Neuroscience and Biobehavioral Reviews 134 (2022) 104549

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Impact of social isolation on the oxytocinergic system: A systematic review and meta-analysis of rodent data

Júlia S. Krimberg [a,b], Francisco S. Lumertz [c], Rodrigo Orso [c], Thiago W. Viola [c], Rosa Maria M. de Almeida [a,b,*]

[a] Neuroscience Graduate Program, Basic Health Sciences Institute (IBCS), Universidade Federal of Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500, Centro Histórico, Porto Alegre, RS, 90050-170, Brazil
[b] Experimental Psychology, Neuroscience and Behavior Laboratory (LPNeC), Psychology Institute, Universidade Federal of Rio Grande do Sul (UFRGS), Rua Ramiro Barcellos, 2600, Room 216. Santa Cecília, Porto Alegre, RS, 90035-003, Brazil
[c] Developmental Cognitive Neuroscience Lab (DCNL), Brain Institute of Rio Grande do Sul, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Av. Ipiranga, 6690, Building 63, Jardim Botânico, Porto Alegre, RS, 90619-900, Brazil

ARTICLE INFO

Keywords:
Oxytocin
Oxytocin receptor
Oxytocinergic system
Social isolation
Stress
Behavior

ABSTRACT

Social isolation (SI) stress results from a combination of intrinsic and environmental factors and is associated with a variety of negative developmental outcomes. Oxytocin (OXT) might play a role in the consequences of SI in the brain and periphery. We conducted a systematic review and meta-analysis to compile data about the effects of SI in the oxytocinergic system of rats and mice, and its relation to behavioral alterations. Five databases (EMBASE, PsychNet, PubMed, Scopus, and Web of Science) were searched in March 2021, using ("Social Isolation" AND (mouse OR rat) AND (oxytocin OR oxytocin receptor)). This review followed the PRISMA guidelines, including registration in PROSPERO, and risk of bias assessment. The twelve articles included in this review indicated that SI was associated with decreased OXTR levels, resulting in behavioral alterations like increased aggression and anxiety-like behavior, hyperactivity, and diminished social behaviors and memory. No significant effects on OXT levels were observed. Administration of synthetic OXT or its agonists partially decreases those unwanted behaviors to similar levels of control animals.

1. Background

Social contact is a natural reinforcer for humans and rodents and is essential for optimal neurodevelopment. In humans, previous evidence has indicated that social contact during the developmental period can modulate cognition, behavior, and emotions throughout lifespan (Silvers, 2022). Specifically, maladaptive social environments can be extremely aversive especially during childhood and adolescence, which may lead to cognitive impairments, anxiety, depression, impulsiveness, and addictive behaviors (Lockhart et al., 2018; Lupien et al., 2009; Silvers, 2022; Trezza et al., 2014). Many of those findings translate to rodents (Burke et al., 2017; Burke and Miczek, 2014; Dafny and Yang, 2006; Lapiz et al., 2003; Lupien et al., 2009; Makinodan et al., 2012; Trezza et al., 2014; Walker et al., 2019), where deprivation of social contact during adolescence can generate stress in levels comparable to human chronic stressors in this period, such as severe negative family situations, intense bullying, negligence, abuse and others (Noschang et al., 2021; Robbins, 2016). Thus, social isolation (SI) in animals can serve as a trigger for anxiety and depressive-like behavior phenotypes (Lampert et al., 2017; Tanaka et al., 2019; Vanderschuren and Trezza, 2013), diminished social play (Vanderschuren and Trezza, 2013) and increased locomotor activity, which often cannot be reversed with resocialization (Lapiz et al., 2003; Robbins, 2016).

Alterations in the hypothalamic-pituitary-adrenal axis (HPA-axis), serotoninergic and dopaminergic neurotransmission, as well as limbic system functioning have been reported following animal SI (Lapiz et al., 2003; Mumtaz et al., 2018; Robbins, 2016; Vanderschuren and Trezza, 2013). Moreover, brain regions such as the hippocampus and prefrontal cortex (PFC) are highly affected by long-term periods of isolation (Lapiz et al., 2003; Mumtaz et al., 2018; Robbins, 2016). However, the most

* Corresponding author at: Experimental Psychology, Neuroscience and Behavior Laboratory (LPNeC), Psychology Institute, Universidade Federal of Rio Grande do Sul (UFRGS), Rua Ramiro Barcellos, 2600, Room 216. Santa Cecília, Porto Alegre, RS, 90035-003, Brazil.
E-mail addresses: juliaskrimberg@gmail.com (J.S. Krimberg), fsindermannlumertz@gmail.com (F.S. Lumertz), rodrigo_orso@hotmail.com (R. Orso), thiago.wendt.viola@gmail.com (T.W. Viola), rosa.almeida@ufrgs.br (R.M.M. de Almeida).

https://doi.org/10.1016/j.neubiorev.2022.104549
Received 30 October 2021; Received in revised form 29 December 2021; Accepted 20 January 2022
Available online 21 January 2022

consistent physiological outcomes induced by social deprivation are impairments in the reward system through the mesolimbic, mesocortical, and mesoamygdaloid dopaminergic pathways (Lapiz et al., 2003; Mumtaz et al., 2018; Robbins, 2016). In addition to dopamine, the neuromodulator peptide Oxytocin (OXT) appears to also play a significant role in the social reward system, as its receptors have been found in reward-related brain areas such as the PFC, the ventral tegmental area (VTA), the amygdala, and the nucleus accumbens (NAcc) (Bowen and Neumann, 2017; Jurek and Neumann, 2018; Leong et al., 2018; Love, 2014; Shamay-Tsoory and Abu-Akel, 2016; Vanderschuren and Trezza, 2013).

OXT is known to act in the peripheral and central nervous systems, impacting an array of behavioral and physiological functions such as reproduction (Insel et al., 1997), maternal care (Marlin and Froemke, 2017), pain tolerance (Boll et al., 2018; Marlin and Froemke, 2017), social memory (Cilz et al., 2018; Mumtaz et al., 2018), pro-social behavior (Shamay-Tsoory and Abu-Akel, 2016), and stress response (Sippel et al., 2017). Considering that OXT also plays a role in the regulatory function of the HPA-axis (Lien et al., 2017; Tang et al., 2019), chronic or acute stressors could impact the oxytocinergic system through positive feedback. However, research on OXT has also focused on the development of new treatments for anxiety, depression, addiction, and even aggressive behavior (Neumann, 2007; Sippel et al., 2017), which are psychopathological conditions associated with altered responses to stress and reward.

The impact of OXT on the central nervous system is not limited to positive outcomes. Studies have also reported an increase in aggressive behavior after the administration of the peptide, which seems to be linked to the bonding facilitating aspect of OXT. In humans, intranasal administration of oxytocin prior to competitive tasks where participants were divided into groups of unknown individuals has led to an increased predisposition to cheat to benefit their group (Shalvi and De Dreu, 2014). Similarly, in models using rats, it is seen that OXT administration increases maternal aggression as a response to threat (Ferris et al., 1992; Grillon et al., 2013). It appears that as bonding through OXT administration increases, so does defensive instincts, inducing and modulating anxiety and aggressive behaviors (Grillon et al., 2013).

Comprehending how social behavior can induce alterations in OXT is essential to further reveal the neurobiological underpinnings associated with OXT circuitry and the development of behavioral alterations throughout life. For this reason, the objective of this systematic review was to compile and synthesize information on the effects of SI on the oxytocinergic system (central and peripheral) of rats and mice, and its behavioral outcomes when compared to group-housed animals. Furthermore, we assessed the methodology used in the studies that could cause divergent results. A meta-analysis was also performed to statistically assess the overall alterations of OXT and its receptors (OXTR).

2. Method

2.1. Eligibility criteria

The PICOS strategy was used according to the review objectives to accurately define the eligibility criteria, where: P (population) is mice or rats; I (intervention) is mice or rats who underwent social isolation; C (compare) is mice and rats who did not undergo social isolation; O (outcome) are oxytocin and behavioral measures; and S (study design) is experimental studies. Therefore, our inclusion criteria were studies in which (a) samples consisted of rats and/or mice; (b) social isolation was used as a stress protocol; (c) no other disease / disorder model protocol was implemented; and (d) oxytocin or its receptors were measured or manipulated. Our exclusion criteria were: (a) books or chapters of books, conference reports, dissertation or thesis, non-peer reviewed articles and reviews of any kind; and (b) administration of drugs or compounds other than OXT, OXTR agonist, or OXTR antagonist (studies which also had non-medicated groups, or only medicated with the mentioned compounds were included and partial results were assessed). No limits were established for the year of publication or language.

