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Brief versus long maternal separation in lactating rats: Consequences on maternal behavior, emotionality, and brain oxytocin receptor binding

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Abstract

Maternal separation is a widely used animal model to study early life adversity in offspring. However, only a few studies have focused on the impact of disrupting the maternal bond from the mother's perspective. Such studies reveal alterations in behavior, whereas the underlying neuroendocrine mechanisms remain largely unknown. In this study, we compared the consequences of daily brief maternal separation (BMS; 15 min) versus long maternal separation (LMS; 180 min) during the first week postpartum with respect to behavioral and neuroendocrine changes in lactating Sprague–Dawley dams. Mothers were tested for their maternal care before and after separation, maternal motivation to retrieve pups, as well as anxiety-related and stress-coping behaviors. In addition, we analyzed their basal plasma corticosterone levels and oxytocin receptor binding in selected brain regions of the limbic system and maternal network. LMS dams showed higher levels of behavioral alterations compared to BMS and non-maternally separated (NMS) dams, including increased licking and grooming of the pups and decreased maternal motivation. Anxiety-related behavior was not affected by either separation paradigm, whereas passive stress-coping behavior tended to increase in the LMS group. Plasma corticosterone concentrations were not different between groups. Oxytocin receptor binding was higher in the medial preoptic area and tended to be higher in the prelimbic cortex of LMS dams, only. Our results demonstrate that especially daily prolonged maternal separation impacts on the mothers' behavior and oxytocin system, which suggests that enhanced oxytocin receptor binding could be a compensatory mechanism for potentially decreased central oxytocin release due to limited pup contact.

KEYWORDS
maternal separation, medial preoptic area, oxytocin receptor, passive stress-coping behavior, prelimbic cortex

1 | INTRODUCTION

The maternal bond is the most important and long-lasting social bond in nature. The mother–child dyad is in fact interconnected, and it is critical to maintain a positive and intact bond for the mental and physical well-being of both subjects.[1] In recent decades, several studies have been conducted to investigate the consequences of a disturbed mother-offspring bond focusing on the development of the offspring; hence, the maternal separation paradigm became a well-known animal model for early life stress.[2–4] Maternal separation is performed daily for short (15–30 min) or long periods of time (180–360 min) and is typically applied during the first postnatal days after giving birth. In rodents, maternal separation acts as chronic stressor on the offspring,[5] impacting the offspring's behavior and brain.[6–9] Indeed, it has been linked to increased emotionality[10] (for review see[11]), cognitive impairment,[6] altered stress responses,[12,13] and epigenetic changes in the adult central nervous system.[14,15] Considering that the postpartum period is of potentially high-risk to develop psychiatric illnesses such as postpartum depression, it is surprising that only few studies investigated the effects of a disturbed mother–child bond from the mother's perspective.[16–18] Those studies show that maternal separation affects the mothers' maternal care, and anxiety- and depressive-like behavior.[19–24] For example, daily 180-min separation during the first 2 weeks postpartum increases passive stress-coping behavior in the forced swim test[25] and anhedonia in the sucrose preference test.[26] In other studies, the total removal of the offspring increases passive stress-coping behavior of the mother 1 month later[27] and impairs cognition.[28] However, many inconsistencies are still found when it comes to the phenotypic alterations following offspring separation, contributing to an unclear picture of separation-effects. Therefore, the reproduction of known data can help to confirm experimental setups and animal models used.

Even fewer studies have examined neuroendocrine alterations, mainly focusing on the oxytocin (OXT) and the hypothalamic corticotropin-releasing factor (CRF) systems demonstrating, for example, that prolonged separation of 20 h daily from lactation day 1 (LD1) to LD4 decreases the OXT+ neurons activity in the supraoptic nucleus (SON) of the mothers.[22] To our knowledge, no study has investigated the mechanisms of the OXT system to compensate for the decreased activity of OXT+ neurons following pup separation. Therefore, we aimed to reproduce some behavioral alterations and to add new insight into the neuroendocrine adaptations of the separated mothers, specifically over the first postpartum week, a time-window characterized by higher intensities and incidences of maternal responses.[29]

The OXT system plays an important role in the onset and maintenance of maternal bonding and behavior.[30] OXT is primarily synthesized in and released from the hypothalamic paraventricular nucleus (PVN) and SON. During the peripartum period, the activity of the OXT system is upregulated as it is critical for the expression of maternal behavior, maternal memory, and emotion modulation.[31–37] Furthermore, OXT receptor (OXTR) binding is increased during the postpartum period[38] and is involved in decreased anxiety-related behavior. Moreover, increased OXT system activity also dampens the stress response through modulation of the hypothalamic–pituitary–adrenal (HPA) axis,[39,40] which is triggered by the CRF system eliciting increased plasma adrenocorticotropic hormone and corticosterone levels. Increased brain CRF system activity has severe behavioral effects including impaired maternal behavior.[41] Therefore, it is not surprising that one of the peripartum adaptations is a marked decrease of the CRF system activity.[41] It is evident that a fine-tuning of both systems —increased OXT signaling in parallel to decreased HPA axis activity— is required for adequate maternal behavior.

To shed more light on a potential involvement of both systems on the phenotype of the separated mother (as recently reviewed in[42]), we compared the most frequently used maternal separation paradigms, that is, daily separation for 15 min (brief maternal separation, BMS) versus 180 min (long maternal separation, LMS) in lactating Sprague–Dawley rat mothers. In addition, we included non-maternally separated (NMS) dams as a control group. We investigated whether brief versus long maternal separation during the first week postpartum differentially affects the mothers' behavior and physiology, that is, maternal care, maternal motivation, anxiety-related behavior, passive stress-coping behavior, and OXTR binding as well as basal plasma corticosterone levels and adrenal gland weight. This study sheds new light on potential compensatory mechanisms in the OXT system that are triggered by offspring separation.

2 | MATERIALS AND METHODS

2.1 | Animals

The experiments were conducted on female Sprague–Dawley rats (Charles River Laboratories), weighing 230–250 g on arrival. The rats were kept under standard laboratory conditions (change of bedding once per week, 12:12 h light/dark cycle, lights on at 7 a.m., room temperature 22 ± 1°C, relative humidity 55 ± 5%) with access to standard rat chow (Ssniff-Spezialdiäten GmbH) 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 × 40 cm × 20 cm) for 10 days. From potential pregnancy day 18 onwards, pregnant females were single housed for undisturbed delivery either in standard laboratory cages (experiment A), or in observational cages (plexiglass, 38 cm × 22 cm × 35 cm; experiments B, C). On the day of delivery (LD0), litters were reduced to eight pups. All experiments were performed in accordance with the European Union Directive (2010/63/EU) and were approved by the Government of Unterfranken. According to the 3-R principles, all efforts were made to minimize the number of rats and their suffering.

2.2 | Maternal separation procedure

Dams were randomly assigned to one of the following three experimental groups: NMS (control group), BMS (15-min separation), or LMS (180-min separation). Separations occurred daily from 10.00 a.m. to 1.00 p.m. in LMS and from 12.45 p.m. to 1.00 p.m. in BMS mothers.

