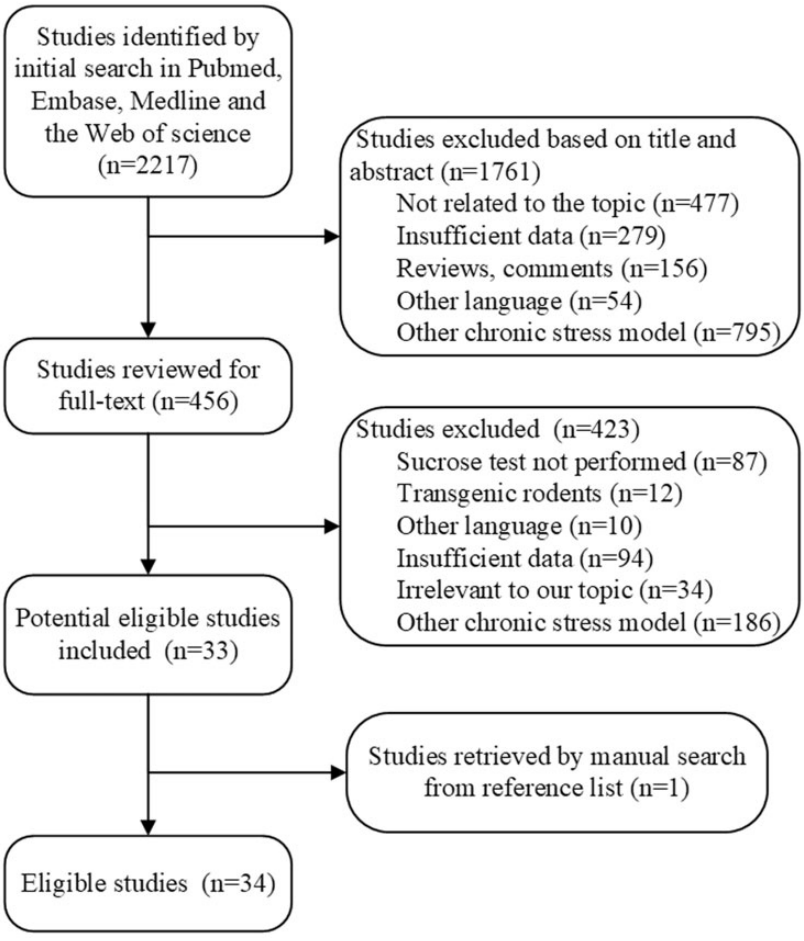

Figure 1. Flow chart of selection process for eligible studies.

remaining 456 articles were reviewed. Ultimately, 33 articles were selected. One article was identified by manually checking reference lists, and therefore a total of 34 articles[20–22,24–54] were enrolled in this meta-analysis.

Study characteristics

The pooled analysis involved 57 studies in the 34 enrolled publications according to different CRS model designs and included seven rodent species/strains (i.e., Sprague–Dawley (SD) and Wistar rats and Kunming, C57BL/6J, ICR, athymic nude, and BALB/c mice). An overwhelming majority of the studies established the CRS-induced depression model in male rodents, while only 3.5% of the studies (2/57) selected female rodents as the research subjects. Almost all studies successfully modeled depression by CRS on the basis of SPT results; however, different CRS designs (e.g., duration and intensity) and SPT protocols (e.g., test onset time, training protocols, water and food deprivation period, sucrose concentrations, and testing period) were used in the included

studies. Rodent characteristics and details of CRS designs are summarized in Table 1; details of SPT protocols are summarized in Table 2.

The validity of using CRS to model depression

Pooled analyses were performed based on the availability of mean, SE, SD, or SEM, and sample size (*n*) data for each stress and control group. SPT results were directly extracted from graphs or figures using a digitizing software and are shown in Table 3.

The pooled analysis of SPT results from the included studies indicated a significant induction of anhedonic-like behavior in CRS model groups of C57BL/6J mice (Figure 2), SD rats (Figure 3), Wistar rats (Figure 4), Kunming mice, ICR mice, athymic nude mice, and BALB/c mice (Figure 5). Further analysis indicated substantial statistical heterogeneity between studies. These results are summarized in Table 4.

The pooled analysis of SPT results demonstrated a stronger effect of restraint stress on rats than mice. Notably, the pooled analysis showed that SD rats (SMD = −3.956 [−4.286, −3.626], $p < 0.001$, $I^2 = 83.8\%$) and Wistar rats (SMD = −3.531 [−3.960, −3.102], $p < 0.001$, $I^2 = 80.0\%$) exhibited greater susceptibility to restraint stress than C57BL/6J mice (SMD = −2.80 [−3.221, −2.380], $p < 0.001$, $I^2 = 90.4\%$). Furthermore, the total effect size in SD rats was higher than in Wistar rats.

Additionally, the meta-analysis demonstrated differential sensitivity to restraint stress of varied durations. In C57BL/6J mice, the total effect size indicated the instability and invalidity in the induction of anhedonic-like behavior after 1 week of CRS exposure (SMD = −0.954 [−2.037, 0.128], $p = 0.084$, $I^2 = 97.1\%$). A longer CRS exposure protocol resulted in a sufficient effect size, with a higher SMD value found after 3 weeks (SMD = −3.389 [−4.122, −2.655], $p < 0.001$, $I^2 = 88.80\%$) than after 2 weeks (SMD = −2.396 [−3.196, −1.597], $p < 0.001$, $I^2 = 91.7\%$). Four weeks of CRS exposure (SMD = −3.613 [−4.467, −2.759], $p < 0.001$, $I^2 = 84.5\%$) resulted in a stronger behavioral effect than 3 weeks of CRS exposure. In SD and Wistar rats, only 1 week of exposure successfully recapitulated the anhedonic-like behavior according to the SPT results. Interestingly, a 10-day CRS protocol resulted in stronger behavioral effects than a 2-week protocol in SD rats. Similarly, the effect of 2-week CRS exposure was stronger than 3-week exposure in Wistar rats.

Regarding heterogeneity tests, the single group heterogeneity was low for SD rats after 10-day CRS exposure ($I^2 = 14.20\%$). However, high heterogeneity was observed in the other groups. In SD rats, heterogeneity in the single group with 3-week exposure ($I^2 = 86.5\%$) was higher than that of the group with 2-week exposure ($I^2 = 71.3\%$). For C57BL/6J mice, longer exposure protocols resulted in decreased heterogeneity (1-week: $I^2 = 97.10\%$; 2-week: $I^2 = 91.70\%$; 3-week: $I^2 = 88.80\%$; and 4-week: $I^2 = 84.50\%$).