2.2. Information sources

This study followed the PRISMA guidelines for reporting systematic reviews and meta-analysis (Page et al., 2021a; 2021b). Searches were conducted between October 17th, 2020, and March 8th, 2021, using five databases: EMBASE, PubMed, PsychNet, Scopus, and Web of Science. The general search terms used were (1) Rat or Mouse, (2) Oxytocin or oxytocin receptor, and (3) social isolation, the full strategy for each database is shown in Table 1.

2.3. Selection and data collection processes

Our search resulted in 284 records (see Fig. 1). The research tool Rayyan was used to combine all studies, remove duplicates (n = 151), and for the independent reviewers (JSK and FSL) to simultaneously screen the 140 abstracts. JSK and FSL had a concordance rate of 92 %, divergencies were assessed by a senior third reviewer (RMMdeA). The steps were repeated to assess the 27 resulting articles, this time the full text was analyzed according to the same inclusion and exclusion criteria previously stated.

Based on the analysis of the full texts, 12 articles were selected for the data extraction phase, with a concordance rate between judges of 96 %. The data extracted from the articles were: (1) author and year of publication; (2) species used; (3) strain of the animals; (4) sex of the animals; (5) age at the beginning of isolation; (6) length of complete isolation; (7) total length of isolation (period completely isolated *plus* period where

Table 1
Search strategies used for EMBASE, PsychNet, PubMed, Scopus, and Web of Science.

Database		Search strategy
EMBASE		'social isolation' AND ('oxytocin receptor' OR 'oxytocin') AND ('rat' OR 'mouse')
PsychNet		(Any Field: "social isolation") AND (Any Field: "OXYTOCIN" OR Any Field: "OXYTOCIN RECEPTOR*") AND (Any Field: "RAT*" OR Any Field: "MOUSE" OR Any Field: "MICE")
PubMed	#1	(Social Isolation[mh] OR Isolation, Social[tw] OR Isolations, Social[tw] OR Social Isolations[tw] OR Social Isolation[tw])
	#2	(Oxytocin[mh] OR Syntocinon[tw] OR Pitocin [tw])
	#3	(Receptors, Oxytocin[mh] OR Oxytocin Receptor [tw] OR Oxytocin Receptors[tw])
	#4	(Rats[mh] OR Rat*[tw] OR Rattus[tw] OR Rattus norvegicus[tw] OR Rats, Norway[tw] OR Rats, Laboratory[tw] OR Laboratory Rat[tw] OR Laboratory Rats[tw] OR Rat, Laboratory[tw])
	#5	(Mice[mh] OR Mus[tw] OR Mouse[tw] OR Mus musculus[tw] OR Mice, House[tw] OR Mus[tw] OR Mouse[tw] OR Mus musculus[tw] OR Mice, House [tw] OR House Mice[tw] OR Mouse, House[tw] OR House Mouse[tw] OR Mus domesticus[tw] OR Mus musculus domesticus[tw] OR domesticus, Mus musculus[tw] OR Mice, Laboratory[tw] OR Laboratory Mice[tw] OR Mouse, laboratory[tw] OR Laboratory OR Mouse[tw] OR Mouse, Swiss[tw] OR Swiss Mouse[tw] OR Swiss Mice [tw] OR Mice, Swiss[tw])
	#6	#1 AND (#2 OR #3) AND (#4 OR #5)
Scopus		(('social isolation') AND (oxytocin OR 'Oxytocin Receptors') AND (rat* OR mice))
Web of Science	#1	ALL="social isolat*"
	#2	ALL = (Oxytocin* OR "oxytocin receptor*")
	#3	ALL = (Rat* OR Mice)
	#4	#1 AND #2 AND #3

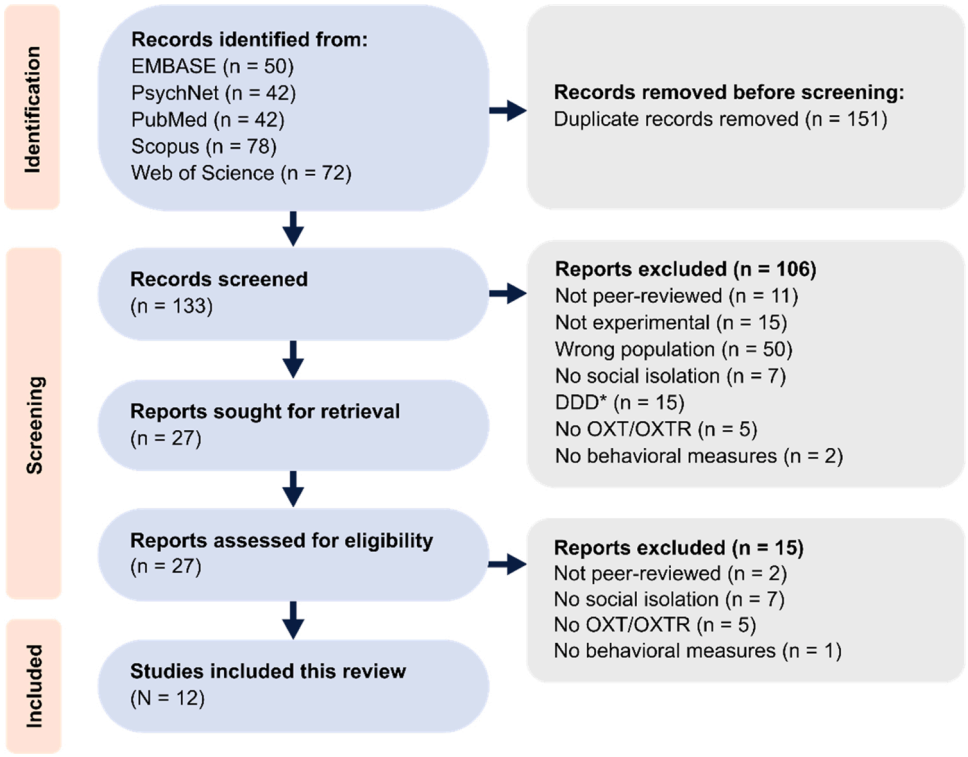

Fig. 1. flowchart indicating the number of findings per database, and the detailed selection process until the inclusion of the final 12 studies, as suggested by the PRISMA 2020 guidelines. *DDD: Disease, disorder, and/or drugs interferences.

animals left isolation for testing); (8) behavioral measures/tests; (9) biological measures/tests; (10) age at euthanasia; (11) behavioral outcomes; (12) biological outcomes; (13) drugs administered and dosage; (14) cerebral areas investigated; (15) method of biological analysis. Both reviewers (JSK and FSL) extracted the data from the articles in identical spreadsheets in to compare, confirm and combine the data. When studies used pharmaceuticals other than OXT, OXT agonist, or OXT antagonist, we assessed only results from the vehicle sample. To allow statistical analysis of the outcomes, the mean, standard error of the mean and number of subjects per group per analysis were extracted from the text or graphs, using the free version of the software GetData Graph Digitalizer. The qualitative data was extracted and later coded as it follows:

- Species: (0) rat; and (1) mice;
- Sex: (0) male; and (1) female;
- Length of isolation, in days: (0) 1–14; (1) 15–28; (3) 29–42; (4) 43–58; (5) 59–72; and (6) 73–86;
- Age at sacrifice, in PND: (0) 1–14; (2) 21–45; (3) 46–60; and (4) 61–90;
- Biomarker: (0) OXT; and (1) OXTR
- Behaviors analyzed: (1) anxiety-like; (2) Social; (3) Locomotor Activity; and (4) Aggression.
- Tissue: (0) Medial Pre-frontal Cortex (mPFC); (1) PVN; (2) PVNap; (3) PVNproper; (4) SON; (5) NAcc; (6) NAcc anterior; (7) VTA; (8) BNST; (9) BNST anterior; (10) BNST posterior; (11) Lateral Septum (LS) ventral; (12) Central Amygdala (CeA); (13) Ventromedial Nucleus of the Hypothalamus; (14) Hippocampus; (15) Striatum; and (16) plasma;
- Time between last test and tissue extraction: (0) not informed; (1) less than 24 h; and (2) more than 24 h.
- OXT/OXTR Outcome: (0) no alterations; (1) increased compared to control subjects; and (2) decreased compared to control subjects.
- Behavioral Outcome: (0) no alterations; (1) increased compared to control subjects; and (2) decreased compared to control subjects.

The risk of bias (RoB) was assessed by two independent reviewers (JSK and FSL) using SYRCLE's tool (high, low, or unknown risk of bias) (Hooijmans et al., 2014), and later compared and combined. Based on the considerations by Saper (2009) and Saper and Sawchenko (2003) regarding antibody and immunohistochemistry specificity, we added as a source of bias the nondisclosure of information regarding the methods and materials used when performing immunohistochemical analysis. This assessment was performed using the criteria presented in Saper (2005), which indicates three basic elements that should be informed when reporting immunohistochemical studies: 1) Complete information on the antibody, including the commercial name of the product, manufacturer, and product code; 2) How has the specificity of the antibody been characterized; and 3) What controls are necessary for immunostaining?.