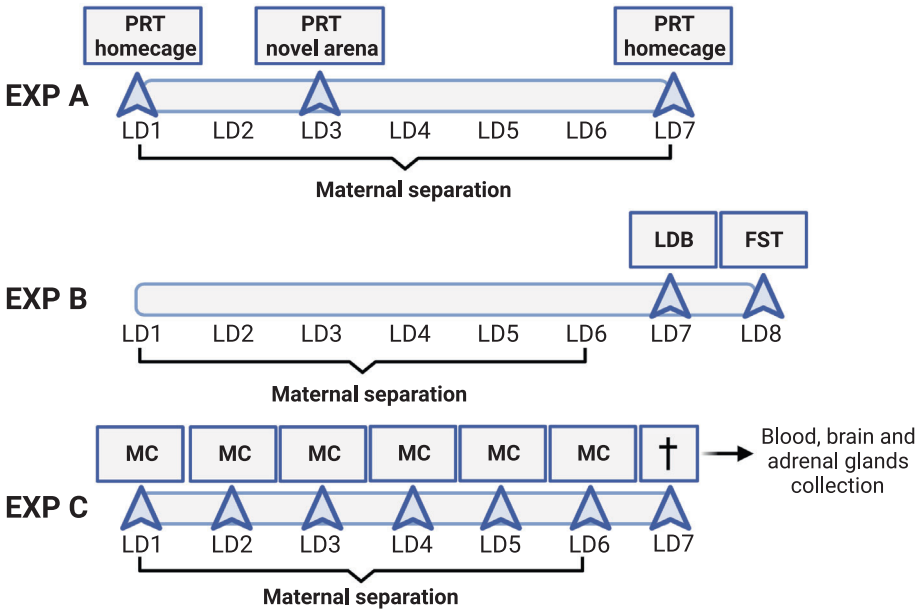

FIGURE 1 Experimental design and timeline. Experiment A was designed to study maternal motivation in two different settings. Experiment B aimed to reveal effects of maternal separation on anxiety-related and passive stress-coping behavior. Experiment C was designed to monitor maternal care before and after separation, and to collect basal samples from blood, brain, and adrenal glands. Abbreviations: EXP, experiment; FST, forced swim test; LD, lactation day; LDB, light–dark box; MC, maternal care; PRT, pup retrieval test.

During the separation, litters were kept in boxes containing their home cage bedding on a heating pad under constant temperature (32°C). Mothers were left undisturbed in their home cage during the separation procedure. At the end of the separation protocol, pups were returned to their home cage and placed in the corner opposite of the mother.

2.3 | Experimental schedule

For an overview of experiments A–C, see Figure 1.

2.3.1 | Experiment A

From LD1 to LD7, rat mothers underwent the maternal separation protocol except on LD3 when all three groups were tested in the pup retrieval test (PRT) in a novel arena. In addition, the pup retrieval test was performed in the home cage on LD1 and LD7 in the BMS and LMS groups after ending the separation. Number of rats were: NMS = 9; BMS = 9; LMS = 8.

2.3.2 | Experiment B

From LD1 to LD6, a different cohort of rat mothers underwent the maternal separation protocol. On LD7, all three groups were tested in the light dark box (LDB) and on LD8 in the forced swim test (FST). Number of rats were: NMS = 8; BMS = 8; LMS = 7.

2.3.3 | Experiment C

From LD1 to LD6, a different cohort of rat mothers underwent the maternal separation protocol. Maternal care was monitored from LD1 to LD6 before and after the reunion with the pups. The bodyweight of mothers and of whole litters was taken from all groups at 5.00 p.m. from LD1 to LD6. On LD7, all rats were sacrificed, and blood, brains and adrenal glands were collected for further analysis. Number of rats were: $n = 7$ in each group.

2.4 | Test for maternal motivation

Maternal motivation to retrieve pups is an essential maternal behavior ensuring the survival of the offspring until their reproductive maturity.[43] The motivation was tested in the PRT, either in the home cage or in a novel arena, and the number of pups retrieved every 10-s noted.

2.4.1 | Home cage

Maternal motivation to retrieve pups into the nest after placing them back into the home cage was recorded immediately after reunion following the separation paradigms.[20] Pups were placed in the corner opposite to the mother and the mother's behavior was videotaped for later analysis by an experimenter blind to the treatment. The latency to retrieve each of the eight pups during the 10-min test was analyzed.

2.4.2 | Novel arena

The PRT was performed between 10.00 a.m. and 12.00 p.m. and lasted 15 min. One hour prior to testing, pups were removed from the mother into separate boxes containing bedding from their home cage under controlled temperature conditions (32°C). For the PRT, pups were placed in a novel arena (54 cm × 34 cm × 60 cm) covered with

bedding from their home cage following a specific scheme of placement as previously described.[44] Trials were video recorded for subsequent analysis by an experimenter blind to the treatment. The latency to retrieve each of the eight pups during the 15-min test was analyzed. Before each test, the arena was cleaned with tap water and dried thoroughly.

2.5 | Test for anxiety-related behavior

Anxiety-related behavior was tested between 9.00 a.m. and 12.00 p.m. in the LDB.[45] The arena was divided into a light (40 cm × 50 cm, 180 lux) and a dark compartment (40 cm × 30 cm, 0 lux) connected via an opening (7.5 cm × 7.5 cm). At the beginning of the test, dams were placed in the center of the light box and their behavior was recorded for 10 min for later analysis by an experimenter blind to the treatment with EthoVision XT (Noldus). Behaviors analyzed were: time spent in the light box, numbers of transitions from the light to the dark box and locomotor activity in the whole arena. Before each test, the arena was cleaned with tap water and dried thoroughly.

2.6 | Test for passive stress-coping

Passive stress-coping behavior was tested in the FST.[46] Between 9.00 a.m. and 12.00 p.m., rat mothers were forced to swim for 10 min in a cylindrical tank (50 cm high, 30 cm diameter) filled with tap water (23 ± 1°C) to a depth that rats could not touch the bottom with their hind paws or tail. Trials were recorded for later analysis using the software JWatcher (https://www.jwatcher.ucla.edu) by an experimenter blind to the treatment. The total time spent floating (passive stress-coping, indicative of depressive-like behavior[47]) was analyzed.

2.7 | Observation of maternal care

Maternal care in the home cage was monitored and manually scored by an experimenter blind to the treatment according to an established protocol.[44,48–50] Observation of maternal care was performed from 9.00 a.m. to 10.00 a.m. and from 1.00 p.m. to 2.00 p.m. every 2 min for 10 s prior to the maternal separation (T1) and after reunion with the pups (T2) leading to a total count of 60 observation points per dam and per day. The scored behaviors to determine the quality of maternal care were licking and grooming (LG) of the pups and arched-back nursing (ABN).[51]

2.8 | Blood collection and corticosterone ELISA

Between 10.00 a.m. and 12.00 p.m., dams in their home cage were transported to a separate room, flash-anesthetized with isoflurane (in preparation for perfusion; see below), the thorax opened, and blood was collected immediately from the right atrium of the heart in EDTA-coated tubes (0.5 M, pH 7.4; Sarstedt), which were maintained on ice until further processing. Blood samples were centrifuged for 10 min at 9,391 *g*, plasma was collected and processed with the ELISA kit for corticosterone analysis following the protocol of the manufacturer (Tecan IBL International GmbH).