Discussion

The CRS model is widely used to recapitulate depression features due to its relative simplicity. However, it is frequently criticized for its perceived lack of efficacy. We performed a meta-analysis of studies that used CRS protocols to evaluate anhedonic-like behavior in rodents. As the primary endpoint of this study, we attempted to identify strain-dependent susceptibilities to CRS on the basis of a core symptom of depression, anhedonia.

CRS is one of the most extensively used stress paradigms in laboratory animals to model human psychological stress. CRS

Table 1. Primary characteristics of the included studies.

Study	Sex	Animal strain	Restraint duration	Period/ day	Depression-like behavioral testing	Water and food consumption	Body weight	Corticosterone determination	Catecholamine determination
Yin et al., 2020	male	C57BL/6 mice	1 week	6 hours	sucrose preference test				
Yin et al., 2020	male	C57BL/6 mice	2 weeks	6 hours	sucrose preference test				
Yin et al., 2020	male	C57BL/6 mice	3 weeks	6 hours	sucrose preference test; forced swimming test				
Liang et al., 2015	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; open field test; elevated-plus maze; novel object recognition; object placement test		√	√	√
Kim et al., 2018	male	C57/BL6 mice	3 weeks	2 hours	sucrose preference test; forced swimming test; tail suspension test		√	√	
Castañeda et al., 2015	male	Sprague–Dawley rats	1 week	2.5 hours	sucrose consumption test		√		
Castañeda et al., 2015	male	Sprague–Dawley rats	2 weeks	2.5 hours	sucrose consumption test; active avoidance behavior; immobility; active behaviors (i.e., climbing and swimming)		√		
Hashikawa-Hobara et al., 2015	male	C57BL/6 mice	15 days	3 hours	sucrose preference test; open field test; forced swimming test			√	
Moreno et al., 2020	male	Sprague–Dawley rats	3 weeks	2 hours	sucrose preference test; forced swimming test; social interaction test		√		
Eiland et al., 2012	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; forced swimming test; elevated-plus maze			√	
Eiland et al., 2012	female	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; forced swimming test; elevated-plus maze			√	
Chiba et al., 2012	male	Wistar rats	1 week	6 hours	sucrose preference test	√	√		
Chiba et al., 2012	male	Wistar rats	2 weeks	6 hours	sucrose preference test	√	√		
Chiba et al., 2012	male	Wistar rats	3 weeks	6 hours	sucrose preference test	√	√		
Chiba et al., 2012	male	Wistar rats	4 weeks	6 hours	sucrose preference test; open field test; forced swimming test; elevated-plus maze	√	√	√	
Aboul-Fotouh et al., 2013	male	Wistar rats	1 week	6 hours	sucrose preference test	√	√		

(continued)

Table 1. Continued.

Study	Sex	Animal strain	Restraint duration	Period/ day	Depression-like behavioral testing	Water and food consumption	Body weight	Corticosterone determination	Catecholamine determination
Aboul-Fotouh et al., 2013	male	Wistar rats	2 weeks	6 hours	sucrose preference test	√	√		
Sawsan Aboul-Fotouh et al., 2013	male	Wistar rats	3 weeks	6 hours	sucrose preference test	√	√		
Aboul-Fotouh et al., 2013	male	Wistar rats	4 weeks	6 hours	sucrose preference test	√	√		
Tsuchimine et al., 2020	male	BALB/c mice	3 weeks	6 hours	sucrose preference test; forced swimming test; tail suspension test		√	√	
Tsuchimine et al., 2020	male	C57BL/6J mice	3 weeks	6 hours	sucrose preference test; forced swimming test; tail suspension test		√	√	
Cheng et al., 2017	male	Sprague–Dawley rats	9 weeks	6 hours	sucrose preference test; open field test		√		
Chen et al., 2020	male	C57BL/6 mice	2 weeks	4–8 hours	sucrose preference test; forced swimming test		√		√
Shilpa et al., 2017	male	Wistar rats	10 days	2 hours	sucrose preference test; open field test; forced swimming test; spatial learning and memory test; elevated-plus maze		√		
Wang et al., 2017	male	Sprague–Dawley rats	1 week	6 hours	sucrose preference test				
Wang et al., 2017	male	Sprague–Dawley rats	2 weeks	6 hours	sucrose preference test				
Wang et al., 2017	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test				
Wang et al., 2017	male	Sprague–Dawley rats	4 weeks	6 hours	sucrose preference test				
Liu et al., 2018	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; locomotor activity test; forced swimming test; open field test; elevated plus-maze		√		
Li et al., 2019	male	C57BL/6J mice	3 weeks	2 hours	sucrose preference test; forced swimming test; social interaction test; tail suspension test; novel object recognition			√	

(continued)

Table 1. Continued.

Study	Sex	Animal strain	Restraint duration	Period/ day	Depression-like behavioral testing	Water and food consumption	Body weight	Corticosterone determination	Catecholamine determination
Luo et al., 2015	male	Sprague–Dawley rats	10 days	4 hours	sucrose preference test; forced swimming test		√	√	
Pan et al., 2019	male	Sprague–Dawley rats	3 weeks	3 hours	sucrose preference test; open field test; forced swimming test	√	√	√	
Li et al., 2019	male	C57BL/6J mice	3 weeks	2 hours	sucrose preference test; forced swimming test; novelty-suppressed feeding; tail suspension test				
Zhu et al., 2019	male	C57BL/6J mice	4 weeks	6 hours	sucrose preference test			√	√
Zhao et al., 2017	male	Kunming mice	3 weeks	6 hours	sucrose preference test; open field test; forced swimming test; tail suspension test				
SH Park et al., 2018	male	C57BL/6J mice	1 week	3 hours	sucrose preference test; open field test; forced swimming test		√		
SH Park et al., 2018	male	C57BL/6J mice	2 weeks	3 hours	sucrose preference test; open field test; forced swimming test		√	√	√
Han et al., 2014	male	ICR mice	2 weeks	2 hours	sucrose preference test; light/dark exploration; forced swimming test; tail suspension test				
Wang et al., 2021	male	C57BL/6J mice	30 days	6 hours	sucrose preference test; open field test; forced swimming test; tail suspension test		√	√	
Zhou et al., 2021	male	Wistar rats	4 weeks	6 hours	sucrose preference test; open field test; forced swimming test; novelty-suppressed feeding		√		
MJ Park et al., 2018	male	C57BL/6J mice	3 weeks	3 hours	sucrose preference test; open field test; forced swimming test; tail suspension test; social interaction test; dominance tube test	√		√	
Wang et al., 2019	male	Kunming mice	4 weeks	4 hours	sucrose preference test; forced swimming test; tail suspension test		√	√	√
Liu et al., 2016	male	Sprague–Dawley rats	1 week	6 hours	sucrose preference test		√		

(continued)

Table 1. Continued.

