Psychoneuroendocrinology 186 (2026) 107777

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Long-term offspring loss in lactating rats: Neurobiological and emotional consequences in a novel animal model

Luisa Demarchi [1], Alice Sanson, Anna-Lena Boos, Oliver J. Bosch [*]

Department of Behavioural and Molecular Neurobiology, Regensburg Center for Neuroscience, University of Regensburg, 93053 Regensburg, Germany

ARTICLE INFO

Keywords:
Maternal bond
Oxytocin
Corticotropin-releasing factor
Stress-coping behavior
Hypothalamic-pituitary-adrenal Axis

ABSTRACT

The maternal bond is a vital social connection that supports the survival and well-being of both the caregiver and offspring. Disruption of this bond, particularly following offspring loss, can result in profound trauma with long-lasting consequences. While considerable research has focused on the impact of maternal separation on offspring development, the biological effects of offspring loss on the mother remain largely unexplored. In this study, we examined the long-term effects of offspring loss on neuroplasticity, the oxytocin (OXT) and corticotropin-releasing factor (CRF) systems, and stress-coping behaviors in Sprague-Dawley rat mothers. We examined two groups of lactating mothers: (I) a control group, in which dams remained with their pups until natural weaning, and (II) a separated group, in which all offspring were removed on lactation day 1 and the mothers experienced offspring loss until the time corresponding to weaning (19 days). Our results reveal that pup removal increased oxytocin receptor binding–most prominently in the ventromedial hypothalamus (VMH)–and reduced dendritic spine density in this brain region, without altering estrogen receptor α or calbindin cell expression. Separated mothers additionally showed elevated plasma corticosterone levels and increased passive stress-coping behaviors in the forced swim test. Remarkably, passive stress-coping behavior was rescued by central CRF receptor blockade but not by central OXT treatment, indicating that the CRF system plays a critical role in the distress response to offspring loss. These findings establish a novel rat model to investigate the neurobiological consequences of maternal stress following offspring loss.

1. Introduction

The maternal-infant bond is among the strongest social bonds in mammals (Rilling and Young, 2014). In humans, disruption of this bond such as through offspring loss, elicits grief-like reactions in bereaved mothers, encompassing a range of emotional, cognitive, and behavioral responses, including depression and altered stress response. These dysregulations can progress to prolonged grief disorder (PGD) (O'Connor, 2019; Alves et al., 2022; McCarthy et al., 2010). Despite the clinical relevance, the neurobiological consequences of offspring loss on the maternal brain -particularly in relation to neuroplasticity and the oxytocin (OXT) and corticotropin-releasing factor (CRF) systems- remain poorly understood. Rodent studies have been instrumental in characterizing maternal bonding. Reduced maternal care in rats negatively impacts the offspring development and acts as emotional trauma (for review see (Nemeroff, 2016)). Importantly, repeated offspring separation has been shown to induce significant behavioral consequences in mothers (Demarchi et al., 2023; Baracz et al., 2020; Bolukbas et al., 2020; Alves et al., 2020). However, repeated separations do not fully model the experience of complete offspring loss -a scenario more analogous to human maternal grief. Only a limited number of studies have specifically investigated permanent offspring loss in rat mothers (Pawluski et al., 2009; Rincon-Cortes and Grace, 2021) (for review see (Demarchi et al., 2021)). Previous work has shown that permanent pup removal induces long-lasting negative affective states in dams, characterized by enhanced passive stress-coping behavior, reduced social motivation, and persistent neurobiological alterations, including disrupted mesolimbic dopamine function, with some effects partially mitigated by social buffering (Pawluski et al., 2009; Rincon-Cortes and Grace, 2021). Our previous work demonstrates that offspring loss immediately after birth alters maternal brain function and stress-coping behavior during the first postpartum week, including heightened passive

* Correspondence to: Department of Behavioural and Molecular Neurobiology, Regensburg Center of Neuroscience, University of Regensburg, Universitaetsstr. 31, 93053 Regensburg, Germany.
E-mail addresses: ldemarchi@fordham.edu (L. Demarchi), alice.sanson@ur.de (A. Sanson), anna-lenaboos@gmx.de (A.-L. Boos), oliver.bosch@ur.de (O.J. Bosch).
[1] Current address: Department of Biological Sciences, Fordham University, Bronx, NY, USA.

https://doi.org/10.1016/j.psyneuen.2026.107777
Received 30 October 2025; Received in revised form 26 January 2026; Accepted 30 January 2026
Available online 1 February 2026

stress-coping, altered neuronal activity, and decreased OXT receptor (OXT-R) binding in the central amygdala (CeA) (Demarchi et al., 2024). Building on these findings, we hypothesized that prolonged offspring loss over 19 days in rats could cause enduring neurobiological changes paralleling aspects of human grief (Demarchi et al., 2021), with particular emphasis on the CRF and OXT systems. Disruption of social bonds in rodents affects neurotransmitter systems, neuroplasticity and behavior (Shirenova et al., 2023; Dimonte et al., 2023). For example, in the monogamous and biparental prairie vole (*Microtus ochrogaster*) partner loss enhances CRF system signaling, reduces OXT signaling specifically in the nucleus accumbens (NAcc), and alters microglial activity and morphology in a brain region- and sex-specific manner (Bosch et al., 2009, 2016, 2018; Pohl et al., 2021).

The CRF system, a key mediator of stress and depression, and has been extensively studied in both humans and rodents. Dysregulated CRF system activity is strongly linked to mood disorders (Fernandez Macedo et al., 2013; Binder and Nemeroff, 2010; Klampfl et al., 2016, 2018; Sanson et al., 2025; Carpenter et al., 2004). CRF ligands (CRF, Urocortin 1–3) and their receptors (CRF-R1 and CRF-R2) are widely expressed both peripherally and centrally, and regulate physiological, autonomic, and behavioral responses to stress (Binder and Nemeroff, 2010; Deussing and Chen, 2018). Elevated CRF levels in the cerebrospinal fluid and increased CRF-R binding have been reported in depressed patients (Pandey et al., 2019), consistent with upregulated CRF system activity (Arborelius et al., 1999; Davis et al., 2018). In rats, CRF overexpression induces depressive-like symptoms, altered hypothalamic-pituitary-adrenal (HPA) axis function, and behavioral changes (Flandreau et al., 2012), while chronic stress can lead to CRF system sensitization (Herman et al., 2016). OXT, another key neuropeptide in social bonding, is essential for maternal bonding (Marlin et al., 2015; Sanson and Bosch, 2022; Carcea et al., 2021), and other forms of social affiliation (Menon and Neumann, 2023). OXT-R are widely distributed throughout the brain, where OXT signaling modulates stress responses and facilitates social behavior (Jurek and Neumann, 2018). OXT is thought to have antidepressant-like properties, partly by dampening HPA axis reactivity (Arletti and Bertolini, 1987; Windle et al., 1997; Neumann et al., 2000a), making it a candidate target for grief- and depression-related disorders (Menon and Neumann, 2023; Jurek and Neumann, 2018) including postpartum depression (Rashidi et al., 2022).

Neuroplasticity is another important factor in grief and depression. Alterations in dendritic spine density, a key structural marker of plasticity, have been implicated in depression (Ho and King, 2021), though the mechanisms remain incompletely understood (Price and Duman, 2020). Neuroplasticity and emotional processing are also regulated by estrogen receptor ESR1 by influencing dendritic spine formation and OXT-R expression (Sheppard et al., 2019; Young et al., 1998; Jaric et al., 2019). ESR1 is expressed at high density in calbindin-immunoreactive (ir+) cells of the ventromedial hypothalamus (VMH) (Mori et al., 2008), and calbindin-ir+ cells are reduced in the occipital cortex of post-mortem depressed patients (Maciag et al., 2010).

We hypothesized that early loss of offspring would lead to enduring changes in the maternal stress-response, neuroendocrine systems and neuroplasticity. To test this, we studied lactating female Sprague-Dawley rats that experienced one day of motherhood followed by 19 days of offspring loss. We examined stress-coping behavior, HPA axis reactivity, OXT-R binding, dendritic spine density, and ESR1- and calbindin-ir+ cells expression in key limbic and maternal brain regions. This work provides novel insights into the neurobiology of maternal distress and highlights the potential therapeutic relevance of targeting central CRF-R signaling.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats were obtained from Charles River Laboratories (Sulzfeld, Germany) and kept under standard laboratory conditions (12:12 h light/dark cycle, lights on at 7 a.m., room temperature 22 ± 1°C, relative humidity 55 ± 5°C) with access to standard rat chow (ssniff-Spezialdiäten GmbH, Soest, Germany) and water *ad libitum*. After 7 days of habituation, females were mated with sexually experienced male Sprague-Dawley rats in standard laboratory cages (Eurostandard Type IV, 60 cm x 40 cm x 20 cm) for 10 days. Pregnancy was confirmed by the presence of spermatozoa in vaginal smears collected daily during the mating period, with the day of sperm detection designated as gestational day 1. From pregnancy day 18 on, pregnant females were single-housed for undisturbed delivery in observational cages (plexiglass, 38 cm x 22 cm x 35 cm); all rats delivered 8-15 pups within 3-4 days after being single-housed. The studies were conducted in accordance with the ARRIVE guidelines, the European regulations of animal experimentation (European Directive 2010/63/EU) and were approved by the local government of Unterfranken (Bavaria, Germany). According to the 3-Rs principles, all efforts were made to minimize the number of animals and their distress or suffering.

2.2. Study design

We investigated two primary groups of lactating females: (I) control mothers, which remained with their pups until natural weaning, and (II) separated mothers, which had all offspring removed on lactation day 1 and experienced offspring loss for 19 days (Fig. 1). The day of parturition was designated as lactation day 0 (LD0), irrespective of the time of delivery. All pups were removed on the following day (LD1) at 10:00 AM. The 19-day offspring loss period was selected because it spans the entire pre-weaning phase in rats, a critical period during which the mother-offspring bond is maintained and lactation-associated neuroendocrine adaptations are fully established in rats (Numan and Young, 2016). Our study followed a stepwise approach. First, we assessed the effects of prolonged offspring loss on OXT-R binding, ESR1- and calbindin-ir$^+$ cells in brain regions implicated in maternal behavior. This analysis provided an initial characterization of neuroendocrine adaptations within the maternal brain and helped identify target regions for subsequent investigation. Next, we evaluated dendritic spine density and behavioral outcomes, incorporating an additional group of *virgin* females to distinguish experience-dependent plasticity in maternal neural circuits.