2.9 | Brain sampling and OXTR autoradiography

After blood sampling (see above), dams underwent cardiac perfusion with ice-cold 1× PBS, 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, that is, agranular insular cortex (AIP), accessory olfactory nuclei (AOB), bed nucleus of the stria terminalis (BNST), central amygdala (CeA), lateral septum dorsal (dLS) and ventral (vLS), medial preoptic area (MPOA), nucleus accumbens shell (NAcc shell), prelimbic cortex (PL), ventral medial hypothalamus (VMH), six sections per rat were collected on SUPERFROST microscope slides and stored at −20°C until further processing. The OXTR autoradiography was performed following an established protocol.[44,52] Briefly, the ornithin vasotocin analog ([^{125}I]-OVTA [d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Orn^8,[^{125}I]Tyr^9-NH$_2$]; Perkin Elmer) 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 2× in Tris (50 mM, pH 7.4), covered with the tracer solution (50 mM tracer, 10 mM $MgCl_2$, 0.1% BSA) for 60 min, washed 3× in Tris/$MgCl_2$ buffer for 7 min, each, followed by 30-min spinning in Tris/$MgCl_2$. Finally, slides were dipped into water and air dried before being exposed to Biomax MR films (Kodak) for 15 days. The films were scanned using the EPSON Perfection V800 Scanner (Epson GmbH), and the optical density of each region of interest was analyzed using ImageJ[53] by subtracting the background activity as previously described.[54] The analyses were performed simultaneously for six sections per rat and per region in the left hemisphere.

2.10 | Adrenal gland collection

After brain removal, adrenal glands were collected and stored on ice in 1× PBS. Adrenal glands were dissected from the surrounding fat and weighed to calculate the adrenal gland's relative weight (adrenal gland weight/bodyweight).

2.11 | Statistical analyses

All statistical analyses were performed with GraphPad PRISM 9 (GraphPad Software). Normality and homoscedasticity were verified (Shapiro–Wilk or Kolmogorov–Smirnov test and Brown-Forsythe test, respectively) and analysis of outliers was run via the ROUT method. Maternal motivation, maternal behavior, and bodyweight were analyzed using a two-way RM ANOVA (factors: time × treatment) followed by Sidak post-hoc multiple comparisons if main effects were found. Latency to retrieve the first pup in the homecage was analyzed using the

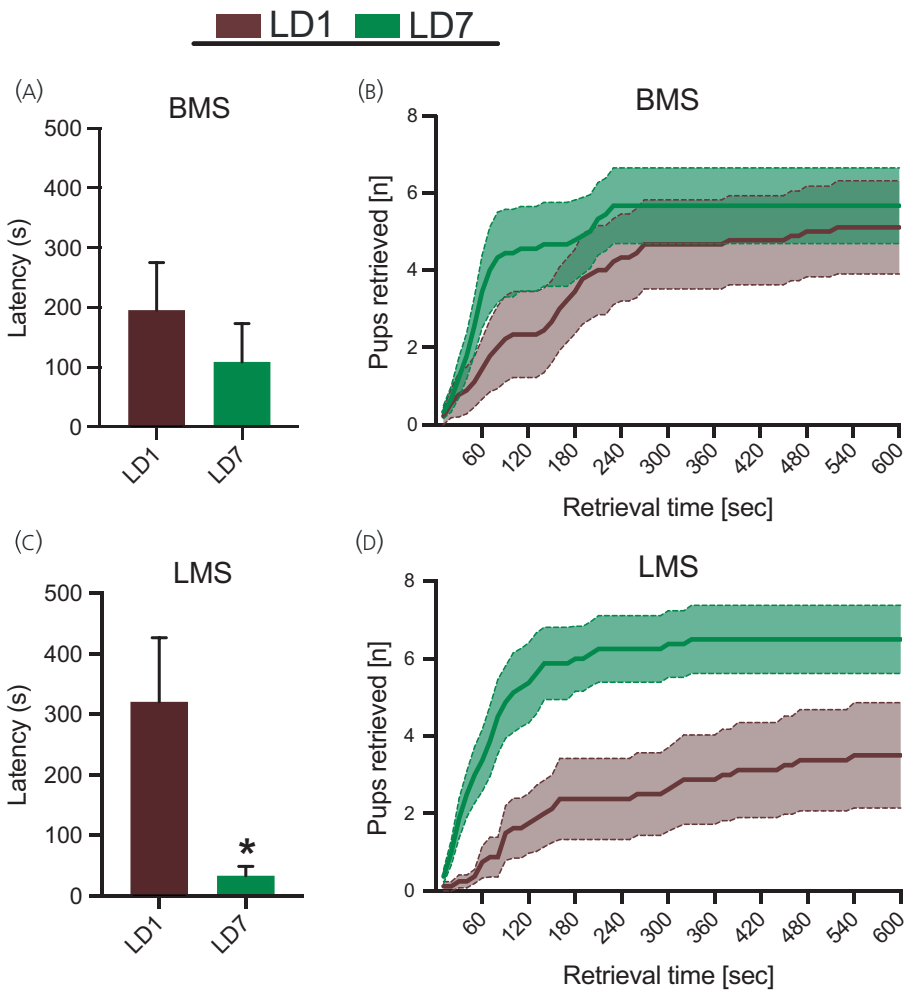

FIGURE 2 Maternal motivation in the home cage on LD1 and LD7. (A) Time until retrieval of first pup in BMS (Mann–Whitney test). (B) Number of retrieved pups during the 600-s test in BMS group on LD1 (brown line) versus LD7 (green line) (two-way RM ANOVA). (C) Time until retrieval of first pup in LMS (Mann–Whitney test). (D) Number of retrieved pups during the 600-s test in LMS group on LD1 (brown line) versus LD7 (green line) (two-way RM ANOVA). Number of animals: BMS = 9, LMS = 8. Dashed lines represent SEM values. *$p < .05$ LD1 versus LD7.

Mann–Whitney test. Latency to retrieve the first pup on LD3, behavioral parameters analyzed in the LDB and FST, OXTRs binding, and adrenal gland weight were analyzed using a one-way ANOVA. Plasma corticosterone concentration data did not meet the homoscedasticity and a Welch's ANOVA was run. Additional size effects between groups were calculated using the Cohen's *d* coefficient and eta squared h^2. Data are presented as mean ± SEM; $p < .05$ was considered significant and a trend was accepted up to $p = .08$.

3 | RESULTS

3.1 | Experiment A

3.1.1 | LMS impaired maternal motivation in the home cage

In the BMS group, no differences were found in the latencies to retrieve the first pup between LD1 and LD7 (Mann–Whitney U = 28, n1 = n2 = 9, $p = .296$ two-tailed; Figure 2A).

In BMS dams, the main effect of time on number of pups retrieved was significant (two-way RM ANOVA [F {59, 944} = 16.13, $p < .0001$, $h^2 = 0.16$]; Figure 2B) but the main effect of LD1 and LD7 on the number of pups retrieved was not significant (two-way RM ANOVA [F {1, 16} = 0.7047, $p = .414$, $h^2 = 0.03$]; Figure 2B). In BMS dams, there was not a significant interaction effect (two-way RM ANOVA; factors time x treatment [F {59, 944} = 0.7729, $p = .894$, $h^2 = 0.007$]; Figure 2B).