Study	Sex	Animal strain	Restraint duration	Period/ day	Depression-like behavioral testing	Water and food consumption	Body weight	Corticosterone determination	Catecholamine determination
Liu et al., 2016	male	Sprague–Dawley rats	2 weeks	6 hours	sucrose preference test		√		
Liu et al., 2016	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; open field test; forced swimming test; elevated-plus maze		√		
Ampuero et al., 2015	male	Sprague–Dawley rats	10 days	2 hours	sucrose preference test; novelty-suppressed feeding; spontaneous motor activity; elevated plus maze; tail suspension test; forced swim test		√		
Ampuero et al., 2015	male	Sprague–Dawley rats	10 days	2 hours	sucrose preference test; novelty-suppressed feeding; spontaneous motor activity; elevated plus maze; tail suspension test; forced swim test		√		
Zhu et al., 2014	female	C57BL/6J mice	4 weeks	1 hour	sucrose preference test; open field test; forced swimming test; novelty-suppressed feeding; tail suspension test; elevated-plus maze; locomotor activity test	√			
Seewoo et al., 2020	male	Sprague–Dawley rats	13 days	2.5 hours	sucrose preference test; forced swimming test; elevated-plus maze		√		
Zhang et al., 2020	male	athymic nude mice	2 weeks	8 hours	sucrose preference test; tail suspension test				√
Aboul-Fotouh et al., 2014	male	Wistar rats	1 week	4 hours	sucrose preference test		√		
Aboul-Fotouh et al., 2014	male	Wistar rats	2 weeks	4 hours	sucrose preference test		√		
Aboul-Fotouh et al., 2014	male	Wistar rats	3 weeks	4 hours	sucrose preference test		√		
Aboul-Fotouh et al., 2014	male	Wistar rats	4 weeks	4 hours	sucrose preference test; open field test; social interaction test; forced swim test		√	√	√

(continued)

Table 1. Continued.

Study	Sex	Animal strain	Restraint duration	Period/ day	Depression-like behavioral testing	Water and food consumption	Body weight	Corticosterone determination	Catecholamine determination
Zhang et al., 2011	male	Sprague–Dawley rats	1 week	6 hours	sucrose preference test		√		
Zhang et al., 2011	male	Sprague–Dawley rats	2 weeks	6 hours	sucrose preference test		√		
Zhang et al., 2011	male	Sprague–Dawley rats	3 weeks	6 hours	sucrose preference test; forced swimming test		√	√	

protocols are simple and require less time, cost, and labor than CUMS. This study evaluated the validity of CRS in rodent models by analyzing effects on anhedonic-like behavior. After undergoing CRS for at least 1 week, there was decreased responsiveness to sucrose consumption analogous to anhedonia, the core symptom of MDD. However, there were methodological differences between the CRS protocols, including in restraint conditions, duration, and handling.

A comparative study demonstrated that increasingly severe movement restrictions led to greater behavioral stress responses.[49] Our pooled analysis of SPT results confirmed that duration of CRS exposure contributed to anhedonia-like behavioral responses, especially in C57BL/6J mice. Other differences in experimental procedures, including light/dark phase, water and food deprivation, presence of a foreign object, and novel noises and odors in the housing may simultaneity function as the stimuli, thereby potentially altering endocrine physiology and the development of depressive-like behaviors.

Rodents naturally avidly consume sweet foods and selectively drink sweet liquids when given a free choice of two bottles with separate access to sucrose solution and regular water.[55,56] Sucrose preference is a valid read-out of hedonic behavior, and a reduced sucrose preference ratio in stressed animals relative to controls is indicative of anhedonia.[56] Some studies have measured absolute sucrose consumption as a measure of anhedonia;[57] however, it is unclear how this measure affects reliability. First, the intake volume of sucrose solution can fluctuate considerably in rodents due to weight differences in experimental animals. Second, in some cases the rodents consume a decreased volume of liquid including sucrose solution and regular water. Occasionally, they consume large amounts of both liquids.[56] Thus, in our

Table 2. Summary of the sucrose preference test protocols used in included studies.

Study	Onset time	Training protocol	Water and food deprivation period	Sucrose concentration	Testing period
Yin et al., 2020	the last day of stress	1% sucrose for 2 days followed by 2 days of water	24 hours	1% sucrose	2 hours
Liang et al., 2015	the last day of stress	water for 2 days followed by 2 days of 1% sucrose and water	6 hours	1% sucrose	1 hours
Kim et al., 2018	the day after stress	2% sucrose for 24 hours	NO	2% sucrose	48 hours
Castañeda et al., 2015	NA	1% sucrose and water for 3 hours/day for 7 consecutive days prior to chronic restraint	1 hour	1% sucrose	NA
Hashikawa-Hobara et al., 2015	the day after stress	water for 3 days before the last experimental day	NO	1% sucrose	2 hours
Moreno et al., 2020	NA	NA	NO	1% sucrose	24 hours
Eiland et al., 2012	the last 2 days of stress	NO	NO	2% sucrose	48 hours
Chiba et al., 2012	the last day of stress	NO	NO	1% sucrose	24 hours
Aboul-Fotouh et al., 2013	NA	1% sucrose solution the week before stress	NO	1% sucrose	24 h
Tsuchimine et al., 2020	the last day of stress	2% sucrose and water for 1 week	NO	2% sucrose	24 hours
Cheng et al., 2017	the day after stress	1% sucrose for 24 hours followed by 24 hours of water and 1% sucrose	24 hours	1% sucrose	1 hours
Chen et al., 2020	the day after stress	water and 1% sucrose for 2 days	12 hours	1% sucrose	12 hours
Shilpa et al., 2017	14 days after stress	water and 1% sucrose for 2 days	18 hours	1% sucrose	2 hours
Wang et al., 2017	at least 12 hours after stress	1% sucrose	23 hours	1% sucrose	1 hour

(continued)

Table 2. Continued.