2.3. Experimental groups

Rats were randomly assigned to one of three experimental groups: (I) control lactating mothers (primiparous) that remained with their pups until weaning (*LD20* group), (II) separated mothers whose litters were removed on LD1 (*LD1+19* group; primiparous) , and (III) *virgin* females (*virgin* group; nulliparous). To standardize litter size, each litter was adjusted to eight pups of mixed sexes on the day of delivery (LD0). In the *LD1+19* group, all pups were removed on LD1 at 10:00 a.m., after which the mothers were left undisturbed in their home cages. To control for the effects of single housing, *virgin* females were housed individually for the

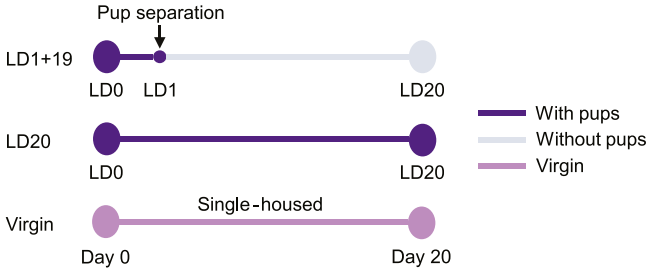

Fig. 1. Schematic timelines summarizing the maternal separation paradigm. Abbreviations: LD, lactation day.

same duration as separated mothers.

2.4. Experimental design

All experiments were conducted in independent cohorts, and no animals were used in more than one experiment.

Experiment 1: a first cohort of rats followed the protocol described above and was sacrificed on LD20 without further manipulations (Fig. 1). Brains were collected for: (i) OXT-R binding analysis (*LD20* = 6; *LD1+19* = 6), (ii) immunofluorescence analysis (*LD20* = 7; *LD1+19* = 5), and (iii) dendritic spine analysis (*LD20* = 4; *LD1+19* = 4; *virgin* = 5).

Experiment 2: a second cohort (*LD20* = 15; *LD1+19* = 15; *virgin* = 9) was tested for locomotor and anxiety-like behavior in the light-dark box test (LDB) on LD19 and in the open field test (OF) on LD20. Immediately following the final behavioral test, rats were sacrificed.

Experiment 3: a third cohort (*LD20* = 13; *LD1+19* = 14; *virgin* = 8) was evaluated for stress-coping behavior in the single-session forced swim test (FST) on LD20. Within 5 min of test completion, rats were sacrificed, and trunk blood was collected for plasma corticosterone (CORT) analysis. Experiments 2 and 3 were conducted in separate cohorts to avoid confounding effects of repeated behavioral testing and stress exposure. Experiment 2 assessed anxiety-like behavior and locomotor activity using the LDB and OF tests, which require repeated testing under low-stress conditions. In contrast, Experiment 3 employed the FST, a stressful paradigm that robustly activates the HPA axis and would have interfered with behavioral and endocrine measures if conducted concurrently. Plasma CORT levels were measured exclusively in Experiment 3, as blood collection was timed to assess acute HPA axis activation following the FST. CORT was not assessed in Experiment 2 because the behavioral paradigms used are mild stressors and do not reliably elicit robust endocrine responses, and because prior behavioral testing would have confounded interpretation of acute stress-induced CORT levels.

Experiments 4 and 5: two additional cohorts were prepared following the same protocol. On LD15, all rats underwent stereotaxic implantation of an intracerebroventricular (icv) guide cannula for acute substance infusion. On LD19, they completed the 15-min pre-test session of the two-session forced swim test (FST). On LD20, they were tested in a 10-min session following infusion of either vehicle (VEH) or a pharmacological treatment. In Experiment 4, the CRF-R1/2 antagonist D-Phe was administered (*LD20 VEH* = 10; *LD1+19 VEH* = 8; *LD1+19 D-Phe* = 9), whereas in Experiment 5, synthetic OXT was infused (*LD20 VEH* = 8; *LD1+19 VEH* = 8; *LD1+19 OXT* = 7). In both experiments, all rats were sacrificed immediately after the final behavioral test and brains taken to verify cannula placement.

2.5. Brain analyses

2.5.1. OXT-R autoradiography

Rats were deeply anesthetized with isoflurane and underwent cardiac perfusion with ice-cold 1x PBS and were decapitated. Brains were flash-frozen in n-methylbutane, and stored at −20 °C until cutting into coronal sections of 16 µm using a cryostat (CM3050S, Leica Microsystem GmbH). For each brain region of interest ((bed nucleus of the stria terminalis (BNST), nucleus accumbens shell (NAcc shell), medial preoptic area (MPOA), accessory olfactory bulbs (AOB), central amygdala (CeA), agranular insular cortex (AIP), ventromedial hypothalamus (VMH), lateral septum (LS), prelimbic cortex (PL)), six slices per rat were collected on SUPERFROST microscope slides and stored at −20 °C until further processing. These regions were selected based on their established roles in maternal behavior, stress regulation, and oxytocin signaling, as well as prior evidence indicating their sensitivity to maternal experience and offspring loss. The OXT-R autoradiography was performed following an established protocol (Oliveira et al., 2021). Briefly, the ornithin-vasotocin analogue [125I]- OVTA [d(CH2)5[Tyr (Me)2,Thr4,Orn8,[125I]Tyr9-NH2]; Perkin Elmer, USA) was used as a tracer. First, the slides were thawed and allowed to dry thoroughly at room temperature. The tissue was shortly fixed via 0.1 % PFA, washed 2x in Tris (50 mM, pH 7.4), covered with the tracer solution (50 mM tracer, 10 mM MgCl2, 0.1 % BSA) for 60 min, washed 3x in Tris / MgCl2 buffer for 7 min, each, followed by 30 min spinning in Tris / MgCl2. Finally, slides were dipped into water and air dried before being exposed to Biomax MR films (Kodak, Cedex, France) for 15 days. The films were scanned using the EPSON Perfection V800 Scanner (Epson GmbH, Munich, Germany), and the optical density of each region of interest was analyzed using ImageJ (Schneider et al., 2012) by subtracting the background activity as previously described (Oliveira et al., 2021; Bosch and Neumann, 2008). The analyses were performed simultaneously for 6 slices per rat and per region.

2.5.2. Immunofluorescence staining

Rats were deeply anesthetized with isoflurane prior to transcardial perfusion with ice-cold 1x PBS and subsequently with 4 % paraformaldehyde (PFA) dissolved in 1x PBS. Brains were removed, fixed overnight in 4 % PFA, and subsequently incubated in 30 % sucrose in 1x PBS until the brain sank. After fixation and cryoprotection in sucrose, brains were flash-frozen in n-methylbutane, and stored at −20 °C until cutting into coronal sections of 16 µm using a cryostat (CM3050S, Leica Microsystem GmbH, Nussloch, Germany). Six consecutive slices containing the VMH region were collected per rat on SUPERFROST microscope slides and stored at −20 °C until further processing. Slides were washed in 1x PBS, permeabilized with 0.3 % Triton, blocked with 5 % normal goat serum (Vector Laboratories, Newark, USA), and incubated overnight at 4 °C with primary antibodies (Merck Millipore, rabbit anti ER alpha 1:2000; Merck, mouse anti-NeuN 1:1000; Synaptic Systems, chicken anti-calbindin 1:2000). The next day, slides went through 1 h incubation with secondary antibodies (Alexa Fluor 488-conjugated anti-rabbit IgG 1:800, Alexa Fluor 594-conjugated anti-mouse IgG 1:800 and Alexa Fluor 649-conjugated anti-chicken IgG 1:800) at room temperature. All slides were finally mounted using ROTI®Mount FluorCare DAPI (Carl Roth, Karlsruhe, Germany). Immunofluorescence analyses were performed to assess ESR1 and calbindin expression in the ventromedial hypothalamus, using NeuN as a neuronal marker.

2.5.3. Golgi staining for spine density visualization

The spine density was assessed in *virgin*, *LD20* and *LD1+19* groups to investigate neuronal plasticity. Firstly, rats were sacrificed with CO_2 and brains were collected. The Rapid GolgiStainTM Kit (FD NeuroTechnologies, Columbia, USA) was used and applied according to the manufacturer's protocol. Brains were cut into 100 µm slices using a cryostat (CM3050S, Leica Microsystem GmbH) at −28° and placed on gelatin-coated microscope slides (FD NeuroTechnologies, Columbia, USA). After complete drying, Golgi staining was performed, and sections were stored at room temperature in the dark before acquiring images using a brightfield microscope (Leica Thunder DM6 B, Camera Leica DFC9000 GT). ImageJ (Schneider et al., 2012) was used to count the spines of secondary dendrites in the PL, AIP, BLA and VMH. Secondary dendrites from different neurons (between 4 and 12, depending on the brain region) were chosen from 10X overview preview images followed by acquiring 40X focus images for analysis. A region of interest (ROI) corresponding to the selected dendritic segment was defined for quantitative analysis, brightness and sharpening function were adjusted. Spines were counted on a 25–30 μm^2 section of the dendrite (each dendrite belonging to a different neuron) and the absolute spine density was calculated by using the following formula (Gu et al., 2014): (n = number of spines; µm = dendritic length): $\frac{n-1}{\mu m}$.

2.6. Behavioral experiments

Prior to all behavioral testing between 9:00 a.m. and 12:00 p.m., rats were transferred to the experimental room and allowed to acclimate for

at least 30 min.