LMS dams displayed significantly lower latencies to retrieve the first pup on LD7 (Mann–Whitney $U = 12$, n1 = n2 = 8, $p = .033$ two-tailed; Figure 2C). There was a significant main effect of time (two-way RM ANOVA; [F {1.97, 27.65} = 17.23, $p < .0001$, $h^2 = 0.12$]; Figure 2D) and of lactation day on the number of pups retrieved (two-way RM ANOVA; [F {1, 14} = 6.646, $p = .022$, $h^2 = 0.25$]; Figure 2D). In LMS dams, an interaction effect was found when comparing LD1 and LD7 (two-way RM ANOVA; factors time x treatment [F {59, 826} = 1.402, $p = .028$, $h^2 = 0.009$]; Figure 2D).

3.1.2 | LMS impaired maternal motivation in a novel arena on LD3

The treatment groups differed in the latency to retrieve the first pup (one-way ANOVA [F {2, 22} = 3.978, $p = .034$, $h^2 = 0.27$]; Figure 3A).

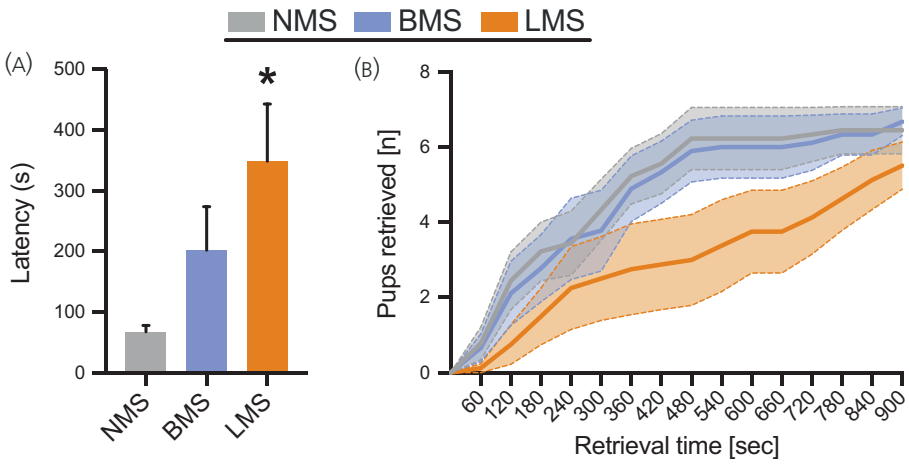

FIGURE 3 Maternal motivation in a novel arena on LD3. (A) Time until retrieval of first pup (one-way ANOVA). (B) Number of retrieved pups during the 900-s test (two-way RM ANOVA). Number of animals: NMS = 9, BMS = 9, LMS = 8. Dashed lines represent SEM values. *$p < .05$ LMS versus NMS.

Post-hoc Sidak multiple comparisons test revealed that the time taken to retrieve the first pup was significantly greater in LMS than NMS dams ($p = .029$, 95% CI = [−538.9, −23.57]). However, neither NMS and BMS ($p = .445$) nor BMS and LMS ($p = .374$) differed from each other.

The main effect of 10-s intervals of time on the number of retrieved pups was significant (two-way RM ANOVA [F {2.90, 66.89} = 44.73, $p < .0001$, $h^2 = 0.33$]; Figure 3B) but the main effect of treatment groups was not significant (two-way RM ANOVA [F {2, 23} = 2.155, $p = .138$, $h^2 = 0.08$]; Figure 3B). There was no interaction effect comparing NMS, BMS and LMS dams (two-way RM ANOVA [F {178, 2047} = 0.866, $p = .891$, $h^2 = 0.01$]; Figure 3B).

3.2 | Experiment B

3.2.1 | BMS and LMS had no effect on anxiety-related behavior

On LD7, no significant differences between the groups were found in the time spent in the light box of the LDB as a measure of anxiety-related behavior (one-way ANOVA [F {2, 18} = 0.113, $p = .895$, $h^2 = 0.01$]) (NMS: 42.6 ± 2.3 s; BMS: 44.5 ± 4.6 s; LMS: 41.9 ± 4.6 s) nor in transitions between the compartments (F [2, 18] = 1.169, $p = .333$, $h^2 = 0.15$) (NMS: 10.1 ± 1.3; BMS: 10.8 ± 3.5; LMS: 13.6 ± 2.2) or locomotion during the LDB test (F [2, 18] = 0.94, $p = .409$, $h^2 = 0.09$) (NMS: 4436 ± 389 cm; BMS: 5188 ± 727 cm; LMS: 4163 ± 307 cm).

3.2.2 | LMS tended to increase passive stress-coping behavior

On LD8, time spent floating during the FST over the first 5-min test tended to differ between groups (one-way ANOVA [F {2, 20} = 2.875, $p = .079$, $h^2 = 0.22$]) (NMS: 20.93 ± 4.47 s; BMS: 12.46 ± 2.87 s; LMS: 28.78 ± 6.71 s). Cohen's d coefficient revealed the strongest effect size between LMS and BMS groups ($d = 1.06$); however, this did not reach statistical significance. Over the full 10-min test, no significant differences were found between groups (one-way ANOVA [F {2, 20} = 2.083, $p = .150$, $h^2 = 0.17$]).

3.3 | Experiment C

3.3.1 | BMS and LMS increased LG behavior

The main effect of time (two-way RM ANOVA [F {1.839, 18.3} = 27.64, $p < .0001$, $h^2 = 0.36$]; Figure 4A) and of treatment (two-way RM ANOVA [F {1, 10} = 25.69, $p = .0005$, $h^2 = 0.01$]; Figure 4A) on the total mean LG frequency was significant. LG differed between the groups depending on time and treatment (two-way RM ANOVA; factors time x treatment [F {2, 20} = 12.69, $p = .0003$, $h^2 = 0.17$]; Figure 4A). The post-hoc Sidak multiple comparisons revealed that from T1 (before separation) to T2 (after separation), BMS ($p = .037$) and LMS dams ($p = .002$) showed increased LG compared to the NMS group.

ABN did not differ depending on time (two-way RM ANOVA [F {2, 20} = 2.686, $p = .093$, $h^2 = 0.15$]; Figure 4B) or treatment (two-way RM ANOVA [F {1, 10} = 1.645, $p = .228$, $h^2 = 0.04$]; Figure 4B). In addition, when calculating the delta LG frequencies between T2 (after separation) and T1 (before separation), there was a main time effect (two-way ANOVA [F {5, 90} = 15.97, $p < .0001$, $h^2 = 0.12$]; Figure 4C), a main treatment effect (two-way ANOVA [F {2, 90} = 212.3, $p < .0001$, $h^2 = 0.62$]; Figure 4C) and an interaction effect (two-way ANOVA, factors time x treatment [F {10, 90} = 9.553, $p < .0001$, $h^2 = 0.14$]; Figure 4C). Post-hoc Sidak multiple comparisons revealed increased LG in BMS versus NMS on LD1 ($p = .006$), LD2 ($p = .0003$), LD3 ($p < .0001$), LD5 ($p < .0001$), and LMS versus NMS from LD1 to LD6 ($p < .0001$, each; Figure 4C), but no differences were observed when comparing BMS and LMS. No significant effect was found when calculating the delta ABN frequencies between T2 and T1 (Figure 4D).