2.4. Meta-Analysis

A meta-analysis was conducted using the Random Effects Model (RE Model) and a multilevel approach to create the forest plots, using a 2-level hierarchical data structure model. The samples within and between studies were nested together according to the biomarker (OXT or OXTR) and the structure of the brain analyzed, or the behavioral measure (locomotor activity or aggressive, anxiety-like, social behavior), and the effect size of the results was determined using the standardized mean difference (SMD), calculated using Cohen's D. Influence analysis, Q statistic, and I^2 were also performed to detect outliers, heterogeneity, and variability. All analysis were performed using the 'metafor' package (2.4−0) from the statistical software R 4.0.0.

3. Results

3.1. Studies' characteristics

Individual characteristics and results of the studies are presented at Tables 2 and 3, sorted from newest (2021) to oldest (2005) publication

Table 2
All time/length measures are expressed in days. F: Females; M: Males; *partial sample; #: OXT analysis; &: OXTR analysis.

Authors (year)	Risk of Bias	Species (strain)	Sample	Sex	Age at start of isolation	Length of Isolation	Behavioral Measure (PND)	Euthanasia	Cerebral Areas	Method of Analysis
Huang et al. (2021)	High	Mouse (C57BL/6 N)	~70*	M	PND21	42	PND60~70 Reciprocal social interaction test Three Chambers Test Marble burying Open Field Test Elevated Plus-Maze Resident Intruder Assay	63~70	Medial Pre-frontal Cortex Ventral Tegmental Area Nucleus Accumbens	Immunohistochemistry[&] Western Blotting[&]
Goh et al. (2020)	Low	Rat (Lister Hooded)	80*	M	PND22	Adolescents: 14 Adults 65−69	Locomotor Activity (51−57) Non-spatial Novel Object Discrimination (52−58) Object-based novel location discrimination (59−65) Social interaction and concomitant ultrasonic vocalizations, (66−70)PPI (74 −78) Fear-motivated contextual freezing responses (79−83) Bowl digging attentional set-shift task (79−125)	PND 36 Or PND 87-91	Hippocampus and Striatum	Multiplex Analysis[#]
Tan et al. (2020)	Low	Mouse (Swiss)	20*	M	42−49 PND	42	Two-mouse social isolation test (84−91)	PND 84-91	–	–
Tan et al. (2019)	High	Mouse (Swiss)	160	M	Cohort1: PND56−70Cohort 2: PND49 −56	42	Two-mouse social isolation test		–	–
Tanaka et al. (2019)	Low	Rat (Long-Evans)	90	M & F	PND 23	~60	Social Preference Test or Social Approach Test (PND 70−84)	PND 70-84	Paraventricular nucleus Supraoptic Nucleus	Immunohistochemistry[#]
Oliveira et al. (2019)	Low	Rat (Wistar)	48	M & F	PND 21	53	Female Intruder Test Male Resident-intruder Test + Elevated Plus-Maze (PND 78), Social Preference Test (PND 86), and Social Discrimination Test (PND 88) (Cohort 2)	PND 74 (cohort 1) PND 88		Receptor Autoradiography[#, &]
Harvey et al. (2019)	High	Rat (Sprague-Dawley)	72	M & F	PND21	51	Elevated Plus-Maze (PND76)	PND78		Blood Immunoassay[#]
Han et al. (2018)	Low	Mouse (C57BL/6 N)	34*	M	PND42	35	One of the following: Forced Swimming Test Sucrose Preference Test Open Field Test Elevated Zero-Maze Test	PND77		qPCR[&] Mipsc[&]
Neal et al. (2018)	High	Rat (Long-Evans)	16*	M	PND28−31	40	Behavioral observation (social group) Social Investigation Task (PND61−64) Problem-solving Escape Task (PND~63−67)	PND68-71		Blood Immunoassay[#] Immunohistochemistry[#]
Gilles and Polston (2017)	Unsure	Rat (Sprague-Dawley)	88	M & F	PND21	82−86	Social Play (PND33−38) Forced Swim Test (PND60−64) Sucrose Preference Test (PND79−81)	PND103-107		Immunohistochemistry[#]
Tanaka et al. (2010)	High	Rat (Long-Evans)	16	M & F	23	17 (IHC) 19−25 (behavioral)	Elevated Plus Maze (38−41) Social Recognition Test (42−48)	40 (IHC) 42−48	PVN	Immunohistochemistry[#]
Nair (2005)	Unsure	Rat (Sprague-Dawley)	48	M		35	Startle test		Nucleus Accumbens (Shell) Lsi, Bed Nucleus of the Stria Terminals (lateral &) BNSTmpl, BlA, CeA and MeA)	Receptor Autoradiography[&]

Table 3
Summary of findings indicating alterations on (a) behavior and cognition; (b) and (c) Oxytocin levels when isolated animals are compared to control/grouped animals. Where (▲) indicates an increase; (▼) indicates a decrease, (●) indicates no change; and (n/m) indicates "not measured". * compared to unmedicated isolated animals.

Authors (year)	(a) Behavior and Cognition	(b) Oxytocin Indicators	(c) Pharmacological Approach
Huang et al. (2021)	▲repetitive behaviors ▲ anxiety-like behavior ▲ aggressive behavior ▼ direct and indirect social contact ▼ locomotor activity	● OXTR in the VTA, and NAcc ▼ OXTR in the mPFC	OXTR antagonist (L-368,899) in the mPFC mimics the effect of social isolation
Goh et al. (2020)	▲ aggressive behavior ▲ locomotor activity ▼ Prosocial behavior ▼ Associative Memory (fear) ▼Reversal learning	● OXT in the Hippocampus and Striatum	n/m
Tan et al. (2020)	▲aggressive behavior ● Social behaviors● Locomotor activity	n/m	OXTR antagonist (L-368,899; 10 mg/kg) did not affect behavior
Tan et al. (2019)	▲ aggressive behavior ▼ social behavior	n/m	OXT ▼ aggression; ▼ locomotor activity and ▲ social behavior* OXTR antagonist (L-368,899): ▼ aggression* OXTR agonist (L-368,899) + OXT: ▼ aggression; ▼ locomotor activity; ▲ social behavior* OXTR agonist: ▼ aggression; ▲ social behavior*
Tanaka et al. (2019)	▼ social preference (especially for females)	▼ OXT cell activation the PVN and SON (females)	n/m
Oliveira et al. (2019)	▲ aggressive behavior ● anxiety-like behavior ● social preference ▼ memory (social discrimination)	▲ OXT mRNA in the PVN (▲ females) ● OXT mRNA in the SON ● OXTR binding in the BNST ▼ OXTR binding in the NAcc (▲ f emales)	n/m
Harvey et al. (2019)	● Anxiety-like behavior	▼ OXT (plasma)	n/m
Han et al. (2018)	▲ depression-like behavior (immobility and sucrose consumption) ▲ anxiety-like behavior Both presented after 4 weeks of isolation, but not before	● mIPSC amplitude in the medial CeA ● OXTR mRNA in the PVN ▼ OXTR mRNA in the CeA (after the 5th week of isolation) ▼ mIPSC frequency in the medial CeA	OXT in the medial CeA: ▼ depressive-like and anxiety-like behaviors;▼ GABAergic inhibition; ▲ mIPSC frequency in the medial CeA
Neal et al. (2018)	● problem solving● social investigation	▲ OXT (plasma)	n/m
Gilles and Polston (2017)	▲ aggressive behavior ▲ social play ▲ depression-like behavior	● OXT-ir in SON and PVN	n/m
Tanaka et al. (2010)	▲ anxiety-like behavior (males) ▼ memory (discrimination)	● OXT-ir in te PVNpml or PVNmpv ▼ OXT-ir in the PVNmpd (females)	n/m
Nair (2005)	● startle test response	● OXTR binding in the ILS	n/m

year. Most papers (n = 8; 66.7 %) used rats in their experimentation, the strains used were Sprague-Dawley (n = 3; 37.5 %), Long-Evans (n = 3; 37.5 %), Wistar (n = 1; 12.5 %), and Lister-Hooded (n = 1; 12.5 %). Mice was used by four studies (33.3 %), C57BL/6 N (n = 2; 50 %) and Swiss (n = 2; 50 %) mice were chosen. Although males and females were investigated in some articles (n = 5; 41.7 %), most of them used male-only samples (n = 7; 58.3 %). The sample sizes accounted for varied from 16 to 160 (the sample sizes stated correspond to the number of animals in the groups analyzed in this review; other groups in the studies, when present, were not accounted), with a median of 70 animals. The sample size of Tanaka and collegues (2010) could not be identified. Different methodologies were observed to be used to direct or indirectly assess the animal's OXT levels (Table 2). As studies may have applied more than one method, compound, or measurement, the sum of studies from each described approach might vary.