2.6.1. Light dark box (LDB)

On LD19, rats from experiment 2 were tested in the LDB to assess anxiety-like behavior (Crawley and Goodwin, 1980). The apparatus consisted of a light compartment (40 x 50 cm, 180 lux) and a dark compartment (40 x 30 cm, 0 lux) connected by a 7.5 x 7.5 cm opening. At the start of the test, rats were placed in the center of the light box, and behavior was recorded for 10 min using EthoVision XT (Noldus, Wageningen, the Netherlands) by an experimenter blind to group assignment. The following parameters were analyzed: (a) percentage of time spent in the light box, (b) latency to re-enter the light compartment, and (c) locomotor activity. The arena was cleaned with tap water and thoroughly dried between trials to remove olfactory cues while avoiding residual odors from cleaning agents that could influence subsequent behavior.

2.6.2. Open-field (OF)

On LD20, rats from experiment 2 were tested in the OF to evaluate locomotor activity and anxiety-like behavior (Gould et al., 2009). The apparatus was an empty rectangular arena (80 x 80 cm). Rats were placed in one corner and allowed to explore freely for 10 min. Behavior was recorded using EthoVision XT, with analysis performed by an experimenter blind to treatment. The following measures were scored: (a) locomotion, (b) velocity, and (c) number of center entries. The arena was cleaned with tap water and thoroughly dried between trials to minimize olfactory carryover effects.

2.6.3. Forced swim test (FST)

Two versions of the FST were employed to assess passive stress-coping behavior. *Rationale:* we used a single-session protocol to capture baseline group differences without pre-exposure, whereas the two-session protocol provided higher sensitivity to drug effects -particularly relevant because animals in experiment 4–5 underwent surgery (see below).

2.6.3.1. Single-session FST. On LD20, rats from experiment 3 were tested in a single 10-min FST session. Each rat was placed in a cylindrical tank (50 cm high, 30 cm diameter) filled with tap water (23 ± 1 °C) at a depth preventing contact with the bottom. Behavior was recorded and analyzed using JWatcher (https://www.jwatcher.ucla.edu) by an experimenter blind to group assignment. The total time spent immobile (floating) was quantified as an index of passive stress-coping (Slattery and Cryan, 2014).

2.6.3.2. Two-session FST. In experiments 4 and 5, rats were tested using the two-day Porsolt FST, the standard paradigm for evaluating antidepressant treatments (Slattery and Cryan, 2014). These animals had previously undergone stereotaxic surgery (see details below) and were tested 5 days postoperatively. On LD19, rats underwent a 15-min pre-test session. On LD20, they were exposed to a 10-min test session following intracerebroventricular infusion of vehicle or a pharmacological agent. The two-session design was chosen to stabilize baseline immobility at the pre-test and to maximize the dynamic range for detecting drug-induced reductions at the test while controlling for post-surgical variability. All other experimental conditions and behavioral analyses were identical to those described for the single-session FST.

2.7. Stereotaxic surgery

On LD15, rats of experiments 4 and 5 were implanted with a stainless-steel guide cannula (21-G, length 12 mm), above the right lateral cerebral ventricle (1.0 mm posterior, 1.6 mm lateral to bregma, 1.8 mm ventral) (Paxinos and Watson, 1998). Surgeries were performed under isoflurane anesthesia, following previously described procedures (Klampfl et al., 2013). The guide cannula was closed using a 25-G stainless steel stylet to prevent occlusion. To minimize post-surgical discomfort, all rats received a subcutaneous injection of buprenorphine (0.05 mg/kg, Bayer Vital GmbH, Leverkusen, Germany) prior to surgery. Following surgery, rats were monitored continuously until recovery from anesthesia and then returned to their home cages. Rats were observed daily for general health, body weight, and signs of discomfort or infection throughout the recovery period.

2.8. Drug administration

On LD20, rats of experiment 4 and 5 received a single acute icv infusion 10 min prior to the second session of the two-day forced swim test. Icv administration was chosen to assess system-level contributions of CRF and OXT signaling to stress-coping behavior, rather than region-specific mechanisms. Depending on the treatment group, animals were infused with vehicle (VEH; 5 µL sterile Ringer's solution, pH adjusted to 7.4; Braun, Melsungen, Germany), the human/rat CRF-R1/2 antagonist D-Phe [(D-Phe^{12}, Nle^{21,38}, α-Me-Leu)-CRF (12–41); 10 µg/5 µL; Bachem, Bubendorf, Switzerland], or synthetic OXT (1 µg/5 µL; Tocris, Nordenstadt, Germany). All doses were selected based on previous studies (Klampfl et al., 2013). Infusions were performed using a 25 G stainless-steel infusion cannula (14 mm length) connected to a 10 µL Hamilton micro syringe via PE-50 tubing (50 cm). The infusion cannula was inserted into the pre-implanted guide cannula and held in place with a piece of silicon tubing. Substances were delivered over 30 s, after which the cannula was left in position for an additional 30 s to minimize reflux before removal, as previously described (Neumann et al., 2000a).

2.9. Verification of cannula placement

Rats of experiment 4 and 5 were sacrificed immediately following the last behavioral test with CO_2 and blue dye was injected via the infusion system into the guide cannula. Verification of the infusion site of the icv cannula was observed by dye spread throughout the ventricular system. Only animals with correct verified infusion sites were included in the statistical analyses.

2.10. ELISA for plasma CORT

In experiment 3, rats were sacrificed within 5 min after the single-session FST, and approximately 1 mL of trunk blood was collected in EDTA-coated tubes on ice (Sarstedt, Numbrecht, Germany). Samples were centrifuged at 4 °C (10,000 rpm/12298 rcf) for 10 min. Plasma was aliquoted and stored at −20 °C until analysis. CORT concentrations were quantified using a commercially available ELISA kit (Tecan IBL International GmbH, Hamburg, Germany) following the manufacturer's instructions. All samples were measured in duplicate, and mean values were used for statistical analysis.

2.11. Statistical analysis

All statistical analyses were performed with GraphPad PRISM 9 (GraphPad Software, San Diego, USA). Data were first tested for normality (Shapiro-Wilk or Kolmogorov–Smirnov test) and homogeneity of variance (Brown-Forsythe test). Potential outliers were assessed using the ROUT method (Q = 1 %); no data points were identified or removed. When normality assumption was not met, nonparametric tests were used (Kruskal–Wallis with Dunn's multiple comparisons). Group comparisons of OXT-R binding, ESR1- and calbindin-ir+ cell counts were performed using two-sample Student's *t*-test. Dendritic spine density, plasma CORT concentration, anxiety-like behavior, locomotor activity and stress-coping behavior were analyzed by one-way ANOVA followed by Sidak's post hoc multiple comparisons. Data are expressed as mean ± SEM; and significance was set at $p \leq 0.05$. Due to technical

issues during trunk blood collection, plasma CORT measurements were not obtained for four animals in Experiment 3; behavioral data from these animals were retained, but they were excluded from CORT analyses.

3. Results

3.1. Increased OXT-R binding in the VMH and PL after offspring loss

To determine whether offspring loss alters OXT-R binding within maternal and limbic brain regions, we performed receptor autoradiography in *LD20* and *LD1+19* mothers. Group differences were analyzed using two-sample Student's *t*-tests. *LD1+19* dams exhibited significantly higher OXT-R binding in the ventromedial hypothalamus (VMH; $M = 0.257$, $SD = 0.077$) compared to *LD20* dams ($M = 0.147$, $SD = 0.083$; $t(10) = 2.366$, $p = 0.039$; Fig. 2A). A similar finding was observed in the prelimbic cortex (PL), where OXT-R binding was slightly elevated in *LD1+19* ($M = 0.070$, $SD = 0.005$) relative to *LD20* dams ($M = 0.066$, $SD = 0.002$, $t(10) = 2.203$, $p = 0.052$; Fig. 2B).

No significant group differences were detected in the other regions analyzed (Fig. 2C): BNST ($t(10) = 0.828$, $p = 0.427$); NAcc shell ($t(10) = 1.440$, $p = 0.180$); MPOA ($t(10) = 0.215$, $p = 0.834$); AOB ($t(10) = 0.595$, $p = 0.565$); CeA ($t(10) = 0.061$, $p = 0.952$); AIP ($t(10) = 1.212$, $p = 0.254$); LS ($t(10) = 0.613$, $p = 0.554$)].

3.2. ESR1- and calbindin-ir+ cells in the VMH and PL did not differ between groups

To investigate whether the increased OXT-R binding in the VMH and PL (see above) was associated with changes in ESR1 expression, we quantified ESR1- and calbindin-ir+ cells in the VMH and PL of *LD20* and *LD1+19* mothers. In the VMH, no significant group differences were detected in the proportion of ESR1-ir+ cells ($t(10) = 0.456$, $p = 0.658$; Fig. 3A, B) or of calbindin-ir+ cells ($t(10) = 0.706$, $p = 0.496$; Fig. 3A, C). ESR1-ir+ cells were not detectable in the PL (data not shown).

3.3. Reduced secondary dendritic spine density in the VMH after offspring loss

To assess the impact of offspring loss on neuroplasticity, we quantified secondary dendritic spine density in the VMH, PL, BLA and AIP of *virgin*, *LD20* and *LD1+19* females (Fig. 4A), based on regions identified in our previous study (Demarchi et al., 2024). Statistical analysis

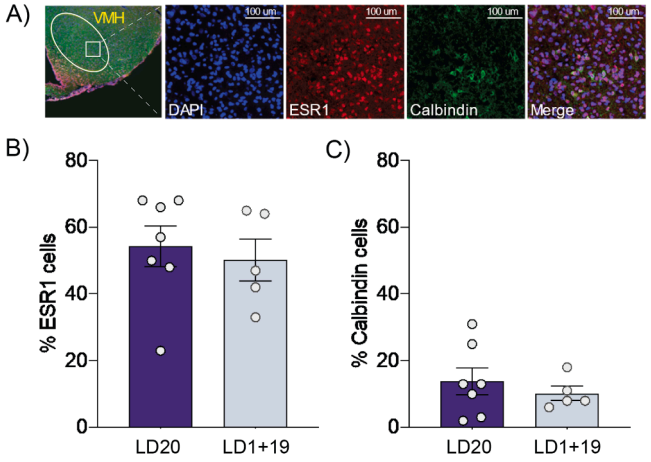

Fig. 3. ESR1- and calbindin-ir+ cells in the VMH. (A) Representative fluorescent images showing DAPI (blue), ESR1 cells (red), calbindin (green) and merged channels in the VMH. Percentage of (B) ESR1- and (C) calbindin-ir+ cells in the VMH. One-way ANOVA. Data are expressed as mean ± SEM.