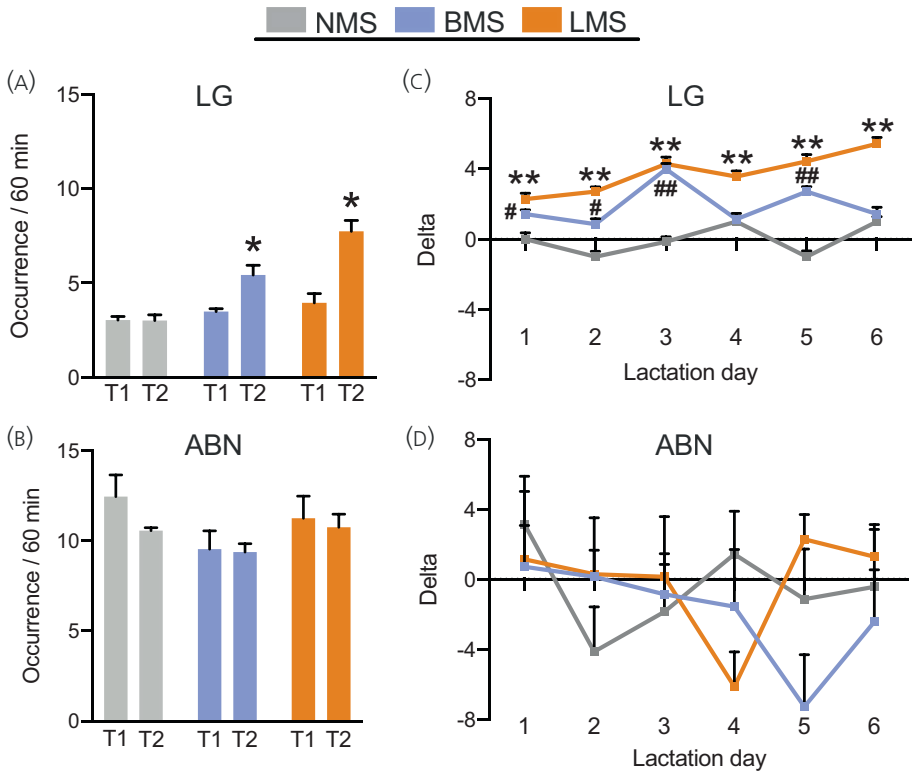

FIGURE 4 Maternal care in the home cage before and after separation from LD1 to LD6. Mean overall frequency of (A) licking and grooming the pups (LG) and (B) arched back nursing (ABN) for 60 min at T1 (preceding the separation) and T2 (immediately after reunion with the pups). Delta scores (T2-T1) for (C) LG and (D) ABN from LD1 to LD6. (A–D) Two-way RM ANOVA, number of animals: $n = 7$ (each group). Results are expressed as mean + SEM. $^{*}p < .05$, $^{**}p < .0001$ LMS versus NMS; T2 versus T1; $^{\#}p < .05$, $^{\#\#}p < .0001$ BMS versus NMS.

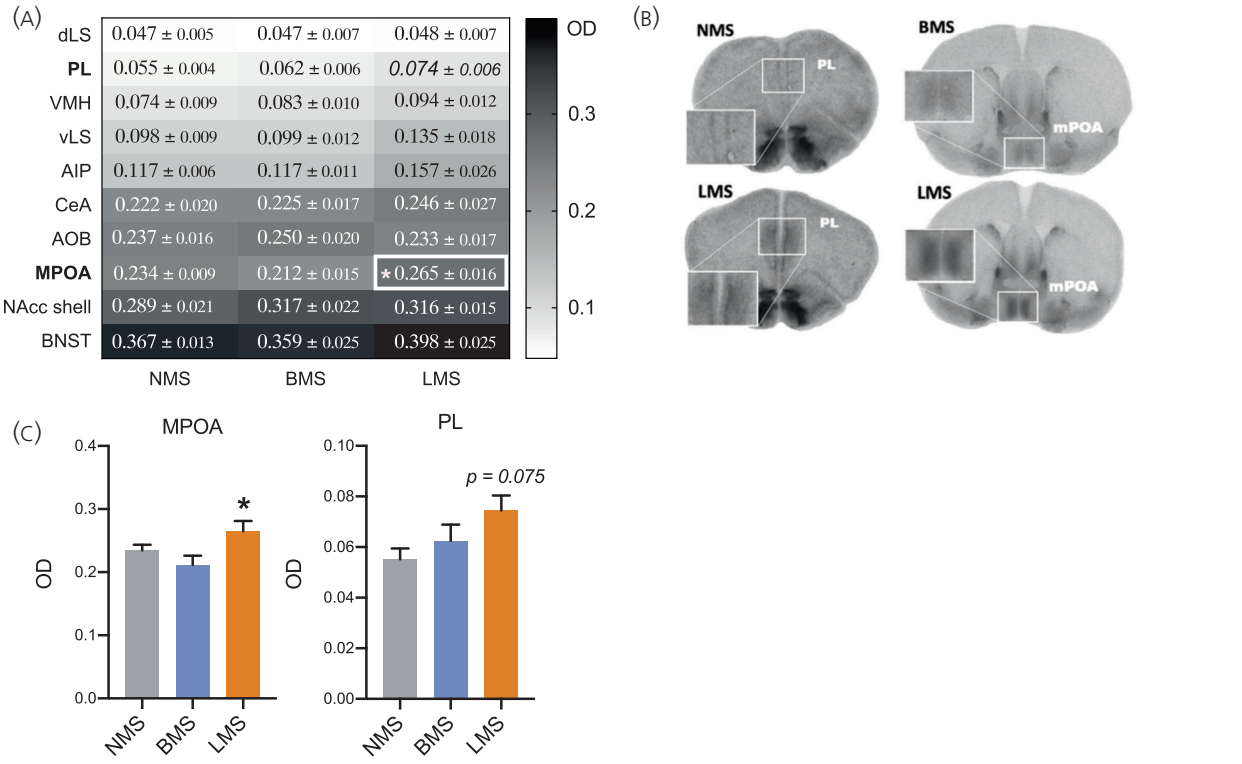

FIGURE 5 Oxytocin receptor (OXTR) binding in brain regions of the limbic system and maternal network on LD7. (A) Overview of gray densities (arbitrary units) for OXTR binding in the analyzed brain areas (PL: numbers in italic represent a trend in LMS versus NMS). (B) Optical gray densities (arbitrary units) for OXTR binding in the MPOA and PL. (C) Representative coronal brain sections of the prelimbic cortex (PL) and medial preoptic area (MPOA) demonstrating differences in OXTR binding. Abbreviations: Agranular insular cortex (AIP), accessory olfactory nuclei (AOB), bed nucleus of the stria terminalis (BNST), central amygdala (CeA), lateral septum dorsal (dLS) and ventral (vLS), medial preoptic area (MPOA), nucleus accumbens shell (NAcc shell), prelimbic cortex (PL), ventral medial hypothalamus (VMH). (A, B) One-way ANOVA, number of animals: $n = 7$ (each group). Results are expressed as mean ± SEM. $^{*}p < .05$ LMS versus BMS.