Study	Onset time	Training protocol	Water and food deprivation period	Sucrose concentration	Testing period
Liu et al., 2018	NA	NA	NO	NA	1 hour
Li et al., 2019	the day after stress	1% sucrose for 2 days	24 hours	1% sucrose	24 hours
Luo et al., 2015	4 days after stress	1% sucrose for 24 hours	12 hours (water)	1% sucrose	4 hours
Pan et al., 2019	NA	1% sucrose for 1 day followed by water and 1% sucrose for 1 day	24 hours	1% sucrose	1 hour
Li et al., 2019	the day after stress	NA	NO	NA	NA
Zhu et al., 2019	the day after stress	1% sucrose for 1 day followed by 1 day of water and 1% sucrose	12 hours	1% sucrose	24 hours
Dan Zhao et al., 2017	NA	water and 1% sucrose	22 hours	1% sucrose	2 hours
SH Park et al., 2018	NA	water for 24 hours	15 hours	1% sucrose	1 hour
Han et al., 2014	the day after stress	2 days of water and 1% sucrose	NO	1% sucrose	48 hours
Wang et al., 2021	the day after stress	NO	NO	0.1% sucrose	24 hours
Zhou et al., 2021	the day after stress	1% sucrose for 1 day followed by 1 day of water and 1% sucrose	23 hours	1% sucrose	1 hour
MJ Park et al., 2018	4 days after stress	water and 1% sucrose for 2 days	NO	1% sucrose	48 hours
Wang et al., 2019	NA	NA	24 hours (water)	1% sucrose	24 hours
Liu et al., 2016	the last day of stress	1% sucrose for 2 days	6 hours	1% sucrose	1 hour
Ampuero et al., 2015	the day after stress	1% sucrose or water for 3 hours daily for 5 days after the beginning of the stress protocol	12 hours	1% sucrose	1 hour
Zhu et al., 2014	NA	1% sucrose	4 hours (water)	1% sucrose	1 hour
Seewoo et al., 2020	2 days after stress	8 hours of water and 1% sucrose	16 hours	1% sucrose	1 hour

(continued)

Table 2. Continued.

Study	Onset time	Training protocol	Water and food deprivation period	Sucrose concentration	Testing period
Zhang et al., 2020	NA	3 days of water and 1% sucrose	15 hours (water)	1% sucrose	8 hours
Aboul-Fotouh et al., 2014	the last day of stress	1% sucrose 2 weeks before stress	NO	1% sucrose	24 hours
Zhang et al., 2011	NA	1% sucrose for 1 day followed by 1 day of water and 1% sucrose	23 hours	1% sucrose	1 hour

NA: not available in the article.

meta-analysis, we chose to use the sucrose preference ratio rather than the absolute sucrose consumption as the indicative measure of SPT. Sucrose preference ratio is a widely accepted parameter for anhedonia-like behavior in depressive rodents.

The test designs differed between the included studies, including test onset time, training protocols, water and food deprivation period, sucrose concentrations, and testing period. According to the recommendations of previous studies in the field of depression, a 1% to 2% (weight/volume) sucrose solution is the optimal concentration to elicit a preference over water. Some of the included studies ignored habituation to sucrose solution and two-bottle conditioning and did not conduct baseline measurements. Food and water deprivation prior to SPT can act as an additional stressor that affects anhedonic behavioral response. It is notable that the time chosen for SPTs is also important because circadian rhythms influence drinking behavior. Accordingly, it is appropriate to adopt a standard protocol for SPT to estimate anhedonic phenotypes in depression models.

Although there was decreased sucrose preference in the stressed groups compared with controls, the duration of CRS can differentially affect anhedonic-like behavior in model animals. Experimental animals present different degrees of decreased sucrose preference (%) depending on CRS duration. For example, sucrose intake tended to decrease in C57BL/6J mice over exposure durations from 1 to 4 weeks. Publication bias was assessed in different rodent species/strains by funnel plot (Figure 6), which indicated marginal effects of publication bias that were mostly attributable to small sample sizes and insufficient reporting of negative data. The trim and fill method allows estimates of an adjusted meta-analysis in the presence of publication bias;[58] thus, we performed a trim and fill

Table 3. Summary of the sucrose preference test results from studies included in the pooled analysis

Study	Animals	Restraint duration	Mean (%)*	SD/SEM/SE**	Number of controls	Mean (%)	SD/SEM/SE	Number of stressed animals
Yin et al., 2020	C57BL/6 mice	1 week	76.752	14.598	7	65.219	17.744	7
Yin et al., 2020	C57BL/6 mice	2 weeks	80.286	11.294	7	60.742	15.481	7
Yin et al., 2020	C57BL/6 mice	3 weeks	86.583	7.265	7	66.155	14.191	7
Liang et al., 2015	Sprague–Dawley rats	3 weeks	91.934	2.212	8	72.053	4.689	8
Kim et al., 2018	C57/BL6 mice	3 weeks	71.321	4.619	6	64.172	4.292	6
Castañeda et al., 2015	Sprague–Dawley rats	1 week	93.836	3.252	8	48.523	7.396	8
Castañeda et al., 2015	Sprague–Dawley rats	2 weeks	93.305	4.123	8	58.704	5.377	8
Hashikawa-Hobara et al., 2015	C57BL/6 mice	15 days	72.179	3.519	7	59.363	4.379	7
Moreno et al., 2020	Sprague–Dawley rats	3 weeks	89.532	1.094	24	69.433	1.915	30
Eiland et al., 2012	Sprague–Dawley rats	3 weeks	94.814	1.182	8	76.053	5.857	8
Eiland et al., 2012	Sprague–Dawley rats	3 weeks	95.11	1.755	8	85.698	5.642	8
Chiba et al., 2012	Wistar rats	1 week	91.01	0.961	11	82.585	4.544	11
Chiba et al., 2012	Wistar rats	2 weeks	88.407	2.394	11	70.975	6.559	11
Chiba et al., 2012	Wistar rats	3 weeks	92.363	1.149	11	81.219	7.627	11
Chiba et al., 2012	Wistar rats	4 weeks	88.231	1.217	11	74.843	7.492	11
Aboul-Fotouh et al., 2013	Wistar rats	1 week	84.335	2.19	8	72.112	3.967	8
Aboul-Fotouh et al., 2013	Wistar rats	2 weeks	84.183	3.476	8	53.894	5.294	8
Aboul-Fotouh et al., 2013	Wistar rats	3 weeks	84.811	3.407	8	51.231	4.079	8
Aboul-Fotouh et al., 2013	Wistar rats	4 weeks	85.064	3.85	8	45.947	3.986	8
Tsuchimine et al., 2020	BALB/c mice	3 weeks	79.005	2.003	5∼10	80.257	2.065	5–10
Tsuchimine et al., 2020	C57BL/6J mice	3 weeks	75.188	3.817	5∼10	45.713	7.823	5–10
Cheng et al., 2017	Sprague–Dawley rats	9 weeks	78.326	9.631	10	49.131	17.429	10
Chen et al., 2020	C57BL/6 mice	2 weeks	80.22	3.512	6∼9	70.797	3.306	6–9
Shilpa et al., 2017	Wistar rats	10 days	83.877	6.361	12	33.687	4.864	12
Wang et al., 2017	Sprague–Dawley rats	1 week	69.544	5.712	12	58.947	3.618	12
Wang et al., 2017	Sprague–Dawley rats	2 weeks	68.909	5.549	12	46.247	5.862	12
Wang et al., 2017	Sprague–Dawley rats	3 weeks	71.519	5.337	12	37.367	5.251	12
Wang et al., 2017	Sprague–Dawley rats	4 weeks	70.578	5.922	12	33.02	5.532	12
Liu et al., 2018	Sprague–Dawley rats	3 weeks	71.03	5.061	8	47.644	6.283	8
Li et al., 2019	C57BL/6J mice	3 weeks	80.382	3.924	9	53.823	4.628	9

(continued)

Table 3. Continued.