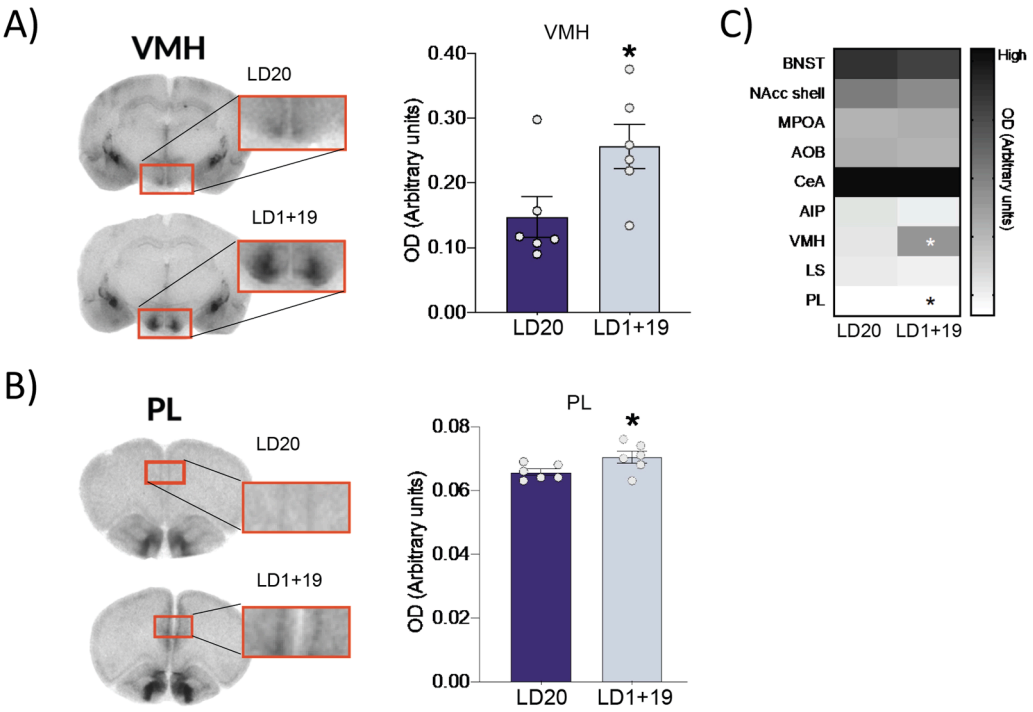

Fig. 2. OXT-R binding in brain regions of the limbic system and maternal network. (A,B) Representative coronal brain sections of the VMH and PL showing differences in OXT-R binding. Optical density (arbitrary units) for OXT-R binding in the (A) VMH and (B) PL. (C) Overview heatmap depicting relative OXT-R binding levels, expressed as optical density (arbitrary units) derived from autoradiographic signal intensity across analyzed brain regions; darker shading indicates higher binding (* $p \leq .05$ versus *LD20*). Abbreviations: AIP, agranular insular cortex; AOB, accessory olfactory nuclei; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; LS, lateral septum; MPOA, medial preoptic area; NAcc, nucleus accumbens; PL, prelimbic cortex; VMH, ventral medial hypothalamus. Student's *t*-test. Data are expressed as mean ±SEM. * $p \leq .05$ versus *LD20*.

revealed a significant group effect in the VMH (one-way ANOVA: $F(2, 10) = 8.734$; $p = 0.006$; Fig. 4B). Post hoc analyses showed that *LD1+19* dams exhibited significantly lower spine density compared to *virgin* ($p = 0.024$) and *LD20* mothers ($p = 0.009$). In contrast, no significant differences were observed in the other regions examined. No differences in spine density were found for the PL (*virgin*: 0.64 ± 0.07; *LD20*: 0.53 ± 0.04; *LD1+19*: 0.62 ± 0.04; one-way ANOVA: $F(2,15) = 0.590$, $p = 0.356$), the BLA (*virgin*: 0.61 ± 0.03; *LD20*: 0.64 ± 0.009; *LD1+19*: 0.61 ± 0.02; one-way ANOVA: $F(2,16) = 0.158$, $p = 0.855$), and the AIP (*virgin*: 0.62 ± 0.05; *LD20*: 0.69 ± 0.04; *LD1+19*: 0.68 ± 0.04; one-way ANOVA: $F(2,15) = 0.599$, $p = 0.562$).

3.4. Anxiety-like behavior was not affected by offspring loss

To examine whether offspring loss altered anxiety-like behavior, *virgin*, *LD20*, and *LD1+19* rats were tested in the LDB on LD19. Groups differed in latency to re-enter the light compartment (Kruskal–Wallis test, $p = 0.045$), with *LD20* dams tending to be faster (48.6 s ± 15.9 s) than *virgin* females (213.5 s ± 77.5 s; Dunn's test, $p = 0.058$). *LD1+19* dams did not differ from either group (121.9 s ± 31.9 s).

No group differences were observed in the time spent in the light compartment (*virgin*: 34.0 s ± 6.3 s; *LD20*: 36.6 s ± 3.6 s; *LD1+19*: 42.0 s ± 5.1 s; one-way ANOVA: $F(2,36) = 0.676$, $p = 0.515$) or in locomotor activity within the LDB (*virgin*: 3778 cm ± 326 cm; *LD20*: 3733 cm ± 194 cm; *LD1+19*: 3851 cm ± 312 cm; one-way ANOVA: $F(2,36) = 0.054$, $p = 0.948$). Thus, *LD1+19* mothers did not differ from either *virgin* or *LD20* mothers in any measure of anxiety-like behavior.

3.5. Locomotor activity was not affected by offspring loss

The same cohort of rats was tested in the OF on LD20. Groups differed significantly in distance travelled (one-way ANOVA: $F(2,36) = 3.773$, $p = 0.032$; Fig. 5A) and in velocity (one-way ANOVA; [$F(2,36) = 3.788$], $p = 0.032$; Fig. 5B). Post hoc comparisons revealed that *virgin* females showed greater locomotion ($p = 0.029$) and velocity ($p = 0.028$) compared with *LD20* dams. *LD1+19* mothers did not differ from either group.

No significant differences were found in the number of center entries (*virgin*: 21.4 ± 2.7; *LD20*: 16.3 ± 1.5; *LD1+19*: 20.5 ± 2.0; one-way ANOVA: $F(2,36) = 1.858$, $p = 0.170$). Overall, offspring loss did not alter locomotor activity.

3.6. Offspring loss increased passive stress-coping behavior and induced a virgin-like stress response

A different cohort of *virgin*, *LD20* and *LD1+19* rats was tested for passive stress-coping behavior in the single-session FST on LD20. Groups differed significantly in time spent floating (one-way ANOVA: $F(2,32) = 6.233$, $p = 0.005$; Fig. 5C). *LD1+19* dams spent more time floating compared with both *virgin* ($p = 0.019$) and *LD20* ($p = 0.014$) rats. Stress-induced plasma CORT levels, which were collected 15 min after the start of the FST, also differed across groups ($F(2,28) = 13.50$, $p = 0.0001$; Fig. 5D). Post hoc comparisons revealed significantly lower CORT concentrations in *LD20* compared with *virgin* ($p = 0.002$) and *LD1+19* ($p = 0.0001$) rats. *LD1+19* mothers did not differ from *virgin* females ($p > 0.05$), suggesting that offspring loss abolished the lactation-induced blunting of HPA axis activity.

3.7. Central CRF-R1/2 blockade normalized passive-stress coping behavior in LD1+19 mothers

Following a pre-test on LD19, *LD20* and *LD1+19* rats were tested in the FST on LD20 after ICV infusion of VEH or the CRF-R1/2 antagonist D-Phe. Groups differed significantly in floating behavior (one-way ANOVA: $F(2,23) = 5.828$, $p = 0.013$; Fig. 6A). *LD1+19 VEH* mothers spent more time floating than *LD20 VEH* rats ($p = 0.047$), replicating the findings from the single-session FST (Fig. 5C). Importantly, floating time was significantly lower in *LD1+19 D-Phe* rats compared to *LD1+19 VEH* ($p = 0.014$), reaching levels indistinguishable from *LD20 VEH* ($p > 0.05$).

3.8. Central OXT infusion did not alter passive-stress coping behavior in LD1+19 mothers

In a different cohort, *LD20* and *LD1+19* rats were tested in the FST after infusion of VEH or synthetic OXT. Groups differed in floating behavior (one-way ANOVA: $F(2,20) = 5.273$, $p = 0.014$; Fig. 6B). Consistent with previous experiments, *LD1+19 VEH* rats floated more than *LD20 VEH* ($p = 0.014$). However, central OXT infusion did not significantly alter passive stress-coping behavior in *LD1+19* mothers, although a non-significant trend toward reduced floating time was observed ($p = 0.08$).