3.3.2 | BMS and LMS had no effect on basal plasma corticosterone concentrations

The treatment groups did not differ in basal plasma corticosterone concentrations (Welch's ANOVA test; $p = .116$) (NMS: 419.3 ± 121.5 ng/ml; BMS: 150.1 ± 41.3 ng/ml; LMS: 720.3 ± 314.4 ng/ml).

3.3.3 | LMS increased OXTR binding in the MPOA

In the MPOA, OXTR binding differed between treatment groups (one-way ANOVA [F {2, 17} = 3.726, $p = .045$, $h^2 = 0.30$]; Figure 5A–C). Post-hoc Sidak multiple comparisons revealed a significant difference between the BMS and LMS groups ($p = .043$). In the PL region, OXTR binding tended to differ between treatment groups (one-way ANOVA [F {2, 18} = 3.005, $p = .075$, $h^2 = 0.25$]; Figure 5A–C). Cohen's d coefficient revealed the strongest effect size between NMS and LMS groups ($d = 1.16$). No differences in OXTR binding between the treatment groups were found in the other analyzed regions: CeA (F [2, 18] = 0.346, $p = .712$), BNST (F [2, 17] = 0.907, $p = .422$), AIP (F [2, 18] = 1.836, $p = .188$), NAcc shell (F [2, 17] = 0.647, $p = .536$), AOB (F [2, 17] = 0.228, $p = .798$), dLS (F [2, 17] = 0.004, $p = .995$), vLS (F [2, 17] = 2.132, $p = .149$), VMH (F [2, 18] = 0.952, $p = .405$).

3.4 | Adrenal glands and bodyweight

Neither the relative adrenal gland weight on LD7 (one-way ANOVA [F {2, 18} = 0.1257, $p = .883$, $h^2 = 0.01$]) (NMS: 247 ± 7 mg; BMS: 257 ± 13 mg; LMS: 253 ± 19 mg) nor the change in bodyweight over the days in mothers (two-way RM ANOVA; factors time x treatment [F {12, 108} = 0.511, $p = .904$, $h^2 = 0.06$]; data not shown) or litters (two-way RM ANOVA; factors time x treatment [F {10, 90} = 0.286, $p = .983$, $h^2 = 0.03$]; data not shown) differed between the groups.

4 | DISCUSSION

The present study compared the consequences of daily brief versus long maternal separation in the first week postpartum on rat mothers' maternal, emotional, physiological, and neuroendocrine parameters (see Table 1 for an overview of the results). Overall, we were able to demonstrate that LMS had a stronger impact on various factors compared to BMS.

LMS resulted in reduced maternal motivation both in the home cage on LD1 (Figure 2C–D) and in a novel, and more challenging, arena (Figure 3). Interestingly, maternal motivation was normalized after 1 week of separation thereby demonstrating that the mothers might have adapted to the daily separation distress.[55] On the contrary, BMS dams did not show alterations in maternal motivation (Figures 2A,B and 3). These findings are consistent with previous studies demonstrating a slower pup retrieval of dams that had been separated from their pups for 180-min or even 360-min versus 15-min.[20,26,56] Our results reveal that long maternal separation reduced maternal motivation immediately, followed by an improvement over 1 week possibly due to coping strategies. As previously demonstrated by Stolzenberg et al.,[57] the rescue of maternal motivation as a result of repeated distress experiences may even imply epigenetic alterations at the level of chromatin modifications, which in turn change the expression of genes that promote, particularly in the MPOA,[57] one of the main brain regions where pup retrieval is processed.[58,59]

With respect to maternal care, both LMS and BMS mothers showed more LG behavior compared to NMS dams. When analyzing the data day-by-day, it becomes clear that LMS dams tended to increase LG behavior over the course of the lactation days (Figure 4C), peaking on LD6. These findings are supported by the literature's assertion that either a short or protracted maternal separation improves maternal behavior.[21,25,60–65] In fact, it has been suggested that separation stress serves as a catalyst for increased maternal behavior.[66,67] Thus, the enhanced pup-directed care following the reunion is probably an attempt to make up for the pups' lack of care during the separation.[20]

As maternal behavior is mediated by increased brain OXT signaling, among others,[29,31,68] we speculated that maternal separation might interfere with the mothers' brain OXT system.[42] In fact, alteration in OXTR binding has been described in a different maternal separation model, that is, 15-min per day lasting from LD1 to LD22 using Wistar rat mothers.[64] Therefore, we analyzed OXTR binding in several limbic and maternal network regions following 1 week of separation. We found that LMS dams – in comparison to BMS and NMS mothers – had significantly increased OXTR binding in the MPOA and tended to have higher OXTR binding in the PL, but not in any other region analyzed (CeA, BNST, AIP, NAcc shell, OB, LS, VMH; Figure 5A–C). While the MPOA is commonly associated with pup retrieval behavior[69,70] (for reviews see[51,59,71]), little is known about a potential role of the PL in maternal behavior. Recently, the PL has been shown to modulate the reward system through the OXT system.[72] OXT neurons are highly activated during pup nursing and OXT is released not only into the periphery but also within the brain.[73,74] Furthermore, nursing the pups is a reward for the mother that is even stronger than cocaine[75] and pup retrieval requires the dopamine mesolimbic system activity.[76] Thus, we hypothesize that the mother is seeking to nurse the pups, which in turn could lead to their retrieval when outside the nest.

Interestingly, a study by Stamatakis et al.[64] describes effects of maternal separation on OXTR binding similar to ours, that is, increased OXTR binding in the prefrontal cortex and MPOA. In addition, the authors show higher OXTR binding also in the hippocampus, LS, and NAcc shell, which was not found in our study. This discrepancy could be explained by the extended maternal separation distress (22 days vs. 7 days) as well as the different rat strain (Wistar vs. Sprague–Dawley). Furthermore, different technical approaches had been used, that is, analyses of sagittal brain sections[64] while we used coronal sections resulting in a different volumetric area for each brain region. However, Stamatakis et al.[64] also demonstrate that in separated rat dams

TABLE 1 Summary of the behavioral and neuroendocrine results.

Group	Maternal care	PRT LD1 vs. LD7	PRT LD3	Passive stress-coping	Plasma cort	OXTR binding
NMS	=		=	=	=	=
BMS	↑	=	=	=	=	=
LMS	↑	↓	↓	=	=	↑ MPOA

Note: ↑ Increase, ↓ decrease, "=" no difference.
Abbreviations: BMS, brief maternal separation; cort, corticosterone; LD, lactation day; LMS, long maternal separation; MPOA, medial preoptic area; OXTR, oxytocin receptor; NMS, non-maternally separated; PRT, pup retrieval test.

increased OXTR binding is paralleled by more LG of the pups, which is in line with studies on high LG-ABN mothers[77] as well as with our present findings. Indeed, LMS dams exhibited the highest frequency of LG after 1 week of maternal separation (Figure 4A,C). Therefore, we speculate that the increased OXTR binding we found in LMS dams might be part of a compensatory strategy for potentially less brain OXT activity, consistent with other studies showing concomitant opposite central changes in OXT and OXTR levels.[78,79] Indeed, the prolonged separation leads to less interactions with the pups, and consequently less milk ejection reflexes, which in turn might result in reduced brain OXT signaling. On the other hand, daily brief separation may not be sufficient to cause alterations at the level of the OXT system during the first postpartum week. Further research could test this hypothesis, especially as the current literature already suggests that the OXT system is modulated by maternal separation, for example, reduced c-Fos expression in OXT+ neurons of the SON of prolonged separated mothers[22] and less immunoreactive OXT neurons in the PVN of 180-min separated mothers.[20]

While anxiety-related behavior is reduced in lactation[51,80,81] neither BMS nor LMS had any effect compared with NMS. The literature is not consistent as some studies on prolonged maternal separation describe the same lack of effect on anxiety-related behavior,[21,27] whereas other studies indicate higher anxiety in prolonged separated mothers.[56,82,83] Differences in maternal separation procedures, rat strain, and anxiety-testing methods could explain these discrepancies. For example, prolonged separated Sprague–Dawley mothers did not show altered anxiety-related behavior in the elevated plus maze,[21] whereas separated Wistar rats had increased anxiety-related behavior.[56,82,83] Therefore, more research with consistent paradigms is needed to better understand the impact of maternal separation on anxiety-related behavior in lactating mothers.