Study	Animals	Restraint duration	Mean (%)*	SD/SEM/SE**	Number of controls	Mean (%)	SD/SEM/SE	Number of stressed animals
Luo et al., 2015	Sprague–Dawley rats	10 days	87.545	3.929	7	66.305	5.992	7
Pan et al., 2019	Sprague–Dawley rats	3 weeks	92.231	1.686	9∼10	67.16	5.281	9–10
Li et al., 2019	C57BL/6J mice	3 weeks	85.635	2.36	9∼10	56.258	2.541	9–10
Zhu et al., 2019	C57BL/6J mice	4 weeks	83.982	1.991	10	72.434	5	10
Zhao et al., 2017	Kunming mice	3 weeks	84.218	2.867	27	55.042	9.2	27
SH Park et al., 2018	C57BL/6J mice	1 week	73.774	0.761	10	41.51	0.703	10
SH Park et al., 2018	C57BL/6J mice	2 weeks	74.854	0.453	10	47.584	0.659	10
Han et al., 2014	ICR mice	2 weeks	75.804	0.731	12∼15	50.731	2.376	12–15
Wang et al., 2021	C57BL/6J mice	30 days	95.244	1.03	10	83.564	1.522	10
Zhou et al., 2021	Wistar rats	4 weeks	91.114	2.668	8∼9	73.253	8.545	8–9
MJ Park et al., 2018	C57BL/6J mice	3 weeks	81.118	3.217	10	56.41	4.341	10
Wang et al., 2019	Kunming mice	4 weeks	84.056	5.06	10	61.247	5.392	10
Liu et al., 2016	Sprague–Dawley rats	1 week	82.069	3.377	8	58.654	7.205	8
Liu et al., 2016	Sprague–Dawley rats	2 weeks	74.832	6.192	8	51.98	8.556	8
Liu et al., 2016	Sprague–Dawley rats	3 weeks	71.648	4.615	8	48.008	5.741	8
Ampuero et al., 2015	Sprague–Dawley rats	10 days	76.287	2.668	13	66.571	1.542	13
Ampuero et al., 2015	Sprague–Dawley rats	10 days	76.287	2.668	11∼14	50.867	5.277	11–14
Zhu et al., 2014	C57BL/6J mice	4 weeks	77.564	1.693	10∼15	71.113	2.225	10–15
Seewoo et al., 2020#	Sprague–Dawley rats	13 days	78	7	5	69	4	25
Zhang et al., 2020	athymic nude mice	2 weeks	64.909	3.085	5	39.696	2.584	5
Aboul-Fotouh et al., 2014	Wistar rats	1 week	83.716	1.836	8∼10	74.855	3.234	8–10
Aboul-Fotouh et al., 2014	Wistar rats	2 weeks	79.881	3.259	8∼10	67.471	2.182	8–10
Aboul-Fotouh et al., 2014	Wistar rats	3 weeks	77.605	3.26	8∼10	60.641	3.364	8–10
Aboul-Fotouh et al., 2014	Wistar rats	4 weeks	77.109	2.944	8∼10	59.934	2.071	8–10
Zhang et al., 2011	Sprague–Dawley rats	1 week	74.25	3.215	16	57.71	3.682	16
Zhang et al., 2011	Sprague–Dawley rats	2 weeks	75.518	2.63	16	65.114	3.273	16
Zhang et al., 2011	Sprague–Dawley rats	3 weeks	77.964	2.577	16	59.628	3.534	16

*Mean indicates the results of sucrose preference tests, which were calculated according to the following formula: % sucrose preference = [sucrose intake ÷ total fluid intake] × 100.
**SD: standard deviation; SE: standard error; SEM: standard error of mean; in the included studies, the usage of SD, SE, and SEM was not consistent, and they were used in different studies.
#Only the indicated article provided the direct results of sucrose preference tests (%); the results of other studies were extracted directly from graphs or figures using Engauge Digitizer software. Regarding the number of control and stressed animals, narrow ranges of values were provided in several articles, and in these cases, the numbers were defined as the medians of the ranges for the pooled analysis.

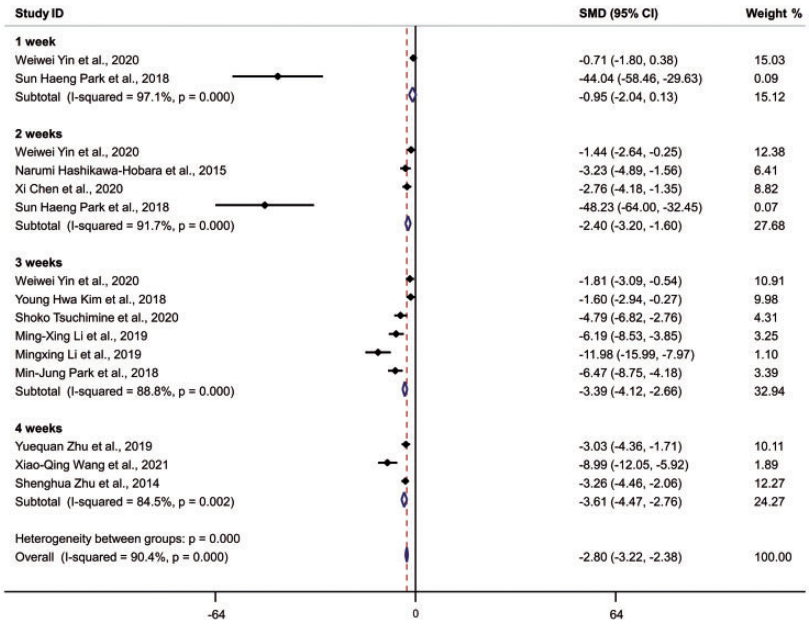

Figure 2. Forest plots of standardized mean difference (SMD) of sucrose preference (%) in C57BL/6J mice following exposure to chronic restraint stress. The effect size was determined by calculating the SMD combined with their 95% confidence intervals. Diamonds indicate SMD values, and the horizontal lines represent 95% confidence intervals.

analysis of the included studies. The results indicated that the presence of publication bias did not greatly affect the pooled analysis of effect size.