4. Discussion

The loss of offspring represents a profound life event with strong effects on physiology and behavior. In humans, such experiences are associated with marked changes in emotional and physical health, including a higher risk for prolonged grief disorders (McCarthy et al., 2010; Kersting and Wagner, 2012; Burden et al., 2016; Huh et al., 2017). To investigate the underlying mechanisms and to identify potential therapeutic targets, appropriate animal models are required. In this study, we build upon our previous work on consequences of short-term separation on rat mothers (Demarchi et al., 2024). We examined the long-term consequences of offspring loss and demonstrate that extended separation manifests in distinct neurobiological and behavioral adaptations. After one day of mothering experience followed by 19 days of constant pup removal, mothers exhibited increased OXT-R binding

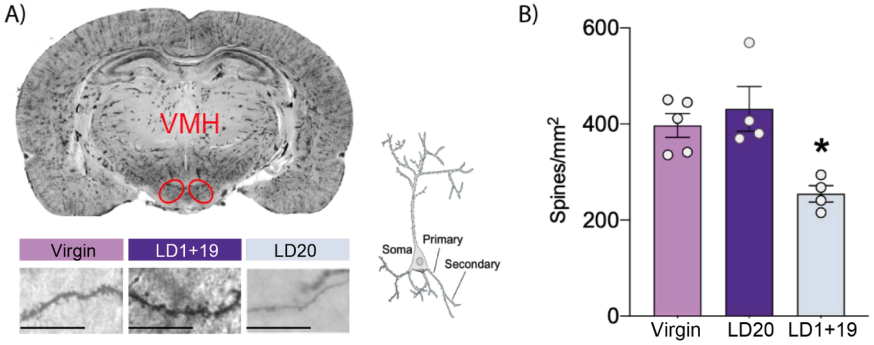

Fig. 4. Secondary dendritic spine density in the VMH. (A) Representative coronal whole brain section with Golgi staining and schematic illustration of the neuronal dendritic arbor (Scale bar 10 μm). (B) Absolute spine density / mm^2 across experimental groups. One-way ANOVA. Data are expressed as mean ± SEM. * $p < .05$ versus all other groups.

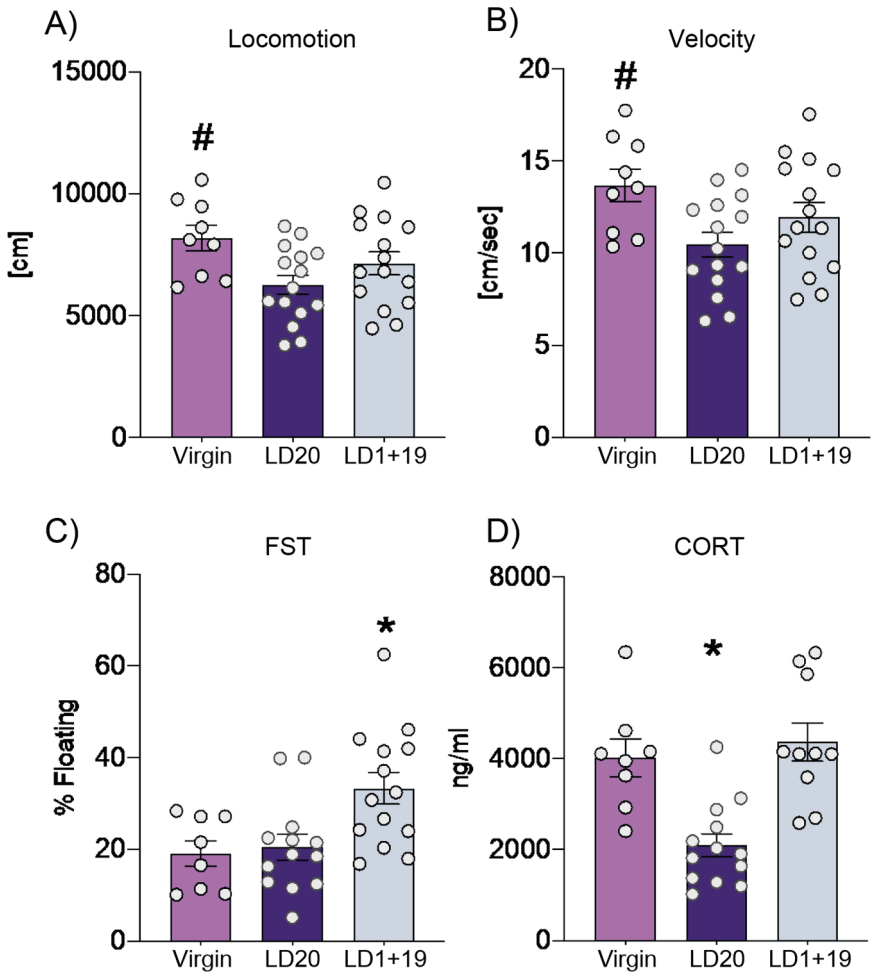

Fig. 5. (A) Locomotor behavior and (B) velocity in the OF, (C) passive stress-coping behavior in the classic FST, and (D) corresponding plasma CORT concentration. (A) Total distance traveled in cm and (B) mean velocity over the 10-min OF test. (C) Percentage of floating during the 10-min classic FST. (D) Plasma CORT concentration 15 min after start of the FST. One-way ANOVA. Data are expressed as mean ± SEM. # $p < .05$ versus *LD20*; * $p < .05$ versus all other groups.

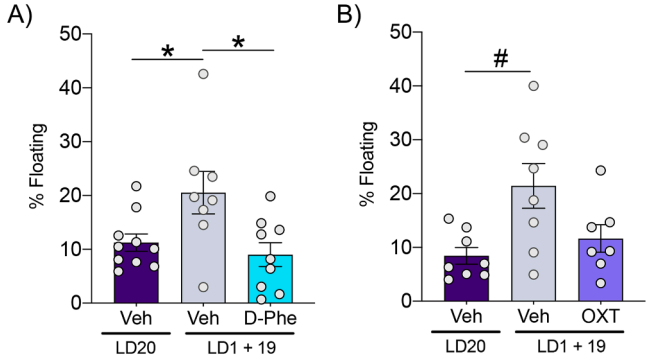

Fig. 6. (A) Passive stress-coping behavior in the FST following central pharmacological manipulation of the brain CRF and (B) OXT systems. (A) Percentage of floating during the 10-min modified FST following icv infusion of VEH or the CRF-R1/2 antagonist D-Phe, and (B) of VEH or synthetic OXT. One-way ANOVA. Data are expressed as mean ± SEM. * $p < .05$ versus all other groups; # $p < .05$ versus *LD20 VEH*.

specifically in the VMH and PL, had reduced dendritic spine density in the VMH, and heightened HPA axis reactivity together with increased passive stress-coping behavior. Importantly, central pharmacological blockade of CRF-R1/2 normalized the impaired behavior, whereas OXT infusion did not, highlighting the prominent role of CRF system activity in mediating maternal distress responses due to pup loss.

4.1. Increased OXT-R binding in the VMH and PL after offspring loss

During the peripartum period, the maternal brain undergoes dynamic adaptations in OXT signaling that support the initiation and maintenance of maternal behaviors (Valtcheva et al., 2023; Ng et al., 2023). In our study, OXT-R binding was significantly elevated in the VMH and PL following offspring loss, contrary to our initial hypothesis that separation would reduce OXT signaling. The VMH, which contains a high density of OXT-Rs and plays a role in maternal care, aggression, and defensive behaviors (Bridges et al., 1999; Hashikawa et al., 2017; Kunwar et al., 2015; Narita et al., 2016; Mitre et al., 2017), has not been studied in the context of pup removal. The PL, a region critical for cognitive and emotional regulation (Marek et al., 2018; Capuzzo and Floresco, 2020), has also been implicated in maternal care, as OXT-R blockade in this area disrupts caregiving behavior (Sabihi et al., 2014). Thus, the upregulation of OXT-Rs in both the VMH and PL may reflect adaptive -yet potentially insufficient- neurobiological adjustments triggered by permanent offspring removal. Previous studies investigating repeated maternal separation also report changes in OXT-R binding in the PL and MPOA (Demarchi et al., 2023; Champagne et al., 2001; Stamatakis et al., 2015), typically characterized by increased OXT-R expression associated with preserved or enhanced maternal behavior and interpreted as adaptive, experience-dependent plasticity. Importantly, these studies did not report alterations in VMH OXT-R

binding. In contrast, the present findings demonstrate increased OXT-R binding in the PL together with a selective recruitment of the VMH following permanent offspring loss, suggesting region-specific adaptations that may reflect the severity and irreversibility of the maternal disruption. Reduced maternal interactions, including fewer opportunities for the milk ejection reflex, likely decrease endogenous OXT release (Li et al., 2020), which has been associated with reduced activity of OXT-positive neurons in the PVN (Liu et al., 2019). Based on this evidence, we propose that permanent offspring loss alters OXT dynamics, with potentially less OXT release leading to the detected OXT-R upregulation thereby serving as a compensatory adaptation. Future studies should examine brain region-specific OXT release and transcriptional regulation to provide a more complete picture of system-wide neuroendocrine adaptations.

4.2. ESR1 and calbindin may not mediate OXT-R changes

Estrogen has been shown to regulate OXT-R expression via ESR1 and to interact with calbindin (Jaric et al., 2019; Wang et al., 2013; Vishnyakova et al., 2021; Kalinowski et al., 2022). To assess whether the OXT-R upregulation observed in our study could be explained by alterations of these factors, we quantified ESR1- and calbindin-ir$^+$ cells in the VMH and PL. No differences were detected in the proportion of ESR1- or calbindin-ir$^+$ cells in the VMH between groups, and ESR1 was not detectable in the PL. These findings suggest that changes in ESR1 or calbindin expression are unlikely to account for the observed increase in OXT-R binding following offspring loss, although a regulatory role cannot be formally excluded. Instead, other neuroendocrine pathways may contribute to the observed receptor alterations. In particular, CRF signaling represents a likely candidate, given its known interactions with the OXT system and its role in stress-related neuroadaptations (Winter and Jurek, 2019).