When tested for passive stress-coping in the FST, which is indicative of a depressive-like phenotype[47] (but also see[84]), LMS but not BMS dams showed a tendency towards increased floating behavior in the first 5 min. Such increased floating behavior in LMS is in line with previous studies.[25,26] The lack of effect in BMS mothers further demonstrates that brief separation from the pups may not be sufficient to increase passive stress-coping, which might be explained by the fact that BMS could represent a safer and more natural early rearing condition than LMS.[60] Interestingly, passive stress-coping in the FST is influenced by brain OXT signaling as shown, for example, by intranasal and intracerebroventricular administration of OXT in male and female rats.[85,86] Hence, altered OXT signaling in separated mothers could also be involved in altered passive stress-coping behavior. However, there is, as yet, no direct evidence for such an effect of OXT in separated rat mothers.

Basal plasma corticosterone levels did not differ between groups, which contrasts with a study where daily repeated brief separation in Sprague–Dawley dams,[26] or the total removal of the offspring, reduced basal corticosterone levels.[87–89] However, we did not test the stress response of the HPA axis, which may have given us a different picture regarding group differences. In a recent study, restraint stress induced higher corticosterone levels in virgin females and in one day separated dams, but not in control lactating dams.[87] Future research could shed more light on the impact of offspring separation on the mother's basal as well as the stress-induced HPA axis response. Since corticosterone is produced by and released from the adrenal glands, we also assessed their relative weight as an increase is thought to be an indicator for chronic stress.[89] However, we could not detect any differences between groups, which is in line with our corticosterone results, but in contrast to previous studies that show increased adrenal gland weight in prolonged separated dams.[26,60] The longer maternal separation procedures (2 weeks vs. 1 week) may account for the discrepancies. In fact, one week of separation might not be a sufficient stimulus, as it has been shown that chronic stress over two weeks induced adrenal gland hyperplasia and hypertropia.[89]

Neither the bodyweight of the mothers nor the overall weight of their litters was different at any timepoint measured. This confirms previous studies demonstrating that the separation itself has no impact on the mothers' bodyweight.[26,60] In addition, a daily 3-h lack of milk ingestion did not affect the weight of pups, suggesting that the responsiveness of BMS and LMS dams to the pups was not altered, which is in line with our data of unaltered ABN (Figure 4B–D). As with many studies, the design of the current study is subject to limitations. A small number of rats were used for behavioral analysis as higher numbers might have revealed a slightly stronger behavioral outcome. Another limitation was the type of behavioral tests: additional anxiety- and depressive-like behavioral tests could have given more information about the phenotypes of the separated mothers. Finally, the duration of maternal separation treatment days could have limited some molecular and/or behavioral outcomes.

In conclusion, our findings confirm previous studies demonstrating that one week of daily prolonged separation from the pups has a significant impact on the mother's maternal behavior. Our results

suggest a potential compensatory mechanism of increased OXTR expression in maternal brain regions due to the limited pup contact, underlining the complicated involvement of the OXT system when it comes to a break of the maternal bond.

AUTHOR CONTRIBUTIONS

Luisa Demarchi: Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft. **Alice Sanson:** Investigation; methodology; writing – review and editing. **Oliver J. Bosch:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

OJB served as an editor for the special issue on the Parental Brain Meeting 2022 but was not involved in the review process of the present study. No other potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Pohl TT, Young LJ, Bosch OJ. Lost connections: oxytocin and the neural, physiological, and behavioral consequences of disrupted relationships. *Int J Psychophysiol*. 2019;136:54-63.
2. Nishi M. Effects of early-life stress on the brain and behaviors: implications of early maternal separation in rodents. *Int J Mol Sci*. 2020;21(19):75-81.
3. Teissier A, Le Magueresse C, Olusakin J, et al. Early-life stress impairs postnatal oligodendrogenesis and adult emotional behaviour through activity-dependent mechanisms. *Mol Psychiatry*. 2020;25(6):1159-1174.
4. Veenema AH, Bredewold R, Neumann ID. Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. *Psychoneuroendocrinology*. 2007;32(5):437-450.
5. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res*. 2000;122:81-103.
6. Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology*. 2007;32(3):256-266.
7. Lehmann J, Feldon J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Rev Neurosci*. 2000;11(4):383-408.
8. Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM. Long-term behavioural and molecular alterations associated with maternal separation in rats. *Eur J Neurosci*. 2007;25(10):3091-3098.
9. Monroy E, Hernandez-Torres E, Flores G. Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. *J Chem Neuroanat*. 2010;40(2):93-101.
10. Tsotsokou G, Nikolakopoulou M, Kouvelas ED, Mitsacos A. Neonatal maternal separation affects metabotropic glutamate receptor 5 expression and anxiety-related behavior of adult rats. *Eur J Neurosci*. 2021;54(2):4550-4564.
11. Wang D, Levine JLS, Avila-Quintero V, Bloch M, Kaffman A. Systematic review and meta-analysis: effects of maternal separation on anxiety-like behavior in rodents. *Transl Psychiatry*. 2020;10(1):174.
12. Abdelwahab LA, Galal OO, Abd El-Rahman SS, El-Brairy AI, Khattab MM, El-Khatib AS. Targeting the oxytocin system to ameliorate early life depressive-like behaviors in maternally-separated rats. *Biol Pharm Bull*. 2021;44(10):1445-1457.
13. Bian Y, Ma Y, Ma Q, et al. Prolonged maternal separation induces the depression-like behavior susceptibility to chronic unpredictable mild stress exposure in mice. *Biomed Res Int*. 2021;2021:6681397-6681311.
14. Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neurosci Biobehav Rev*. 2005;29(8):1335-1346.
15. Weaver IC. Epigenetic programming by maternal behavior and pharmacological intervention. Nature versus nurture: let's call the whole thing off. *Epigenetics*. 2007;2(1):22-28.
16. Brummelte S, Galea LA. Chronic corticosterone during pregnancy and postpartum affects maternal care, cell proliferation and depressive-like behavior in the dam. *Horm Behav*. 2010;58(5):769-779.
17. Brunton PJ, Russell JA. The expectant brain: adapting for motherhood. *Nat Rev Neurosci*. 2008;9(1):11-25.
18. Jones I, Chandra PS, Dazzan P, Howard LM. Bipolar disorder, affective psychosis, and schizophrenia in pregnancy and the post-partum period. *Lancet*. 2014;384(9956):1789-1799.
19. Alves RL, Portugal CC, Summavielle T, Barbosa F, Magalhaes A. Maternal separation effects on mother rodents' behaviour: A systematic review. *Neurosci Biobehav Rev*. 2020;117:98-109.
20. Baracz SJ, Everett NA, Robinson KJ, Campbell GR, Cornish JL. Maternal separation changes maternal care, anxiety-like behaviour and expression of paraventricular oxytocin and corticotrophin-releasing factor immunoreactivity in lactating rats. *J Neuroendocrinol*. 2020;32(6):e12861.
21. Bolukbas I, Mundorf A, Freund N. Maternal separation in rats induces neurobiological and behavioral changes on the maternal side. *Sci Rep*. 2020;10(1):22431.
22. Liu XY, Li D, Li T, et al. Effects of intranasal oxytocin on pup deprivation-evoked aberrant maternal behavior and Hypogalactia in rat dams and the underlying mechanisms. *Front Neurosci*. 2019;13:122.
23. Orso R, Wearick-Silva LE, Creutzberg KC, et al. Maternal behavior of the mouse dam toward pups: implications for maternal separation model of early life stress. *Stress*. 2018;21(1):19-27.
24. Smith JW, Seckl JR, Evans AT, Costall B, Smythe JW. Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. *Psychoneuroendocrinology*. 2004;29(2):227-244.
25. Boccia ML, Razzoli M, Vadlamudi SP, Trumbull W, Caleffie C, Pedersen CA. Repeated long separations from pups produce depression-like behavior in rat mothers. *Psychoneuroendocrinology*. 2007;32(1):65-71.