Most of the articles (n = 8; 66.7 %) chose to perform biomolecular tests to directly assess the oxytocinergic system. Of those, 62.5 % used Immunohistochemistry (n = 5), while receptor autoradiography, Western Blotting, qPCR and Multiplex Analysis were also used by some. Peripheral measurement of OXT through plasma Immunoassay was used in two, one of which used the method to complement its Immunohistochemical assessment. One third of the studies (n = 4) chose an indirect approach to assess the system, administrating OXT antagonist (n = 3; 75 %), OXT (n = 2; 50 %), and/or OXT agonist (n = 1; 25 %). Most studies isolated animals following weaning at 21–23 post-natal days (n = 7), and the duration of the isolation varied between 17 day to 86 days. Han et al. (2018) identified that behavioral alterations only started to happen in the fourth week of isolation, but not before.

The use of behavioral testing was an inclusion criterion for this review, thus, all studies performed at least one behavioral task. Despite the diversity of tasks used, some seemed to be consistently used to assess certain behaviors. Social behavior was assessed by 58.3 % of the studies, while anxiety-like behavior was assessed by 47.7 % and locomotor activity 25 %.

3.2. Behavioral alterations due to social isolation

Out of the twelve studies assessed, nine (75 %) encountered behavioral and/or cognitive alterations in isolated animals when compared to group-housed animals (see Table 3). The most persistent behavioral alteration observed was an increase in aggressive behavior and anxiety-like behavior, found, respectively, by 100 % and 60 % of the studies which tested those matters. Even though social alterations were the most assessed behavioral characteristic, the type of social behavior investigated and the methods used by those were diverse. Three studies tested social preference (42.9 %) (Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019; Huang et al., 2021; Oliveira, Neumann, & de Jong, 2019; Tanaka et al., 2019; Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019; Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019; Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019;

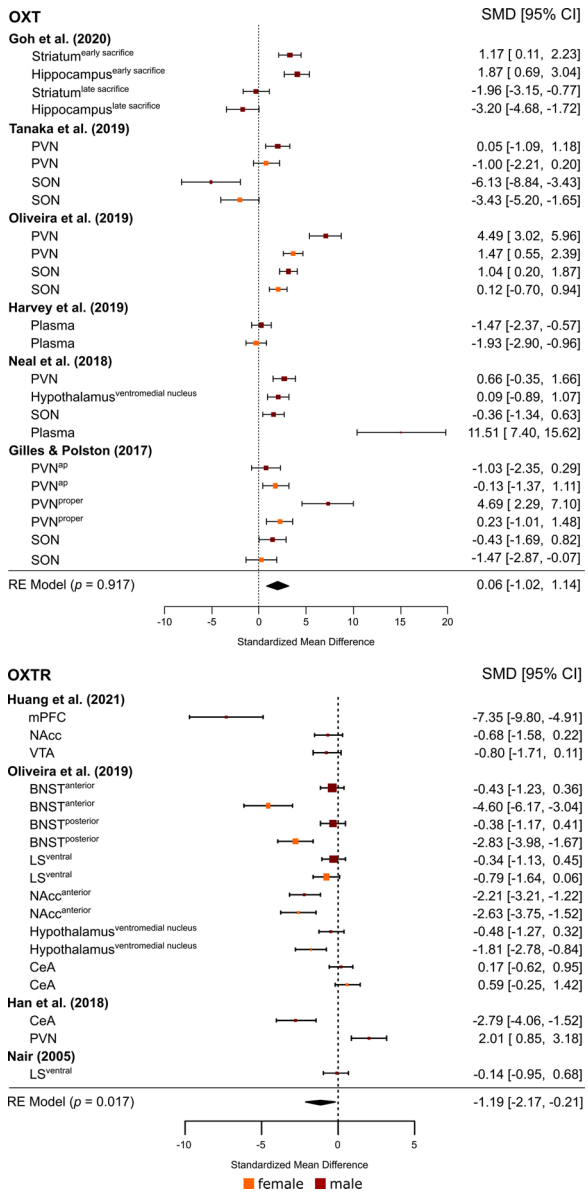

Fig. 2. Effect size of social isolation on OXT and OXTR. Forest plot indicating Standardized Mean Difference (SMD) and 95 % Confidence Interval (CI) using Random Effects Model (RE Model). PVN: Paraventricular Nucleus of the Hypothalamus (ap: anterior parvicellular division); SON: Supraoptic Nucleus; mPFC: medial Pre-frontal Cortex; NAcc: Nucleus Accumbens; VTA: Ventral Tegmental Area; BNST: Bed Nucleus of the Stria Terminals; LS: Lateral Septum; and CeA; Central Amygdala.

Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019; Huang et al., 2021; Oliveira et al., 2019; Tanaka et al., 2019), three studies investigated social interaction (42.9 %), one investigated social approach (14.3 %), and one investigated social play (14.3 %). The impacts of the isolation on social behaviors were not unanimous among studies.

Three studies investigating memory function in socially isolated animals found similar results, indicating a deficit in associative and discriminative memory. Other cognitive functions, such as problem solving, and fear response were investigated by a small number of studies using different methods. The first was assessed by Goh and colleagues (2020) and Neal and their team (2018), and was not significantly different between socially grouped or isolated animals in neither of the studies. Fear response was also investigated by two studies. Nair (2005) found that after a 3-week isolation, animals responded more intensely to the startle sound, while Goh et al. (2020) found a diminished fear response duration in isolated animals using footshocks.

3.3. Oxytocinergic measures

The most common areas investigated by the reviewed studies were the Paraventricular Nucleus of the Hypothalamus (PVN) and Supraoptic Nucleus of the Hypothalamus (SON), both assessed in six out of the eight studies which analyzed tissue samples and assessed cell reactivity via immunohistochemistry immunoreactivity. Out of those, three assessed cell activation, one of which found decreased cell activation in both areas, but only in females (Tanaka et al., 2019). One found decreased OXT cell immunoreactivity (OXT-ir) in one region of the PVN (PVNmpd: medial parvicellular part, ventral zone of the PVN), but not in other regions or the SON (Tanaka et al., 2010); and one did not find differences in OXT-ir (Gilles and Polston, 2017). Furthermore, two studies analyzed mRNA in these areas, the first found an increase in OXT mRNA in the PVM, especially in females while no differences were found in the SON (Oliveira et al., 2019); and a second study evaluated in PVN, where no significant changes were identified (Han et al., 2018).

Studies also proposed novel possible areas of association with SI, such as the mPFC, investigated by Huang et al., 2021 through the infusion of OXTR antagonist L-368,899 in this region 30 min before behavioral tests started, followed by immunohistochemical analysis, finding reduced OXTR expression in this structure in isolated mice. The same study also investigated the presence of OXTR in the VTA and NAcc, and found no significant differences between animals from each housing condition. Oliveira and collegues (2019) studied OXTR binding in the anterior part of the NAcc and found a lowered binding in isolated animals.

The CeA was also the focus of a study investigating depression- and anxiety-like behaviors in mice (Han et al., 2018). It was found that a decrease in OXTR expression occurs in the area; however, this occurs only after the fourth week of isolation (PND~77). Intermediate Lateral Septum (LSi), Hippocampus, and Striatum were also investigated (Goh et al., 2020; Nair, 2005), but did not seem to suffer alterations due to the isolation.

Above, results of the investigations of the central oxytocinergic system alterations were presented. However, two studies (Harvey et al., 2019; Neal et al., 2018) investigated its peripheral levels through blood plasma OXT measurements. Harvey et al. (2019) studied the role of OXT and other hormones in anxiety-like behavior in rats and found reduced levels of OXT in the plasma. In contrast to this finding, Neal and his team (2018) found an increased level of plasma OXT in isolated rats when studying the impact of environmental enrichment (the animals in the enriched environment group were not included in the present analysis, only isolated and group-housed animals were taken into account). However, the latest study result might have been biased due to the protocol, which included a brief isolation before the euthanasia. Corticosterone levels indicate that isolated animals were less stressed at the time of death and this may have altered OXT levels.