4.3. Reduced spine density in the VMH after offspring loss

Neuroplasticity is a central component of maternal adaptations, with dendritic spine remodeling occurring across pregnancy and the postpartum period (Servin-Barthet et al., 2023). In our study, we observed a selective reduction in secondary dendritic spine density within the VMH after offspring loss, whereas no significant changes were detected in other regions examined. Alterations in dendritic spine density have been widely reported in response to stress and depression in both humans and rodents (Workman et al., 2013; Leuner et al., 2014; Wonch et al., 2016; Payne and Maguire, 2019; Becker et al., 2013; Pekarek et al., 2020; Sequeira and Gourley, 2021; Radley et al., 2006; Mitra et al., 2005; Qiao et al., 2016; Holmes et al., 2019). Given the VMH's established role in the regulation of stress reactivity, social interactions, and aggression (Borszcz, 2006; Wilent et al., 2010; Osakada et al., 2024), the observed decrease in spine density may be relevant, rather than directly contribute, to the stress-response dysregulation observed in separated mothers. Importantly, these structural changes were accompanied by increased OXT-R binding in the VMH, suggesting that both cellular and receptor-level adaptations co-occur in this region. One possible interpretation is that reduced spine density may reflect altered excitatory connectivity within VMH circuits, while the concurrent increase in OXT-R binding could shift the balance of neuromodulatory input, thereby potentially reshaping local network responsiveness. However, these interpretations are speculative as causal relationships between structural remodeling, receptor changes, and behavior were not directly tested in the present study. Rather than acting as a simple compensatory mechanism, these parallel adaptations may reflect a reorganization of VMH function following offspring loss, potentially influencing how this region integrates social and stress-related signals. A limitation of the present work is that estrous cycle phases of *virgin* females were not monitored, which could have influenced spine density measurements. Future studies should therefore control for reproductive state and directly test the functional link between OXT-R signaling and structural remodeling in the VMH to clarify how these adaptations interact to shape maternal behavioral responses.

4.4. Anxiety-like and locomotor behaviors were unchanged

Lactation is often associated with reduced anxiety-like behavior, largely mediated by elevated central OXT signaling (Bosch, 2011; Bosch and Neumann, 2012). Consistent with this direction, *LD20* dams showed a trend toward faster re-entry versus *virgin* females, but this did not generalize to other LDB parameters. Crucially, separated mothers (*LD1+19*) did not differ from *LD20* or *virgin* females on any measure, indicating that offspring loss did not produce robust changes in anxiety-related behavior. The absence of locomotor differences across groups further supports that the FST effects were not secondary to altered mobility or general activity. While the lack of pup-derived sensory stimulation in separated mothers could diminish central OXT release -and thereby remove a potential anxiolytic mechanism (Neumann et al., 2000b)- the present data indicates no measurable anxiety-like phenotype in the LDB. Subtle behavioral differences observed in *virgin* females may reflect their period of social isolation, a known driver of heightened anxiety/hyperactivity in rodents (Begni et al., 2020).

4.5. Offspring loss increased passive stress-coping and HPA axis reactivity

Our results show that permanent offspring loss exerted a sustained impact on maternal emotional regulation. In the FST, *LD1+19* mothers consistently exhibited elevated passive stress-coping, as indicated by increased floating time, compared with both *virgin* and *LD20* dams. This outcome replicates and extends previous findings that prolonged maternal separation enhances passive stress-coping in rat dams (Demarchi et al., 2024; Pawluski et al., 2009; Rincon-Cortes and Grace, 2021). The consistent emergence of this phenotype across independent cohorts emphasizes its robustness and supports the validity of this model for probing maternal adaptations to offspring loss. Importantly, locomotor activity did not differ between *LD1+19* and *LD20* dams, confirming that the increase in floating reflects a shift in stress-coping strategy rather than reduced motor capacity or energy availability. In addition, physiological analyses aligned with these behavioral findings. *LD20* mothers displayed the known dampened HPA axis response, consistent with reduced stress reactivity during the postpartum period (Douglas et al., 2003). By contrast, *LD1+19* mothers exhibited CORT levels similar to *virgin* females, indicating that offspring loss disrupts the typical stress-buffering adaptations of lactation. This reversion to a virgin-like endocrine profile suggests both heightened vulnerability to acute stressors and the absence of maternal neuroendocrine mechanisms that normally confer resilience during caregiving. Whether this shift reflects the acute impact of pup loss on HPA axis regulation or the lack of sustained maternal experience remains an open question. Together, these findings demonstrate that offspring loss induces a dual effect: an increase in passive stress-coping behavior and the reactivation of heightened HPA axis activity. This behavioral–endocrine combination provides a mechanistic framework for understanding how the maternal brain adapts–or fails to adapt- after loss and reinforces the translational relevance of this model.

4.6. Central CRF-R1/2 antagonism, but not OXT-R agonism, reversed passive stress-coping

During lactation, activity of the brain CRF system is normally dampened, providing resilience against stress and allowing mothers to maintain adaptive caregiving responses (Klampfl and Bosch, 2019). However, chronic or severe stress around parturition can disrupt this regulation, leading to heightened CRF signaling and increased vulnerability to stress (Darnaudery et al., 2004; Zoubovsky et al., 2020).

Furthermore, the CRF system has been implicated in behavioral adaptations following social bond disruption, as shown in the monogamous prairie voles where partner separation activates CRF-dependent stress responses (Pohl et al., 2019). In line with this, our results demonstrate that icv administration of the CRF-R1/2 antagonist D-Phe normalized the increased passive stress-coping observed in separated mothers during the FST. These findings indicate that offspring loss induces hyperactivity of the CRF system, and that CRF receptor blockade can effectively restore adaptive stress-coping. This interpretation aligns with prior work showing that D-Phe alleviates separation-induced behavioral alterations in both lactating (Bosch et al., 2018) and male prairie voles (Bosch et al., 2009). Given the known functional interactions between CRF and OXT systems (Bosch et al., 2016; Klampfl et al., 2018), we also investigated whether enhancing OXT signaling could counteract the behavioral phenotype. Previous studies in prairie voles demonstrated that local OXT infusion into the NAcc shell rescues separation-induced behavioral deficits (Bosch et al., 2016), and work in lactating rats highlights OXT's regulatory role in stress responses (Jurek and Neumann, 2018; Menon and Neumann, 2023). While CRF ligand expression was not directly quantified in the present study, behavioral rescue by central CRF-R1/2 antagonism demonstrates that CRF receptor signaling is functionally required for the stress-coping phenotype induced by offspring loss. Future studies will assess region-specific CRF expression and release to further localize these effects. However, in our study central OXT infusion did not modify passive stress-coping in separated mothers. This lack of effect does not exclude the OXT system as a relevant modulatory pathway; rather, it suggests that global central infusion may not capture the region-specific actions of OXT. Indeed, targeted manipulations in regions such as the PVN (Wang et al., 2018) or NAcc (Bosch et al., 2016) have produced stronger behavioral effects in other models. The absence of behavioral effects following icv OXT administration may reflect compensatory OXT-R upregulation or the necessity of region-specific OXT signaling, rather than a lack of OXT system involvement per se. Together, our data highlight CRF hyperactivity as a central mechanism in the stress-related consequences of offspring loss, while suggesting that OXT-based interventions may require more precise spatial targeting to be effective. A potential limitation of the pharmacological experiments might be a short post-surgical recovery period of 4-days prior to behavioral testing. However, none of the rats showed abnormalities at the time of testing. All groups underwent identical surgical and recovery procedures, and the robust behavioral rescue produced by CRF-R1/2 antagonism argues against residual surgical stress as the primary driver of the observed effects.

5. Conclusions

This study establishes a translational rat model to investigate the neurobiological consequences of permanent offspring loss. Separated mothers displayed altered stress-coping strategies, elevated HPA axis activity, and structural as well as receptor-level adaptations in limbic brain regions. Specifically, increased OXT-R binding and reduced dendritic spine density were observed in the VMH, identifying this region as a key locus of maternal adaptation to loss. Importantly, pharmacological blockade of CRF-R1/2 signaling restored normal coping behavior, whereas OXT infusion did not, underscoring the CRF system as a primary driver of these outcomes. These findings provide novel insights into the interplay between neuroendocrine signaling, neuroplasticity, and behavioral regulation in the maternal brain. They also suggest that interventions targeting CRF signaling hold particular promise for mitigating the long-term consequences of disrupted maternal experience.

CRediT authorship contribution statement

Luisa Demarchi: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Oliver J. Bosch:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Alice Sanson:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Anna-Lena Boos:** Writing – review & editing, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

Acknowledgements

We thank Dr. J. Pawluski and Dr. B. di Benedetto for scientific discussion. We also thank M. Fuchs, A. Havasi, R. Maloumby, M. Schwarz, M. Breitkopf, L. Saller, E. Rocaboy, S. Pena-Pena and T. Gebhardt for excellent technical help.

This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), through the Neuroscience Graduate Programme "Neurobiology of Emotion Dysfunctions" (GRK 2174, to O.J.B.) and the grant BO1958/8–2 (to O.J.B.).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alves, R.L., et al., 2022. Maternal stress and vulnerability to depression: coping and maternal care strategies and its consequences on adolescent offspring. Transl. Psychiatry. https://doi.org/10.1038/s41398-022-02220-5.

Alves, R.L., Portugal, C.C., Summavielle, T., Barbosa, F., Magalhaes, A., 2020. Maternal separation effects on mother rodents' behaviour: a systematic review. Neurosci. Biobehav Rev. https://doi.org/10.1016/j.neubiorev.2019.09.008.

Arborelius, L., Owens, M.J., Plotsky, P.M., Nemeroff, C.B., 1999. The role of corticotropin- releasing factor in depression and anxiety disorders. J. Endocrinol. https://doi.org/10.1677/joe.0.1600001.

Arletti, R., Bertolini, A., 1987. Oxytocin acts as an antidepressant in two animal models of depression. Life Sci. https://doi.org/10.1016/0024-3205(87)90600-x.

Baracz, S.J., Everett, N.A., Robinson, K.J., Campbell, G.R., Cornish, J.L., 2020. Maternal separation changes maternal care, anxiety-like behaviour and expression of paraventricular oxytocin and corticotrophin-releasing factor immunoreactivity in lactating rats. J. Neuroendocrinol. https://doi.org/10.1111/jne.12861.

Becker, R.O., et al., 2013. Sexual behavior and dendritic spine density of posterodorsal medial amygdala neurons in oxytocin knockout female mice. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2013.07.034.