There was high heterogeneity among studies in the single-group analysis, which suggested difficulties in achieving reproducible effects of the CRS protocol by different research groups. Numerous factors can bring heterogeneity into the pooled results, including the animal strains, animal sex, CRS protocol (e.g., duration, intensity, and housing and restraint conditions), and SPT protocols (e.g., test onset time, training protocols, water and food deprivation period, sucrose concentrations, and testing period), which should be considered when designing CRS protocols for modeling anhedonic-like behavior. Additionally, circadian rhythm and restraint placement are important factors in CRS protocols that should not be overlooked. The restraint placement and time periods used in the included studies are summarized in Table 5. Most of the included studies performed CRS over a fixed daily time period to avoid circadian rhythm fluctuations. Experimental animals were periodically constrained from movement by placing them in tubes of suitable volumes depending on the animal species/strain.

The effectiveness of CRS is not confined to a particular strain/species of animal. Our pooled analysis demonstrated inter-species variability, with rats exhibiting greater susceptibility to restraint stress compared with mice. In terms of murine CRS-induced depression models, BALB/c mice were not commonly used. In 2020, Tsuchimine et al. conducted a comparison of the physiological and behavioral responses to CRS between C57BL/6J and BALB/c mice.[30] The results showed that BALB/c, but not C57BL/6J, mice presented anhedonia-like

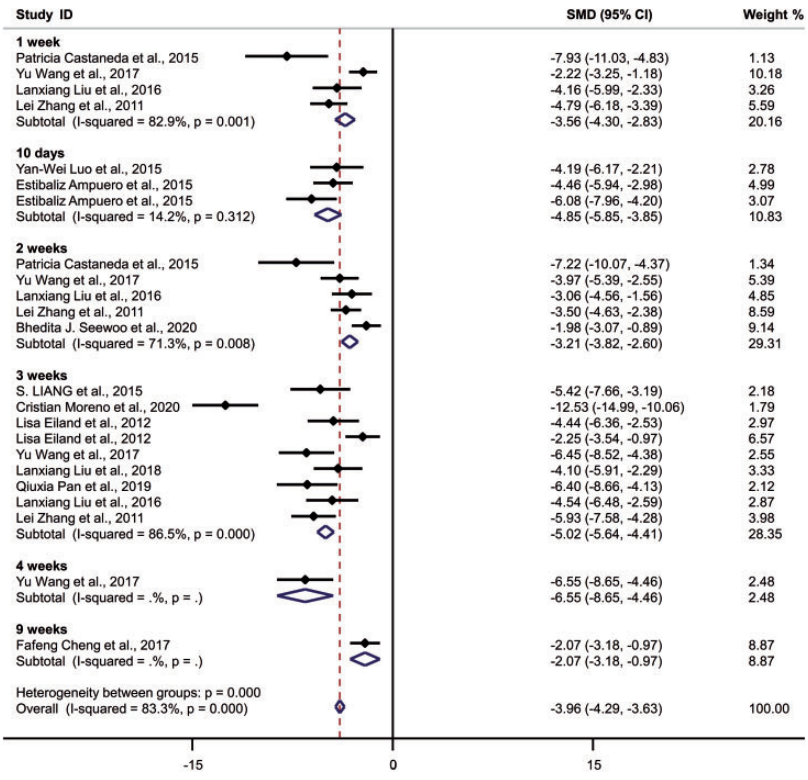

Figure 3. Forest plots of standardized mean difference (SMD) of sucrose preference (%) in Sprague–Dawley rats following exposure to chronic restraint stress. The effect size was determined by calculating the SMD combined with their 95% confidence intervals. Diamonds indicate SMD values, and the horizontal lines represent 95% confidence intervals.

behavior after CRS according to SRT results, indicating a greater behavior stress response in BALB/c than in C57BL/6J mice.

Chronic stress results in a higher magnitude of corticosterone responses, and it has been reported that chronic administration of corticosterone to mice induces anhedonia-like behavior.[59] Consistently, human studies have shown that anhedonia symptoms are associated with higher corticosterone levels in patients with depression.[60] Inter-strain variability in the development of anhedonia-like behavior could be explained by differences in the functionality of the HPA axis. Another explanation for inter-strain variability is differences in the type of immune responses involved including Th1 and Th2 immunity, which may contribute to CRS susceptibility.[61]

An overwhelming majority of the studies established CRS-induced depression models in male rodents, with only 3.5% of the studies (2/57) selecting female rodents as the research subjects. In 2012, Eiland et al. found a significant effect of sex in CRS-induced depression-like behavior, with females exhibiting greater locomotion than males under restraint stress.[29] A similar finding was reported following CRS, in that CRS did not induce distinguishable

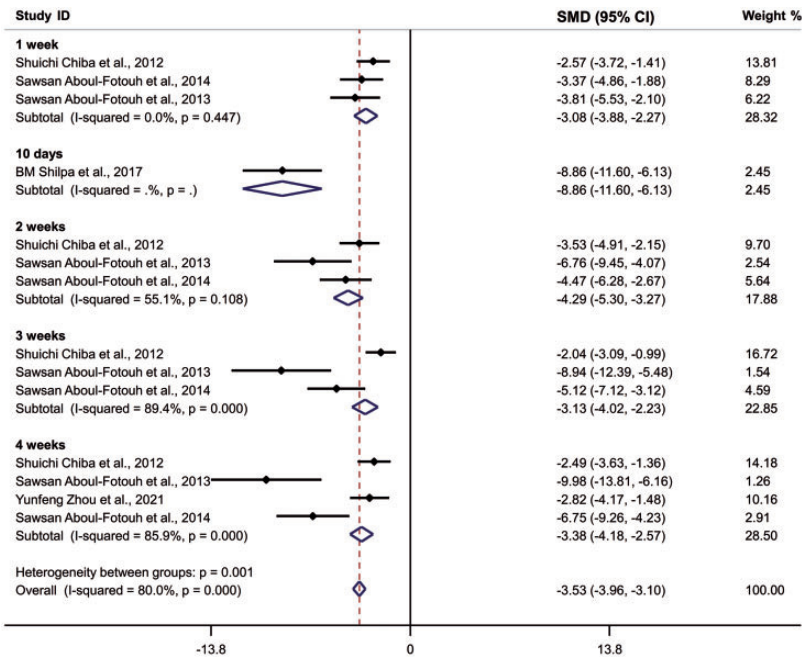

Figure 4. Forest plots of standardized mean difference (SMD) of sucrose preference (%) in Wistar rats following exposure to chronic restraint stress. The effect size was determined by calculating the SMD combined with their 95% confidence intervals. Diamonds indicate SMD values, and the horizontal lines represent 95% confidence intervals.