3.3.1. Pharmacological approaches

We chose to include studies which used the administration of OXT, OXT agonists, or OXT antagonists only in at least one sample group. Two out of the four studies with this approach used OXT to try to manipulate the behavior. Tan et al. (2019) investigated the behavioral response of isolated animals (6 weeks) to six different doses of OXT, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg, and 10 mg/kg, administered intraperitoneally (i.p.) in mice. A dose-dependent curve was performed and, as a result, it was found that the behaviors evaluated were unequally modified by the dosages applied, with 0.3 mg/kg mimicking the group-housed animal's aggressive behavior best, while 1 mg/kg had this result for huddling behavior and locomotor activity, and 3 mg/kg for grooming behavior. Local administration of OXT in the CeA decreased depressive and anxiety-like behaviors in animals isolated for 5 weeks

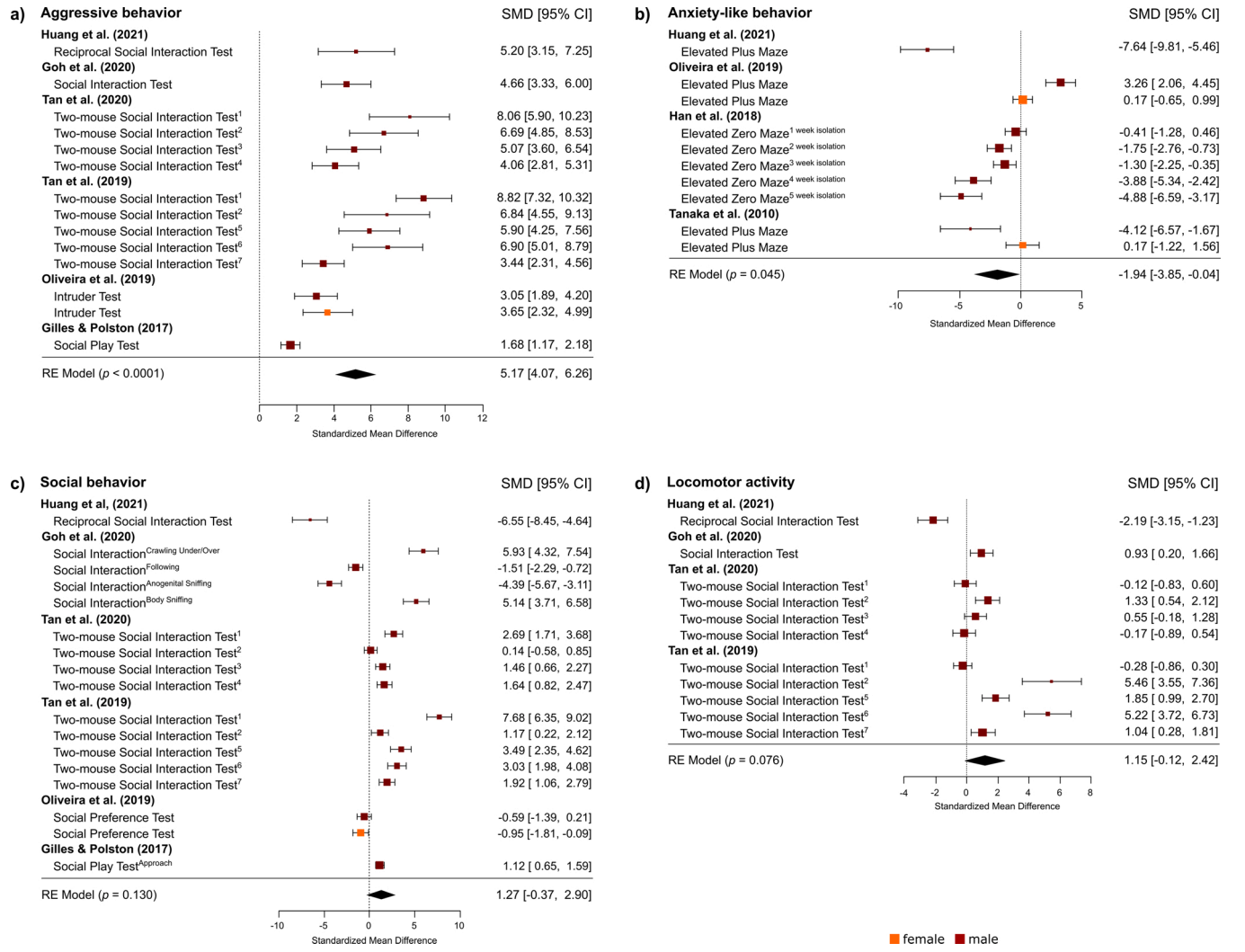

Fig. 3. Effect size of a) Aggressive behavior; b) Anxiety-like behavior; c) Social behavior; and d) Locomotor activity. Forest plot indicating Standardized Mean Difference (SMD) and 95 % Confidence Interval (CI) using Random Effects Model (RE Model).

(Han et al., 2018).

Administration of the OXTR agonist TGOT also had dose-dependent effects on the alterations caused by the isolation (Tan et al., 2019). Although fighting behavior only reached levels similar to group-housed animals with the highest dose administered (10 mg/kg), huddling and grooming behaviors were similar to group-housed animals with 1 mg/kg, the smallest dose administered. The locomotor activity reached levels similar to group-housed animals with 3 mg/kg.

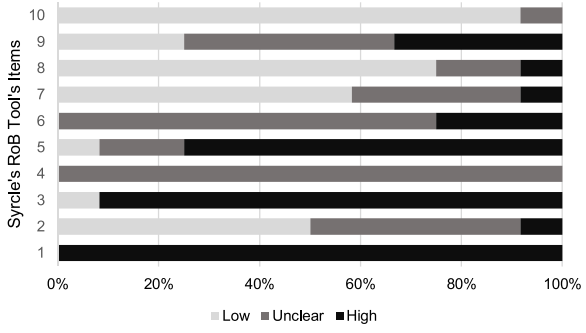

Fig. 4. Risk of Bias Assessment. The SYRCLE's Risk of Bias Assessment Tool was used to determine the level of risk of bias for each of 10 items evaluated by percentage of articles in each classification.

The OXTR antagonist L-368,899 was administered in three studies, in two of those via i.p. in isolated animals at the dose of 10 mg/kg (Tan et al., 2020, 2019), and the third study in grouped animals it was infused into the mPFC at the dose of 10 mM (Huang et al., 2021). While the infusion was able to replicate the behavioral alterations seen in isolates in group-housed animals (Huang et al., 2021), peripheral administration of the antagonist had different outcomes. In one study the agonist did not affect behavior (Tan et al., 2020), in the other it caused a decrease in aggressive behavior, reaching a level close to the grouped animals (Tan et al., 2019). OXTR antagonist (10 mg/kg) administration followed by OXT (3 mg/kg) resulted in a decrease of aggressive behavior and locomotor activity, and an increase in social behavior (huddling and grooming) to similar levels as grouped individuals (Tan et al., 2019), indicating that

3.4. Sex differences

Most of the studies used male samples, while five used male and female animals (Gilles and Polston, 2017; Harvey et al., 2019; Oliveira et al., 2019; Tanaka et al., 2019, 2010), and three of those found significant differences between sexes regarding OXT measures (Oliveira et al., 2019; Tanaka et al., 2019, 2010). Tanaka et al. (2010) found increased anxiety-like behavior, especially in males and a decreased presence of OXT-ir in the medial parvicellular part of the PVN of isolated

females. In a second study, Tanaka et al. (2019) found a more significant decrease on social preference in isolated females, while identifying lowered levels of oxytocin cell activation in the PVN and SON in isolated females, but not isolated males. Oliveira et al. (2019) measured the expression of OXT mRNA in PNV and SON, finding an increased expression in PVN, especially in females. The authors also evaluated OXT binding in the NAcc and BNST, which showed that regardless of the housing situation, females showed higher binding in the NAcc, while males presented higher binding in the BNST. When accounting for housing situation, NAcc binding decreased in isolated animal of both sexes (Oliveira et al., 2019).

3.5. Meta-Analysis

Meta-analysis for the biological material was performed in 9 studies previously included in the systematic review, four of those evaluated OXT levels in multiple brain regions and/or plasma, and another four evaluated OXTR, also in multiple regions. Oliveira and colegues (2019) investigated both markers. Outcomes of pharmacological approaches were not analyzed. Twenty-four effect sizes for OXT were analyzed, resulting in no significant effect of SI on this marker (SMD 0.06; 95 % CI −1.02, 1.14; $p = 0.917$. However, SI did have an impact on OXT receptor levels (SMD −1.19; 95 % CI −2.17, −0.21; $p = 0.017$). Analysis of 18 effect sizes showed that OXTR was reduced in isolated animals (Fig. 2). Sex, species, and time between last behavioral test and tissue collection (<24 h or >24 h) we tested as factors and no significance was found.

Aggressive, anxiety-like, social behavior and locomotor activity were also assessed through a meta-analysis. A total of 14 effect sizes, from six studies, were used for the analysis of aggressive behavior alterations. The effect size of the four experiments presented in Tan et al. (2020) were included, while only five of the seven experiments reported on Tan et al. (2019) were included as re-tested animals were excluded from the analysis. As the data presented in Fig. 3a suggest, this kind of behavior is strongly impacted by SI (SMD 5.17; 95 % CI 4.07, 6.26; $p < 0.0001$). Another behavior that seems to be impacted by the intervention is anxiety-like behavior (Fig. 3b). Four out of five studies which investigated anxiety-like behavior were included in the analysis, Harvey et al. (2019) was not included due to missing data. Analysis using ten effect sizes indicated a slim association between SI and anxiety-like behavior (SMD −1.94; 95 % CI −3.85, −0.04; $p = 0.045$). When the female data were excluded from the analysis, the association remains (SMD -2.50; 95 % CI −4.77, −0.23; $p = 0.031$).