Begni, V., et al., 2020. Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity. PLoS One. https://doi.org/10.1371/journal.pone.0240439.

Binder, E.B., Nemeroff, C.B., 2010. The CRF system, stress, depression and anxiety-insights from human genetic studies. Mol. Psychiatry. https://doi.org/10.1038/mp.2009.141.

Bolukbas, I., Mundorf, A., Freund, N., 2020. Maternal separation in rats induces neurobiological and behavioral changes on the maternal side. Sci. Rep. https://doi.org/10.1038/s41598-020-80087-6.

Borszcz, G.S., 2006. Contribution of the ventromedial hypothalamus to generation of the affective dimension of pain. Pain. https://doi.org/10.1016/j.pain.2006.02.026.

Bosch, O.J., 2011. Maternal nurturing is dependent on her innate anxiety: the behavioral roles of brain oxytocin and vasopressin. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2010.11.012.

Bosch, O.J., et al., 2016. Oxytocin in the nucleus accumbens shell reverses CRFR2-evoked passive stress-coping after partner loss in monogamous male prairie voles. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2015.11.011.

Bosch, O.J., Nair, H.P., Ahern, T.H., Neumann, I.D., Young, L.J., 2009. The CRF system mediates increased passive stress-coping behavior following the loss of a bonded partner in a monogamous rodent. Neuropsychopharmacology. https://doi.org/10.1038/npp.2008.154.

Bosch, O.J., Neumann, I.D., 2008. Brain vasopressin is an important regulator of maternal behavior independent of dams' trait anxiety. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.0807412105.

Bosch, O.J., Neumann, I.D., 2012. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2011.11.002.

Bosch, O.J., Pohl, T.T., Neumann, I.D., Young, L.J., 2018. Abandoned prairie vole mothers show normal maternal care but altered emotionality: potential influence of

the brain corticotropin-releasing factor system. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2017.12.034.
Bridges, R.S., Mann, P.E., Coppeta, J.S., 1999. Hypothalamic involvement in the regulation of maternal behaviour in the rat: inhibitory roles for the ventromedial hypothalamus and the dorsal/anterior hypothalamic areas. J. Neuroendocrinol. https://doi.org/10.1046/j.1365-2826.1999.00322.x.
Burden, C., et al., 2016. From grief, guilt pain and stigma to hope and pride - a systematic review and meta-analysis of mixed-method research of the psychosocial impact of stillbirth. BMC Pregnancy Childbirth. https://doi.org/10.1186/s12884-016-0800-8.
Capuzzo, G., Floresco, S.B., 2020. Prelimbic and infralimbic prefrontal regulation of active and inhibitory avoidance and reward-seeking. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.0414-20.2020.
Carcea, I., et al., 2021. Oxytocin neurons enable social transmission of maternal behaviour. Nature. https://doi.org/10.1038/s41586-021-03814-7.
Carpenter, L.L., et al., 2004. Cerebrospinal fluid corticotropin-releasing factor and perceived early-life stress in depressed patients and healthy control subjects. Neuropsychopharmacology. https://doi.org/10.1038/sj.npp.1300375.
Champagne, F., Diorio, J., Sharma, S., Meaney, M.J., 2001. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.221224598.
Crawley, J., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. https://doi.org/10.1016/0091-3057(80)90067-2.
Darnaudery, M., Dutriez, I., Viltart, O., Morley- Fletcher, S., Maccari, S., 2004. Stress during gestation induces lasting effects on emotional reactivity of the dam rat. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2003.12.001.
Davis, E.G., et al., 2018. Corticotropin-releasing factor 1 receptor haplotype and cognitive features of major depression. Transl. Psychiatry. https://doi.org/10.1038/s41398-017-0051-0.
Demarchi, L., Pawluski, J.L., Bosch, O.J., 2021. The brain oxytocin and corticotropin-releasing factor systems in grieving mothers: what we know and what we need to learn. Peptides. https://doi.org/10.1016/j.peptides.2021.170593.
Demarchi, L., Sanson, A., Bosch, O.J., 2023. Brief versus long maternal separation in lactating rats: Consequences on maternal behavior, emotionality, and brain oxytocin receptor binding. J. Neuroendocr. https://doi.org/10.1111/jne.13252.
Demarchi, L., Sanson, A., Bosch, O.J., 2024. Neurobiological traces of grief: examining the impact of offspring loss after birth on rat mothers' brain and stress-coping behavior in the first week postpartum. Neurosci. Appl. https://doi.org/10.1016/j.nsa.2024.104065.
Deussing, J.M., Chen, A., 2018. The corticotropin-releasing factor family: physiology of the stress response. Physiol. Rev. https://doi.org/10.1152/physrev.00042.2017.
Dimonte, S., et al., 2023. Social isolation from early life induces anxiety-like behaviors in adult rats: Relation to neuroendocrine and neurochemical dysfunctions. Biomed. Pharm. https://doi.org/10.1016/j.biopha.2022.114181.
Douglas, A.J., Brunton, P.J., Bosch, O.J., Russell, J.A., Neumann, I.D., 2003. Neuroendocrine responses to stress in mice: hyporesponsiveness in pregnancy and parturition. Endocrinology. https://doi.org/10.1210/en.2003-0461.
Fernandez Macedo, G.V., Cladouchos, M.L., Sifonios, L., Cassanelli, P.M., Wikinski, S., 2013. Effects of fluoxetine on CRF and CRF1 expression in rats exposed to the learned helplessness paradigm. Psychopharmacology. https://doi.org/10.1007/s00213-012-2859-x.
Flandreau, E.I., Ressler, K.J., Owens, M.J., Nemeroff, C.B., 2012. Chronic overexpression of corticotropin-releasing factor from the central amygdala produces HPA axis hyperactivity and behavioral anxiety associated with gene- expression changes in the hippocampus and paraventricular nucleus of the hypothalamus. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2011.04.014.
Gould, T.D., Dao, D.T., Kovacsics, C.E, 2009. The Open Field Test. In: Gould, T.D. (Ed.), Mood and Anxiety Related Phenotypes in Mice, Neuromethods, 42. Humana Press, pp. 1–20. https://doi.org/10.1007/978-1-60761-303-9_1.
Gu, L., et al., 2014. Long-term in vivo imaging of dendritic spines in the hippocampus reveals structural plasticity. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1464-14.2014.
Hashikawa, K., et al., 2017. Esr1(+) cells in the ventromedial hypothalamus control female aggression. Nat. Neurosci. https://doi.org/10.1038/nn.4644.
Herman, J.P., et al., 2016. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Compr. Physiol. https://doi.org/10.1002/cphy.c150015.
Ho, T.C., King, L.S., 2021. Mechanisms of neuroplasticity linking early adversity to depression: developmental considerations. Transl. Psychiatry. https://doi.org/10.1038/s41398- 021-01639-6.
Holmes, S.E., et al., 2019. Lower synaptic density is associated with depression severity and network alterations. Nat. Commun. https://doi.org/10.1038/s41467-019-09562-7.
Huh, H.J., Huh, S., Lee, S.H., Chae, J.H., 2017. Unresolved bereavement and other mental health problems in parents of the Sewol ferry accident after 18 months. Psychiatry Invest. https://doi.org/10.4306/pi.2017.14.3.231.
Jaric, I., Rocks, D., Greally, J.M., Suzuki, M., Kundakovic, M., 2019. Chromatin organization in the female mouse brain fluctuates across the oestrous cycle. Nat. Commun. https://doi.org/10.1038/s41467-019-10704-0.
Jurek, B., Neumann, I.D., 2018. The oxytocin receptor: from intracellular signaling to behavior. Physiol. Rev. https://doi.org/10.1152/physrev.00031.2017.
Kalinowski, D., Bogus-Nowakowska, K., Kozlowska, A., Rowniak, M., 2022. Expression of calbindin, a marker of gamma-aminobutyric acid neurons, is reduced in the amygdala of oestrogen receptor beta-deficient female mice. J. Clin. Med. https://doi.org/10.3390/jcm11071760.
Kersting, A., Wagner, B., 2012. Complicated grief after perinatal loss. Dialog. Clin. Neurosci. https://doi.org/10.31887/DCNS.2012.14.2/akersting.
Klampfl, S.M., et al., 2018. Maternal stress and the MPOA: Activation of CRF receptor 1 impairs maternal behavior and triggers local oxytocin release in lactating rats. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2018.02.019.
Klampfl, S.M., Bosch, O.J., 2019. Mom doesn't care: when increased brain CRF system activity leads to maternal neglect in rodents. Front Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2019.01.001.
Klampfl, S.M., Brunton, P.J., Bayerl, D.S., Bosch, O.J., 2016. CRF-R1 activation in the anterior- dorsal BNST induces maternal neglect in lactating rats via an HPA axis-independent central mechanism. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2015.11.015.
Klampfl, S.M., Neumann, I.D., Bosch, O.J., 2013. Reduced brain corticotropin-releasing factor receptor activation is required for adequate maternal care and maternal aggression in lactating rats. Eur. J. Neurosci. https://doi.org/10.1111/ejn.12274.
Kunwar, P.S., et al., 2015. Ventromedial hypothalamic neurons control a defensive emotion state. Elife. https://doi.org/10.7554/eLife.06633.
Leuner, B., Fredericks, P.J., Nealer, C., Albin- Brooks, C., 2014. Chronic gestational stress leads to depressive-like behavior and compromises medial prefrontal cortex structure and function during the postpartum period. PLoS One. https://doi.org/10.1371/journal.pone.0089912.
Li, D., et al., 2020. Involvement of hyperpolarization-activated cyclic nucleotide-gated channel 3 in oxytocin neuronal activity in lactating rats with pup deprivation. ASN Neuro. https://doi.org/10.1177/1759091420944658.
Liu, X.Y., et al., 2019. Effects of intranasal oxytocin on pup deprivation-evoked aberrant maternal behavior and hypogalactia in rat dams and the underlying mechanisms. Front Neurosci. https://doi.org/10.3389/fnins.2019.00122.
Maciag, D., et al., 2010. Reduced density of calbindin immunoreactive GABAergic neurons in the occipital cortex in major depression: relevance to neuroimaging studies. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2009.10.027.
Marek, R., Xu, L., Sullivan, R.K.P., Sah, P., 2018. Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. Nat. Neurosci. https://doi.org/10.1038/s41593-018-0137-x.
Marlin, B.J., Mitre, M., D'Amour J, A., Chao, M.V., Froemke, R.C., 2015. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature. https://doi.org/10.1038/nature14402.
McCarthy, M.C., et al., 2010. Prevalence and predictors of parental grief and depression after the death of a child from cancer. J. Palliat. Med. https://doi.org/10.1089/jpm.2010.0037.
Menon, R., Neumann, I.D., 2023. Detection, processing and reinforcement of social cues: regulation by the oxytocin system. Nat. Rev. Neurosci. https://doi.org/10.1038/s41583-023-00759-w.
Mitra, R., Jadhav, S., McEwen, B.S., Vyas, A., Chattarji, S., 2005. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.0504011102.
Mitre, M., et al., 2017. Sex-specific differences in oxytocin receptor expression and function for parental behavior. Gend. Genome. https://doi.org/10.1089/gg.2017.0017.
Mori, H., Matsuda, K., Pfaff, D.W., Kawata, M., 2008. A recently identified hypothalamic nucleus expressing estrogen receptor alpha. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.0806503105.
Narita, K., Murata, T., Matsuoka, S., 2016. The ventromedial hypothalamus oxytocin induces locomotor behavior regulated by estrogen. Physiol. Behav. https://doi.org/10.1016/j.physbeh.2016.05.047.
Nemeroff, C.B., 2016. Paradise Lost: The Neurobiological and Clinical Consequences of Child Abuse and Neglect. Neuron. https://doi.org/10.1016/j.neuron.2016.01.019.
Neumann, I.D., Torner, L., Wigger, A., 2000b. Brain oxytocin: differential inhibition of neuroendocrine stress responses and anxiety- related behaviour in virgin, pregnant and lactating rats. Neuroscience. https://doi.org/10.1016/s0306-4522(99)00433-9.
Neumann, I.D., Wigger, A., Torner, L., Holsboer, F., Landgraf, R., 2000a. Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within theparaventricular nucleus. J. Neuroendocr. https://doi.org/10.1046/j.1365-2826.2000.00442.x.
Ng, H., et al., 2023. Effects of oxytocin ablation on pup rescue, nursing behaviors and response to pup separation in early-to-mid postpartum mice. J. Neuroendocrinol. https://doi.org/10.1111/jne.13247.
Numan, M., Young, L.J., 2016. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2015.05.015.
O'Connor, M.F., 2019. Grief: A Brief History of Research on How Body, Mind, and Brain Adapt. Psychosom. Med. https://doi.org/10.1097/PSY.0000000000000717.
Oliveira, V.E.M., et al., 2021. Oxytocin and vasopressin within the ventral and dorsal lateral septum modulate aggression in female rats. Nat. Commun. https://doi.org/10.1038/s41467-021-23064-5.
Osakada, T., et al., 2024. A dedicated hypothalamic oxytocin circuit controls aversive social learning. Nature. https://doi.org/10.1038/s41586-023-06958-w.
Pandey, G.N., Rizavi, H.S., Bhaumik, R., Ren, X., 2019. Increased protein and mRNA expression of corticotropin-releasing factor (CRF), decreased CRF receptors and CRF binding protein in specific postmortem brain areas of teenage suicide subjects. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2019.04.015.
Pawluski, J.L., Lieblich, S.E., Galea, L.A., 2009. Offspring-exposure reduces depressive-like behaviour in the parturient female rat. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2008.08.001.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotactic Coordinates. Academic Press, San Diego.