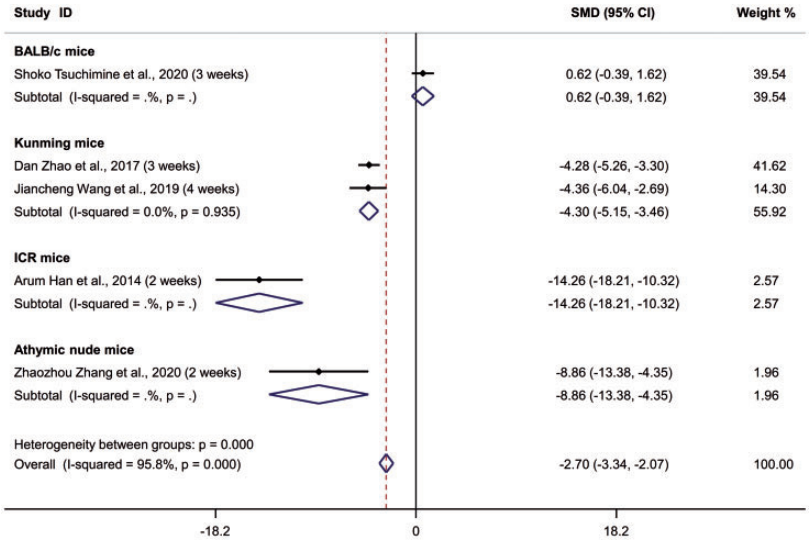

Figure 5. Forest plots of standardized mean difference (SMD) of sucrose preference (%) in mice of other strains following exposure to chronic restraint stress. The effect size was determined by calculating the SMD combined with their 95% confidence intervals. Diamonds indicate SMD values, and the horizontal lines represent 95% confidence intervals.

Table 4. Meta-analyses of sucrose preference tests and heterogeneity assessments.

			Sucrose preference test outcomes				heterogeneity	
Animal strain	Restraint duration	Number of studies	SMD	95% confidence interval	p value	Weight(%)	I2	p value
C57BL/6 mice	1 week	2	−0.954	(−2.037, 0.128)	0.084	15.12	97.10%	<0.001
	2 weeks	4	−2.396	(−3.196, −1.597)	<0.001	27.68	91.70%	<0.001
	3 weeks	6	−3.389	(−4.122, −2.655)	<0.001	32.94	88.80%	<0.001
	4 weeks	3	−3.613	(−4.467, −2.759)	<0.001	24.27	84.50%	<0.001
	overall	15	−2.8	(−3.221, −2.380)	<0.001	100.00	90.40%	<0.001
Sprague–Dawley rats	1 week	4	−3.565	(−4.299, −2.830)	<0.001	20.16	82.90%	0.001
	10 days	3	−4.849	(−5.851, −3.847)	<0.001	10.83	14.20%	0.312
	2 weeks	5	−3.211	(−3.820, −2.601)	<0.001	29.31	71.30%	0.008
	3 weeks	9	−5.025	(−5.644, −4.405)	<0.001	28.35	86.50%	0
	4 weeks	1	−6.554	(−8.650, −4.459)	<0.001	2.48	—	—
	9 weeks	1	−2.073	(−3.181, −0.966)	<0.001	8.87	—	—
	overall	23	−3.956	(−4.286, −3.626)	<0.001	100.00	83.80%	<0.001
Wistar rats	1 week	3	−3.075	(−3.881, −2.269)	<0.001	28.32	0.00%	0.447
	10 days	1	−8.864	(−11.603, −6.125)	<0.001	2.45	—	—
	2 weeks	3	−4.288	(−5.302, −3.274)	<0.001	17.88	55.10%	0.108
	3 weeks	3	−3.127	(−4.024, −2.230)	<0.001	22.85	89.40%	<0.001
	4 weeks	4	−3.375	(−4.178, −2.572)	<0.001	28.50	85.90%	<0.001
	overall	14	−3.531	(−3.960, −3.102)	<0.001	100.00	80.00%	<0.001
Mice of other strains	BALB/c mice	1 (3 weeks)	0.615	(−0.391, 1.622)	0.231	39.54	—	—
	Kunming mice	1 (3 weeks)	−4.282	(−5.262, −3.301)	<0.001	41.62	—	—
		1 (4 weeks)	−4.362	(−6.035, −2.689)	<0.001	14.30	—	—
	ICR mice	1 (2 weeks)	−14.264	(−18.211, −10.317)	<0.001	2.57	—	—
	Athymic nude mice	1 (2 weeks)	−8.861	(−13.376, −4.345)	<0.001	1.96	—	—
	overall	5	−2.703	(−3.336, −2.071)	<0.001	100.00	95.80%	<0.001

SMD: standardized mean difference.

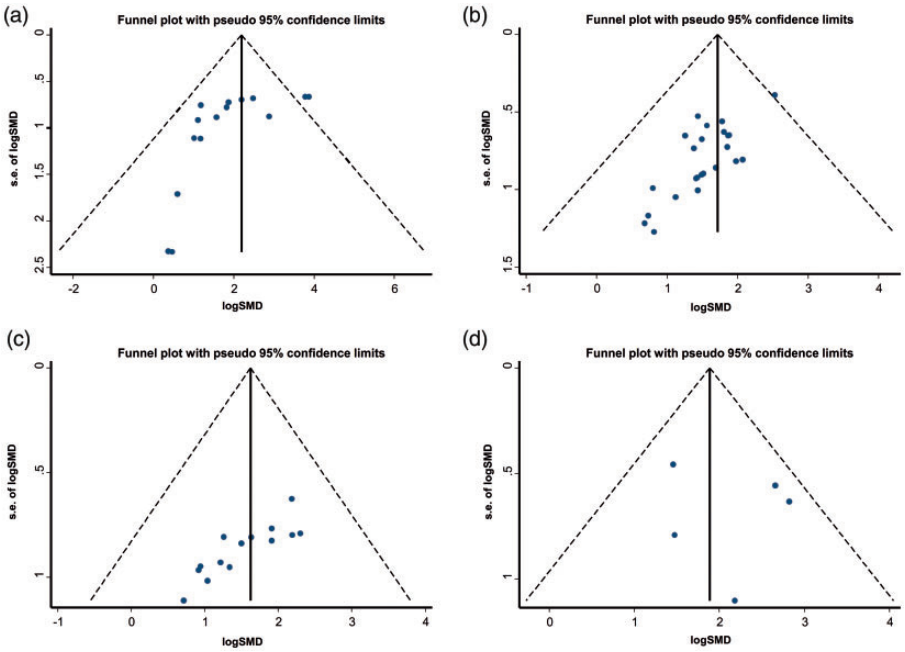

Figure 6. Funnel plots of the effect measure (log|SMD|) versus its precision (standard error [SE] of log|SMD|) of sucrose preference (%) for C57BL/6J mice (a), Sprague–Dawley rats (b), Wistar rats (c) and mice of other strains (d).

Table 5. The restraint placements and time periods used in the included studies.