Social behaviors (Fig. 3c) and locomotor activity (Fig. 3d) were also analyzed using 17 and 11 effect sizes, respectively. No significant differences between the SI and control groups were found in the variables (Social Behavior: SMD 1.27; 95 % CI −0.37, 2.9; $p = 0.130$; Locomotor Activity: SMD 1.15; CI 95 % −0.12, 2.42; $p = 0.076$).

3.6. Reporting bias and certainty assessment

The risk of bias of the 12 studies was assessed through the SYRCLE Risk of Bias tool (Hooijmans et al., 2014) (see Fig. 4). Only two studies (17 %; Tan et al., 2020; Tanaka et al., 2019) were evaluated to have a "low RoB" for most items, while "high RoB" was the most frequent score for five studies (42 %; Gilles and Polston, 2017; Nair, 2005; Neal et al., 2018; Oliveira et al., 2019; Tanaka et al., 2010). Another five studies had "unclear RoB" the most (Goh et al., 2020; Han et al., 2018; Harvey et al., 2019; Huang et al., 2021; Tan et al., 2019).

Overall, the RoB regarding Selection Bias, evaluated through items 1 through 3, appeared to be high, as no studies stated to have referred to perform a random allocation sequencing (item 1), and only one indicated to have adequately concealed allocation during the experiments (item 3). The baseline characteristics of each group were clearly stated in half of the studies (item 2). Performance Bias was assessed through items 4 and 5, and was also considered high by the authors considering 100 % of the studies did not state to have randomly distributed animals through house (item 4), the investigators were mostly not blinded from the knowledge of the grouping situation of the subjects (item 5). While no studies referred to have randomly selected animals for assessments (item 6), the majority of those indicated that the assessor was blinded to the outcome and grouping (item 7). Attrition due to incomplete data (item 8) and other forms of bias (item 10) also appeared to be predominantly low in all selected studies, while reporting bias was mostly unclear (item 9).

The assessment of bias due to unreported information about methods and materials used in immunohistochemical analysis showed that despite all articles which used this technique having informed the product and manufacturer of the antibodies used, only Huang et al. (2021) specified the product code. No studies reported elements 2 and 3 regarding specificity and control for immunostaining.

4. Discussion

The aim of this systematic review and meta-analysis was to compile, summarize, and compare studies' outcomes and methodologies to better understand the effects of SI on the oxytocinergic system, as well as its behavioral repercussions. Consistent with our hypothesis, the collected data indicated a relationship between oxytocinergic system alterations and behavioral alterations caused by SI, as we found a significant decrease in OXT receptor count in multiple brain regions associated with SI (Fig. 2), regardless of the duration of the isolation protocol. However, no significant alterations were found in OXT levels. An increase in aggressive and anxious-like behavior and locomotor activity was commonly reported after SI and was linked to the oxytocinergic pathway once OXT administration was often able to reverse those responses. Overall, these results indicated that the oxytocinergic system is impacted by SI despite length of the isolation, as well as the behavioral outcomes are somewhat mediated by OXT levels.

A wide range of protocols are available for researchers to investigate the different types and particularities of stress. SI protocols study the effect of stress through lack of social contact in a given period of the animal's lifespan, usually post-weaning (Lapiz et al., 2003; Noschang et al., 2021). SI can produce long-lasting to permanent physiological and behavioral alterations due to chronic hyperactivity of the HPA-axis and consequent disruption of negative feedback modulation (Deutschmann et al., 2021; Lampert et al., 2017; Lapiz et al., 2003; Neumann and Slattery, 2016; Noschang et al., 2021; Pisu et al., 2016; Regenass et al., 2018; Stanić et al., 2017). Some of those well-reported alterations, such as increased locomotor activity, anxiety-like and aggressive behavior, and decreased sociability and memory (Noschang et al., 2021), were often present in most of the reviewed studies, even though our meta-analysis only has shown a significant effect of social isolation in anxiety-like behavior and aggression, but not in social or locomotor activity.

However, the effects of SI-induced stress are not restricted to HPA-axis alterations. Among other repercussions, reduced corticosterone (CORT) levels in isolated animals were also associated with decreased levels of OXT (Harvey et al., 2019). In fact, OXT up- and down-regulates the HPA-axis modulating the response to stressors, as well as is regulated by the HPA-axis through the release of corticosterone (Jirikowski et al., 2017; Stanić et al., 2017). After administering CORT, OXT, or CORT + OXT to a group of rats, Stanić et al. (2017) found that the peptide reduced adrenal atrophy and other symptoms caused by CORT administration.

Even though in our meta-analysis OXT levels did not appear to be significantly different between group or isolated-reared animals, many of the behavioral effects of SI were reversed by the administration of synthetic oxytocin or its agonists (Tan et al., 2019). Additionally, OXT antagonist administered in group-housed animals was able to mimic the behavioral effects of the isolation. The OXT receptor (OXTR) count did, however, differ significantly between the groups, indicating that the production of the neuromodulatory hormone is sustained in spite of the

stressful scenario, and that the reduced number of immunoreactive OXTR and therefore the decreased number of synapsis (as occurs when the OXT antagonist is administered), may be responsible for the alterations seen in the isolates. Although the mechanisms for those are still to be described, one can hypothesize that GABA induced inhibition could be behind the OXT-dependent behavioral alterations caused by isolation, as described by Han and colleagues (2018) when studying the medial central amygdala, due to the modulatory effect of OXT on GABAergic circuits.

Because of its anxiolytic and anti-aggression effects in animals (Korkmaz et al., 2020), OXT has emerged as a potential treatment for several disorders. Intranasal OXT dispensers have shown promising results in trials with humans, increasing emotion recognition and expression (Leppanen et al., 2017), as well as for the treatment of psychiatric and neurologic disorders such as depression (Donadon et al., 2021; Panek et al., 2020; Tang et al., 2019), anxiety (Guastella et al., 2009; Panek et al., 2020), addiction (Hansson et al., 2018; Houghton et al., 2021; Lee and Weerts, 2016); and autism (Anagnostou et al., 2014) and dementia (Jesso et al., 2011) symptoms in humans, especially for those experiencing aggressive behavior (de Jong and Neumann, 2017). Exposure to chronic treatment with OXT could potentially partially or completely reverse the OXTR reduction through positive feedback, increasing OXTR mRNA transcription and restoring function (Sue Carter et al., 2020).

Literature is not unanimous about independent variables' (e.g. sex, species and strain) differences in the response to SI (Dumais and Veenema, 2016; Lapiz et al., 2003; Pisu et al., 2016), and regarding OXT and OXTR, our meta-analysis' indicated the inexistence of differences between either of the forementioned factors. Similarly, in our analysis the length of isolation did not appear to impact our outcome variables, despite substantial evidence that different lengths and periods of isolation do impact behavioral and physiological outcomes (Lapiz et al., 2003; Noschang et al., 2021), which indicated those alterations might not be related to OXT or OXTR. However, these results should be accounted carefully considering the variability of protocols used in the reviewed studies.

4.1. Conclusion and limitations

The current systematic and meta-analytic review offers an idea of the state of findings regarding the impacts of stress through SI (social stress) on the oxytocinergic system (highly associated with social functioning and reward). This kind of stress seems to alter the binding of OXT receptors in the brain, reflecting in increased anxiety-like behavior, hyperactivity, aggression, and memory, while the administration of the peptide in its synthetic form or its agonists partially decreases those unwanted behaviors to similar levels of control animals. However, those results should be accounted carefully considering the variability of protocols used in the reviewed studies, as the diversity of methods to assess behavioral and cognitive patterns, as well as sex, strain, and species was evident in this review. To allow this kind of investigation to be performed, it is important for researchers to select the tests and tasks to be used based mainly on similar previous studies. The lack of studies of females is also unfortunate, considering that it precludes the meta-analytical comparison between sexes. Another potential source of bias in the meta-analysis of animal studies is the dismissal of data when group differences are not 'statistically significant', limiting the availability of data to those that reinforce a general hypothesis.

Funding

The first author of this review is contemplated with a research grant by CAPES (en: Coordination for the Improvement of Higher Education Personnel; pt: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The other authors receive funding from CNPq (en: National Council for Scientific and Technological Development; pt: Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Registration

This review was registered in PROSPERO (International prospective register of systematic reviews) under the registration number CRD42020215733.

All quantitative and qualitative data are available under request to the corresponding author.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neubiorev.2022.104549.

References

Anagnostou, E., Soorya, L., Brian, J., Dupuis, A., Mankad, D., Smile, S., Jacob, S., 2014. Intranasal oxytocin in the treatment of autism spectrum disorders: a review of literature and early safety and efficacy data in youth. Brain Res. 1580, 188–198. https://doi.org/10.1016/j.brainres.2014.01.049.