Payne, J.L., Maguire, J., 2019. Pathophysiological mechanisms implicated in postpartum depression. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2018.12.001.
Pekarek, B.T., Hunt, P.J., Arenkiel, B.R., 2020. Oxytocin and Sensory Network Plasticity. Front. Neurosci. https://doi.org/10.3389/fnins.2020.00030.
Pohl, T.T., Jung, O., Di Benedetto, B., Young, L.J., Bosch, O.J., 2021. Microglia react to partner loss in a sex- and brain site-specific manner in prairie voles. Brain Behav. Immun. https://doi.org/10.1016/j.bbi.2021.05.026.
Pohl, T.T., Young, L.J., Bosch, O.J., 2019. Lost connections: Oxytocin and the neural, physiological, and behavioral consequences of disrupted relationships. Int. J. Psychophysiol. https://doi.org/10.1016/j.ijpsycho.2017.12.011.
Price, R.B., Duman, R., 2020. Neuroplasticity in cognitive and psychological mechanisms of depression: an integrative model. Mol. Psychiatry. https://doi.org/10.1038/s41380-019-0615-x.
Qiao, H., et al., 2016. Dendritic Spines in Depression: What We Learned from Animal Models. Neural Plast. https://doi.org/10.1155/2016/8056370.
Radley, J.J., et al., 2006. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. Cereb. Cortex. https://doi.org/10.1093/cercor/bhi104.
Rashidi, M., et al., 2022. Peripartum effects of synthetic oxytocin: The good, the bad, and the unknown. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2022.104859.
Rilling, J.K., Young, L.J., 2014. The biology of mammalian parenting and its effect on offspring social development. Science. https://doi.org/10.1126/science.1252723.
Rincon-Cortes, M., Grace, A.A., 2021. Early Pup Removal Leads to Social Dysfunction and Dopamine Deficit in Late Postpartum Rats: Prevention by Social Support. Front. Glob. Women's. Health. https://doi.org/10.3389/fgwh.2021.694808.
Sabihi, S., Dong, S.M., Durosko, N.E., Leuner, B., 2014. Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. Front. Behav. Neurosci. https://doi.org/10.3389/fnbeh.2014.00258.
Sanson, A., Bosch, O.J., 2022. Dysfunctions of brain oxytocin signaling: Implications for poor mothering. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2022.109049.
Sanson, A., Demarchi, L., Rocaboy, E., Bosch, O.J., 2025. Increased CRF-R1 transmission in the nucleus accumbens shell facilitates maternal neglect in lactating rats and mediates anxiety- like behaviour in a sex-specific manner. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2024.110256.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. https://doi.org/10.1038/nmeth.2089.
Sequeira, M.K., Gourley, S.L., 2021. The stressed orbitofrontal cortex. Behav. Neurosci. https://doi.org/10.1037/bne0000456.
Servin-Barthet, C., et al., 2023. The transition to motherhood: linking hormones, brain and behaviour. Nat. Rev. Neurosci. https://doi.org/10.1038/s41583-023-00733-6.
Sheppard, P.A.S., Choleris, E., Galea, L.A.M., 2019. Structural plasticity of the hippocampus in response to estrogens in female rodents. Mol. Brain. https://doi.org/10.1186/s13041-019-0442-7.
Shirenova, S.D., Khlebnikova, N.N., Narkevich, V.B., Kudrin, V.S., Krupina, N.A., 2023. Nine-month-long social isolation changes the levels of monoamines in the brain structures of rats: a comparative study of neurochemistry and behavior. Neurochem. Res. https://doi.org/10.1007/s11064-023-03858-3.
Slattery, D.A., Cryan, J.F., 2014. The ups and downs of modelling mood disorders in rodents. ILAR J. https://doi.org/10.1093/ilar/ilu026.
Stamatakis, A., et al., 2015. Rat dams exposed repeatedly to a daily brief separation from the pups exhibit increased maternal behavior, decreased anxiety and altered levels of receptors for estrogens (ERalpha, ERbeta), oxytocin and serotonin (5-HT1A) in their brain. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2014.11.016.
Valtcheva, S., et al., 2023. Neural circuitry for maternal oxytocin release induced by infant cries. Nature. https://doi.org/10.1038/s41586-023-06540-4.
Vishnyakova, P.A., et al., 2021. Expression of calbindin and calretinin in the dorsomedial and ventromedial hypothalamic nuclei during aging. Anat. Rec. https://doi.org/10.1002/ar.24536.
Wang, T., et al., 2018. Injection of oxytocin into paraventricular nucleus reverses depressive-like behaviors in the postpartum depression rat model. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2017.09.012.
Wang, C., Jiang, C., Yuan, H., Xiao, C., Gao, D., 2013. Role of calbindin-D28K in estrogen treatment for Parkinson's disease. Neural Regen. Res. https://doi.org/10.3969/j.issn.1673-5374.2013.08.004.
Wilent, W.B., et al., 2010. Induction of panic attack by stimulation of the ventromedial hypothalamus. J. Neurosurg. https://doi.org/10.3171/2009.9.JNS09577.
Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology. https://doi.org/10.1210/endo.138.7.5255.
Winter, J., Jurek, B., 2019. The interplay between oxytocin and the CRF system: regulation of the stress response. Cell Tissue Res. https://doi.org/10.1007/s00441-018-2866-2.
Wonch, K.E., et al., 2016. Postpartum depression and brain response to infants: Differential amygdala response and connectivity. Soc. Neurosci. https://doi.org/10.1080/17470919.2015.1131193.
Workman, J.L., Brummelte, S., Galea, L.A., 2013. Postpartum corticosterone administration reduces dendritic complexity and increases the density of mushroom spines of hippocampal CA3 arbours in dams. J. Neuroendocrinol. https://doi.org/10.1111/j.1365-2826.2012.02380.x.
Young, L.J., Wang, Z., Donaldson, R., Rissman, E.F., 1998. Estrogen receptor alpha is essential for induction of oxytocin receptor by estrogen. Neuroreport. https://doi.org/10.1097/00001756-199803300-00031.
Zoubovsky, S.P., et al., 2020. Chronic psychosocial stress during pregnancy affects maternal behavior and neuroendocrine function and modulates hypothalamic CRH and nuclear steroid receptor expression. Transl. Psychiatry. https://doi.org/10.1038/s41398-020-0704-2.