Study	Animal strain	Restraint placement	Time periods
Yin et al., 2020	C57BL/6 mice	placed in a 50-mL syringe	between 11:00 am and 5:00 pm
Liang et al., 2015	Sprague–Dawley rats	placed in polypropylene cylinders (6-cm inner diameter)	NA
Kim et al., 2018	C57/BL6 mice	placed in a well-ventilated plastic tube	NA
Castañeda et al., 2015	Sprague–Dawley rats	placed in a transparent plexiglass tube (25 × 3 × 8 cm)	between 9:00 am and 12:00 pm
Hashikawa-Hobara et al., 2015	C57BL/6 mice	placed in a modified 50-mL polyethylene tube	starting at 10:00 am
Moreno et al., 2020	Sprague–Dawley rats	Placed in well ventilated and transparent acrylic restrainers (6 × 6 × 18 cm)	between 9:00 am and 11:00 am
Eiland et al., 2012	Sprague–Dawley rats	placed in snuggly-fitted wire mesh restrainers	between 8:00 am and 11:00 am
Chiba et al., 2012	Wistar rats	placed in acrylic cylinders (6.5-cm inner diameter, 20-cm long)	between 9:00 and 15:00
Aboul-Fotouh et al., 2013	Wistar rats	placed in Plexiglas restrainers (25 × 7 cm)	between 8:00 and 14:00

(continued)

Table 5. Continued.

Study	Animal strain	Restraint placement	Time periods
Tsuchimine et al., 2020	BALB/c mice	restrained in a plastic DecapiCone (Braintree Scientific Inc., Braintree, MA, USA)	NA
Cheng et al., 2017	Sprague–Dawley rats	Placed in a plastic restrainer (550-mL water bottle [Nongfu Spring Co. Ltd., Hangzhou, China] or 600-mL water bottle [Danone])	from 8:30am to 9:00 am
Chen et al., 2020	C57BL/6 mice	placed in 50-mL plastic tubes	NA
Shilpa et al., 2017	Wistar rats	placed in rodent immobilization bags	from 10 am to 12 pm
Wang et al., 2017	Sprague–Dawley rats	restrained in a cylinder-shaped wire net (20-cm length and 5-cm diameter)	NA
Liu et al., 2018	Sprague–Dawley rats	Placed in a plastic restrainer (550-mL water bottle [Nongfu Spring Co. Ltd., Hangzhou, China])	between 9:00 and 15:00
Li et al., 2019	C57BL/6J mice	placed in 50-mL conical tubes	Starting at 10:00 am
Luo et al., 2015	Sprague–Dawley rats	placed in a plastic restrainer (350-mL water bottle [Wahaha Co. Ltd., Hangzhou, China])	from 13:00 to 17:00
Pan et al., 2019	Sprague–Dawley rats	Restrained in wooden T-shaped double-binding platforms, including a base platform (20-cm long, 10-cm wide and 2.8-cm thick) and an upper platform (22-cm long and 6.6-cm wide)	from 19:00 to 22:00
Li et al., 2019	C57BL/6J mice	placed in 50-mL conical tubes with ventilation holes	from 10:00 to 12:00
Zhu et al., 2019	C57BL/6J mice	placed in the well-ventilated Plexiglas tubes with an inner diameter of 6 cm	from 09:00 to 15:00
Zhao et al., 2017	Kunming mice	placed in well-ventilated 50-mL conical Plexiglas tubes	from 10:00 to 16:00
SH Park et al., 2018	C57BL/6J mice	placed in a tube (diameter: 30 mm; length: 100 mm [Jeung Do Bio & Plant Co., Ltd., Seoul, Korea])	NA
Han et al., 2014	ICR mice	placed in 50-mL Corning tubes	from 11:00 to 13:00
Wang et al., 2021	C57BL/6J mice	immobilized in a mouse restraint apparatus	NA
Zhou et al., 2021	Wistar rats	placed in acrylic cylinders (6.5 cm in diameter, 20 cm in length)	from 9:00 to 15:00
MJ Park et al., 2018	C57BL/6J mice	placed into 50-mL polypropylene conical tubes	NA
Wang et al., 2019	Kunming mice	placed in acrylic cylinders (inner diameter: 6.5 cm; length: 20.0 cm)	between 9 am and 1 pm
Liu et al., 2016	Sprague–Dawley rats	Placed in a plastic restrainer (550-mL water bottle [Nongfu Spring Co. Ltd., Hangzhou, China])	from 09:00 to 15:00
Ampuero et al., 2015	Sprague–Dawley rats	placed in plastic bags ($18 \times 6 \times 6$ cm)	NA
Zhu et al., 2014	C57BL/6J mice	placed in plastic tubes	Between 09:00 and 11:00
Seewoo et al., 2020	Sprague–Dawley rats	placed in transparent tubes (diameter: 5–6 cm; length: 19–23 cm)	between 12:30 pm and 3:30 pm

(continued)

Table 5. Continued.

Study	Animal strain	Restraint placement	Time periods
Zhang et al., 2020	Athymic nude mice	placed in well-ventilated 50-mL restraint tubes	from 8:00 to 16:00
Aboul-Fotouh et al., 2014	Wistar rats	placed in Plexiglas restrainers (25 × 7 cm)	from 8:00 am to 12:00 pm
Zhang et al., 2011	Sprague–Dawley rats	placed in a locally fabricated wire mesh restrainer with a stainless steel box (15 × 7 × 8 cm)	between 10:30 and 16:30

NA: not available in the article.

anhedonia-like behavior in female C57BL/6J mice, while other studies using male C57BL/6J mice reported a positive effect.[50]

A growing literature suggests sexual dimorphisms in the endocrine and immune systems and in stress resilience.[2] These sex differences are likely attributable to steroid hormones, such as estrogens and androgens, which can modulate the effects of stress on dendritic remodeling and regulate susceptibility to stressful events.[2,62] It was reported that in rats with heart failure induced by myocardial infarction, in contrast to males, females do not develop depression-like behavior or an increase in prefrontal cortex cytokines, and this discrepancy was attributed to the role of estrogens.[63] Thus, the sex of model animals should be considered when designing experiments.

In conclusion, this meta-analysis indicated that the CRS protocol is a reliable and effective rodent model of anhedonic-like behavior. However, there was high heterogeneity in the single subgroup analysis, which may be attributable to the duration and intensity of CRS and to SPF protocols. This work may provide a reference stress duration and intensity for CRS models in specific animal species/strains.

Author contributions

XY conceived and designed the analysis, solved disagreements during study selection and data extraction, and reviewed the manuscript; YM and YX selected the included studies and extracted data; YM analyzed the data, edited the figures and tables, and wrote the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Youth Project of Science and Technology Research Project of Jiangxi Education Department (No. GJJ200251) and the Hunan Cancer Hospital Climb Plan (No. 20210225-1006).

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