Boll, S., Almeida de Minas, A.C., Raftogianni, A., Herpertz, S.C., Grinevich, V., 2018. Oxytocin and pain perception: from animal models to human research. Neuroscience 387, 149–161. https://doi.org/10.1016/j.neuroscience.2017.09.041.

Bowen, M.T., Neumann, I.D., 2017. Rebalancing the addicted brain: oxytocin interference with the neural substrates of addiction. Trends Neurosci. 40 (12), 691–708. https://doi.org/10.1016/j.tins.2017.10.003.

Burke, A.R., Miczek, K.A., 2014. Stress in adolescence and drugs of abuse in rodent models: role of dopamine, CRF, and HPA axis. In: Psychopharmacology, 231. https://doi.org/10.1007/s00213-013-3369-1.

Burke, A.R., McCormick, C.M., Pellis, S.M., Lukkes, J.L., 2017. Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. Neurosci. Biobehav. Rev. 76, 280–300. https://doi.org/10.1016/j.neubiorev.2017.01.018.

Cilz, N.I., Cymerblit-Sabba, A., Young, W.S., 2018. Oxytocin and vasopressin in the rodent hippocampus. Genes Brain Behav. 18 (1), e12535 https://doi.org/10.1111/gbb.12535.

Dafny, N., Yang, P.B., 2006. The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: a review of its locomotor effects. Brain Res. Bull. 68 (6), 393–405. https://doi.org/10.1016/j.brainresbull.2005.10.005.

de Jong, T.R., Neumann, I.D., 2017. Oxytocin and aggression. Brain Imaging in Behavioral Neuroscience, pp. 175–192. https://doi.org/10.1007/7854_2017_13.

Deutschmann, A.U., Kirkland, J.M., Briand, L.A., 2021. Adolescent social isolation induced alterations in nucleus accumbens glutamate signalling. Addict. Biol., e13077 https://doi.org/10.1111/adb.13077.

Donadon, M.F., Martin-Santos, R., Osório, F.L., 2021. Oxytocin effects on the cognition of women with postpartum depression: a randomized, placebo-controlled clinical trial. Prog. Neuropsychopharmacol. Biol. Psychiatry 111 (June 2020). https://doi.org/10.1016/j.pnpbp.2020.110098.

Dumais, K.M., Veenema, A.H., 2016. Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior. Front. Neuroendocrinol. 40, 1–23. https://doi.org/10.1016/j.yfrne.2015.04.003.

Ferris, C.F., Foote, K.B., Meltser, H.M., Plenby, M.G., Smith, K.L., Insel, T.R., 1992. Oxytocin in the amygdala facilitates maternal aggression. Ann. N. Y. Acad. Sci. 652 (1), 456–457. https://doi.org/10.1111/j.1749-6632.1992.tb34382.x.

Gilles, Y.D., Polston, E.K., 2017. Effects of social deprivation on social and depressive-like behaviors and the numbers of oxytocin expressing neurons in rats. Behav. Brain Res. 328, 28–38. https://doi.org/10.1016/j.bbr.2017.03.036.

Goh, J.Y., O'Sullivan, S.E., Shortall, S.E., Zordan, N., Piccinini, A.M., Potter, H.G., et al., 2020. Gestational poly(I:C) attenuates, not exacerbates, the behavioral, cytokine and mTOR changes caused by isolation rearing in a rat 'dual-hit' model for neurodevelopmental disorders. Brain Behav. Immun. 89 (January), 100–117. https://doi.org/10.1016/j.bbi.2020.05.076.

Grillon, C., Krimsky, M., Charney, D.R., Vytal, K., Ernst, M., Cornwell, B., 2013. Oxytocin increases anxiety to unpredictable threat. Mol. Psychiatry 18 (9), 958–960. https://doi.org/10.1038/mp.2012.156.

Guastella, A.J., Howard, A.L., Dadds, M.R., Mitchell, P., Carson, D.S., 2009. A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. Psychoneuroendocrinology 34 (6), 917–923. https://doi.org/10.1016/j.psyneuen.2009.01.005.

Han, R.T., Kim, Y.-B., Park, E., Kim, J.Y., Ryu, C., Kim, H.Y., et al., 2018. Long-term isolation elicits depression and anxiety-related behaviors by reducing oxytocin-induced GABAergic transmission in Central Amygdala. Front. Mol. Neurosci. 11 (August), 1–12. https://doi.org/10.3389/fnmol.2018.00246.

Hansson, A.C., Koopmann, A., Uhrig, S., Bühler, S., Domi, E., Kiessling, E., et al., 2018. Oxytocin reduces alcohol cue-reactivity in alcohol-dependent rats and humans. Neuropsychopharmacology 43 (6), 1235–1246. https://doi.org/10.1038/npp.2017.257.

Harvey, B.H., Regenass, W., Dreyer, W., Möller, M., 2019. Social isolation rearing-induced anxiety and response to agomelatine in male and female rats: role of corticosterone, oxytocin, and vasopressin. J. Psychopharmacol. 33 (5), 640–646. https://doi.org/10.1177/0269881119826783.

Hooijmans, C.R., Rovers, M.M., De Vries, R.B.M., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14 (1), 1–9. https://doi.org/10.1186/1471-2288-14-43.

Houghton, B., Kouimtsidis, C., Duka, T., Paloyelis, Y., Bailey, A., 2021. Can intranasal oxytocin reduce craving in automated addictive behaviours? A systematic review. Br. J. Pharmacol., bph.15617 https://doi.org/10.1111/bph.15617.

Huang, L., Duan, C., Xia, X., Wang, H., Wang, Y., Zhong, Z., et al., 2021. Commensal microbe-derived propionic acid mediates juvenile social isolation-induced social deficits and anxiety-like behaviors. Brain Res. Bull. 166 (December 2020), 161–171. https://doi.org/10.1016/j.brainresbull.2020.12.001.

Insel, T.R., Young, L., Wang, Z., 1997. Central oxytocin and reproductive behaviours. Rev. Reprod. 2 (1), 28–37. https://doi.org/10.1530/ror.0.0020028.

Jesso, S., Morlog, D., Ross, S., Pell, M.D., Pasternak, S.H., Mitchell, D.G.V., et al., 2011. The effects of oxytocin on social cognition and behaviour in frontotemporal dementia. Brain 134 (9), 2493–2501. https://doi.org/10.1093/brain/awr171.

Jirikowski, G.F., Ochs, S.D., Caldwell, J.D., 2017. Oxytocin and steroid actions. In: Current Topics in Behavioral Neurosciences, 35. Springer, Cham, pp. 77–95. https://doi.org/10.1007/7854_2017_9.

Jurek, B., Neumann, I.D., 2018. The oxytocin receptor: from intracellular signaling to behavior. Physiol. Rev. 98 (3), 1805–1908. https://doi.org/10.1152/physrev.00031.2017.

Korkmaz, H., Önal, D., Alışık, M., Erel, Ö., Pehlivanoğlu, B., 2020. The impact of oxytocin on thiol/disulphide and malonyldialdehyde/glutathione homeostasis in stressed rats. Biol. Chem. 401 (11), 1283–1292. https://doi.org/10.1515/hsz-2020-0190.

Lampert, C., Arcego, D.M., de Sá Couto-Pereira, N., dos Santos Vieira, A., Toniazzo, A.P., Krolow, R., et al., 2017. Short post-weaning social isolation induces long-term changes in the dopaminergic system and increases susceptibility to psychostimulants in female rats. Int. J. Dev. Neurosci. 61 (February), 21–30. https://doi.org/10.1016/j.ijdevneu.2017.05.003.

Lapiz, M.D.S., Fulford, A., Muchimapura, S., Mason, R., Parker, T., Marsden, C.A., 2003. Influence of postweaning social isolation in the rat on brain development, contioned behavior, and neurotransmission. Neurosci. Behav. Physiol. 33 (1), 13–29. https://doi.org/10.1023/A:1021171129766.

Lee, M.R., Weerts, E.M., 2016. Oxytocin for the treatment of drug and alcohol use disorders. Behav. Pharmacol. 27 (8), 640–648. https://doi.org/10.1097/FBP.0000000000000258.

Leong, K.-C., Cox, S., King, C., Becker, H., Reichel, C.M., 2018. Oxytocin and rodent models of addiction In: International Review of Neurobiology, 1st ed., 140. Elsevier Inc., pp. 201–247. https://doi.org/10.1016/bs.irn.2018.07.007

Leppanen, J., Ng, K.W., Tchanturia, K., Treasure, J., 2017. Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions. Neurosci. Biobehav. Rev. 78 (April), 125–144. https://doi.org/10.1016/j.neubiorev.2017.04.010.

Lien, Y.J., Chang, H.H., Tsai, H.C., Kuang Yang, Y., Lu, R.B., See Chen, P., 2017. Plasma oxytocin levels in major depressive and bipolar II disorders. Psychiatry Res. 258 (August), 402–406. https://doi.org/10.1016/j.psychres.2017.08.080.

Lockhart, S., Sawa, A., Niwa, M., 2018. Developmental trajectories of brain maturation and behavior: relevance to major mental illnesses. J. Pharmacol. Sci. 137 (1), 1–4. https://doi.org/10.1016/j.jphs.2018.04.008.

Love, T.M., 2014. Oxytocin, motivation and the role of dopamine. Pharmacol. Biochem. Behav. 119, 49–60. https://doi.org/10.1016/j.pbb.2013.06.011.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10 (6), 434–445. https://doi.org/10.1038/nrn2639.

Makinodan, M., Rosen, K.M., Ito, S., Corfas, G., 2012. A critical period for social experience–dependent oligodendrocyte maturation and myelination. Science 337 (6100), 1357–1360. https://doi.org/10.1126/science.1220845.

Marlin, B.J., Froemke, R.C., 2017. Oxytocin modulation of neural circuits for social behavior. Dev. Neurobiol. 77 (2), 169–189. https://doi.org/10.1002/dneu.22452.

Mumtaz, F., Khan, M.I., Zubair, M., Dehpour, A.R., 2018. Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. Biomed. Pharmacother. 105 (December 2017), 1205–1222. https://doi.org/10.1016/j.biopha.2018.05.086.

Nair, H.P., 2005. Central oxytocin, vasopressin, and corticotropin-releasing factor receptor densities in the basal forebrain predict isolation potentiated startle in rats. J. Neurosci. 25 (49), 11479–11488. https://doi.org/10.1523/JNEUROSCI.2524-05.2005.

Neal, S., Kent, M., Bardi, M., Lambert, K.G., 2018. Enriched environment exposure enhances social interactions and oxytocin responsiveness in male long-evans rats. Front. Behav. Neurosci. 12 (September), 1–11. https://doi.org/10.3389/fnbeh.2018.00198.

Neumann, I.D., 2007. Stimuli and consequences of dendritic release of oxytocin within the brain. Biochem. Soc. Trans. 35 (5), 1252–1257. https://doi.org/10.1042/BST0351252.

Neumann, I.D., Slattery, D.A., 2016. Oxytocin in general anxiety and social fear: a translational approach. Biol. Psychiatry 79 (3), 213–221. https://doi.org/10.1016/j.biopsych.2015.06.004.

Noschang, C., Lampert, C., Krolow, R., de Almeida, R.M.M., 2021. Social isolation at adolescence: a systematic review on behaviour related to cocaine, amphetamine and nicotine use in rats and mice. Psychopharmacology 1–36. https://doi.org/10.1007/s00213-021-05777-z.

Oliveira, V.E., de, M., Neumann, I.D., de Jong, T.R., 2019. Post-weaning social isolation exacerbates aggression in both sexes and affects the vasopressin and oxytocin system in a sex-specific manner. Neuropharmacology 156 (October 2018), 107504. https://doi.org/10.1016/j.neuropharm.2019.01.019.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., et al., 2021a. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ, n71. https://doi.org/10.1136/bmj.n71.

Page, M.J., Moher, D., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., et al., 2021b. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ, n160. https://doi.org/10.1136/bmj.n160.

Panek, M., Kawalec, P., Pilc, A., Lasoń, W., 2020. Developments in the discovery and design of intranasal antidepressants. Expert Opin. Drug Discov. 15 (10), 1145–1164. https://doi.org/10.1080/17460441.2020.1776697.

Pisu, M.G., Garau, A., Boero, G., Biggio, F., Pibiri, V., Dore, R., et al., 2016. Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. Neuroscience 320, 172–182. https://doi.org/10.1016/j.neuroscience.2016.02.009.

Regenass, W., Möller, M., Harvey, B.H., 2018. Studies into the anxiolytic actions of agomelatine in social isolation reared rats: role of corticosterone and sex. J. Psychopharmacol. 32 (2), 134–145. https://doi.org/10.1177/0269881117735769.

Robbins, T.W., 2016. Neurobehavioural sequelae of social deprivation in rodents revisited: modelling social adversity for developmental neuropsychiatric disorders. J. Psychopharmacol. 30 (11), 1082–1089. https://doi.org/10.1177/0269881116664450.

Saper, C.B., 2005. An open letter to our readers on the use of antibodies. J. Comp. Neurol. 493 (4), 477–478. https://doi.org/10.1002/cne.20839.

Saper, C.B., 2009. A guide to the perplexed on the specificity of antibodies. J. Histochem. Cytochem. 57 (1), 1–5. https://doi.org/10.1369/jhc.2008.952770.

Saper, C.B., Sawchenko, P.E., 2003. Magic peptides, magic antibodies: guidelines for appropriate controls for immunohistochemistry. J. Comp. Neurol. 465 (2), 161–163. https://doi.org/10.1002/cne.10858.

Shalvi, S., De Dreu, C.K.W., 2014. Oxytocin promotes group-serving dishonesty. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.1400724111.

Shamay-Tsoory, S.G., Abu-Akel, A., 2016. The social salience hypothesis of oxytocin. Biol. Psychiatry 79 (3), 194–202. https://doi.org/10.1016/j.biopsych.2015.07.020.

Silvers, J.A., 2022. Adolescence as a pivotal period for emotion regulation development for consideration at current opinion in psychology. Curr. Opin. Psychol. 44, 258–263. https://doi.org/10.1016/j.copsyc.2021.09.023.

Sippel, L.M., Allington, C.E., Pietrzak, R.H., Harpaz-Rotem, I., Mayes, L.C., Olff, M., 2017. Oxytocin and stress-related disorders: neurobiological mechanisms and treatment opportunities. Chronic Stress 1. https://doi.org/10.1177/2470547016687996.

Stanić, D., Plećaš-Solarović, B., Mirković, D., Jovanović, P., Dronjak, S., Marković, B., et al., 2017. Oxytocin in corticosterone-induced chronic stress model: focus on adrenal gland function. Psychoneuroendocrinology 80, 137–146. https://doi.org/10.1016/j.psyneuen.2017.03.011.

Sue Carter, C., Kenkel, W.M., Maclean, E.L., Wilson, S.R., Perkeybile, A.M., Yee, J.R., et al., 2020. Is oxytocin "nature's medicine"? Pharmacol. Rev. 72 (4), 829–861. https://doi.org/10.1124/pr.120.019398.

Tan, O., Musullulu, H., Raymond, J.S., Wilson, B., Langguth, M., Bowen, M.T., 2019. Oxytocin and vasopressin inhibit hyper-aggressive behaviour in socially isolated mice. Neuropharmacology 156, 107573. https://doi.org/10.1016/j.neuropharm.2019.03.016.

Tan, O., Martin, L.J., Bowen, M.T., 2020. Divergent pathways mediate 5-HT 1A receptor agonist effects on close social interaction, grooming and aggressive behaviour in mice: exploring the involvement of the oxytocin and vasopressin systems. J. Psychopharmacol. 34 (7), 795–805. https://doi.org/10.1177/0269881120913150.

Tanaka, K., Osako, Y., Yuri, K., 2010. Juvenile social experience regulates central neuropeptides relevant to emotional and social behaviors. Neuroscience 166 (4), 1036–1042. https://doi.org/10.1016/j.neuroscience.2010.01.029.

Tanaka, K., Osako, Y., Takahashi, K., Hidaka, C., Tomita, K., Yuri, K., 2019. Effects of post-weaning social isolation on social behaviors and oxytocinergic activity in male and female rats. Heliyon 5 (5), e01646. https://doi.org/10.1016/j.heliyon.2019.e01646.

Tang, A.L., Thomas, S.J., Larkin, T., 2019. Cortisol, oxytocin, and quality of life in major depressive disorder. Qual. Life Res. 28 (11), 2919–2928. https://doi.org/10.1007/s11136-019-02236-3.

Trezza, V., Baarendse, P.J.J., Vanderschuren, L.J.M.J., 2014. On the interaction between drugs of abuse and adolescent social behavior. Psychopharmacology 231 (8), 1715–1729. https://doi.org/10.1007/s00213-014-3471-z.

Vanderschuren, L.J.M.J., Trezza, V., 2013. What the laboratory rat has taught us about social play behavior: role in behavioral development and neural mechanisms. Brain Imaging in Behavioral Neuroscience, pp. 189–212. https://doi.org/10.1007/7854_2013_268.

Walker, D.M., Cunningham, A.M., Gregory, J.K., Nestler, E.J., 2019. Long-term behavioral effects of post-weaning social isolation in males and females. Front. Behav. Neurosci. 13 (April), 1–20. https://doi.org/10.3389/fnbeh.2019.00066.