26. Maniam J, Morris MJ. Long-term postpartum anxiety and depression-like behavior in mother rats subjected to maternal separation are ameliorated by palatable high fat diet. *Behav Brain Res*. 2010;208(1):72-79.
27. Pawluski JL, Lieblich SE, Galea LA. Offspring-exposure reduces depressive-like behaviour in the parturient female rat. *Behav Brain Res*. 2009;197(1):55-61.
28. Pawluski JL, Vanderbyl BL, Ragan K, Galea LA. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or "mothering" alone. *Behav Brain Res*. 2006;175(1):157-165.
29. Bridges RS. Neuroendocrine regulation of maternal behavior. *Front Neuroendocrinol*. 2015;36:178-196.
30. Ishak WW, Kahloon M, Fakhry H. Oxytocin role in enhancing well-being: a literature review. *J Affect Disord*. 2011;130(1–2):1-9.
31. Bosch OJ, Neumann ID. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action. *Horm Behav*. 2012;61(3):293-303.
32. D'Cunha TM, King SJ, Fleming AS, Levy F. Oxytocin receptors in the nucleus accumbens shell are involved in the consolidation of maternal memory in postpartum rats. *Horm Behav*. 2011;59(1):14-21.
33. Ferris CF, Yee JR, Kenkel WM, et al. Distinct BOLD activation profiles following central and peripheral oxytocin Administration in Awake Rats. *Front Behav Neurosci*. 2015;9:245.
34. Lonstein JS, Maguire J, Meinlschmidt G, Neumann ID. Emotion and mood adaptations in the peripartum female:complementary contributions of GABA and oxytocin. *J Neuroendocrinol*. 2014;26(10):649-664.
35. Pedersen CA, Boccia ML. Oxytocin antagonism alters rat dams' oral grooming and upright posturing over pups. *Physiol Behav*. 2003;80(2–3):233-241.
36. Sabihi S, Dong SM, Durosko NE, Leuner B. Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Front Behav Neurosci*. 2014;8:258.
37. Sanson A, Bosch OJ. Dysfunctions of brain oxytocin signaling: implications for poor mothering. *Neuropharmacology*. 2022;211:109049.
38. Insel TR. Regional changes in brain oxytocin receptors post-partum: time-course and relationship to maternal behaviour. *J Neuroendocrinol*. 1990;2(4):539-545.
39. Neumann ID. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog Brain Res*. 2002;139:147-162.
40. Slattery DA, Neumann ID. No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain. *J Physiol*. 2008;586(2):377-385.
41. Klampfl SM, Bosch OJ. Mom doesn't care: when increased brain CRF system activity leads to maternal neglect in rodents. *Front Neuroendocrinol*. 2019;53:100735.
42. Demarchi L, Pawluski JL, Bosch OJ. The brain oxytocin and corticotropin-releasing factor systems in grieving mothers: what we know and what we need to learn. *Peptides*. 2021;143:170593.
43. Numan M, Woodside B. Maternity: neural mechanisms, motivational processes, and physiological adaptations. *Behav Neurosci*. 2010;124(6):715-741.
44. Bosch OJ, Neumann ID. Brain vasopressin is an important regulator of maternal behavior independent of dams' trait anxiety. *Proc Natl Acad Sci USA*. 2008;105(44):17139-17144.
45. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav*. 1980;13(2):167-170.
46. Ebner K, Bosch OJ, Kromer SA, Singewald N, Neumann ID. Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. *Neuropsychopharmacology*. 2005;30(2):223-230.
47. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc*. 2012;7(6):1009-1014.
48. Bayerl DS, Klampfl SM, Bosch OJ. Central V1b receptor antagonism in lactating rats: impairment of maternal care but not of maternal aggression. *J Neuroendocrinol*. 2014;26(12):918-926.
49. Klampfl SM, Neumann ID, Bosch OJ. Reduced brain corticotropin-releasing factor receptor activation is required for adequate maternal care and maternal aggression in lactating rats. *Eur J Neurosci*. 2013;38(5):2742-2750.
50. Klampfl SM, Schramm MM, Stinnett GS, Bayerl DS, Seasholtz AF, Bosch OJ. Brain CRF-binding protein modulates aspects of maternal behavior under stressful conditions and supports a hypo-anxious state in lactating rats. *Horm Behav*. 2016;84:136-144.
51. Bosch OJ. Maternal nurturing is dependent on her innate anxiety: the behavioral roles of brain oxytocin and vasopressin. *Horm Behav*. 2011;59(2):202-212.
52. Oliveira VEM, Lukas M, Wolf HN, et al. Oxytocin and vasopressin within the ventral and dorsal lateral septum modulate aggression in female rats. *Nat Commun*. 2021;12(1):2900.
53. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-675.
54. Baskin DG, Stahl WL. Fundamentals of quantitative autoradiography by computer densitometry for in situ hybridization, with emphasis on 33P. *J Histochem Cytochem*. 1993;41(12):1767-1776.
55. Joushi S, Sheibani V, Esmaeilpour K, Francis-Oliveira J, Taherizadeh Z, Mohtashami Borzadaran F. Maternal separation impairs mother's cognition 1 month beyond the separation. *Int J Dev Neurosci*. 2021;81(7):605-615.
56. Aguggia JP, Suarez MM, Rivarola MA. Early maternal separation: neurobehavioral consequences in mother rats. *Behav Brain Res*. 2013;248:25-31.
57. Stolzenberg DS, Stevens JS, Rissman EF. Experience-facilitated improvements in pup retrieval; evidence for an epigenetic effect. *Horm Behav*. 2012;62(2):128-135.
58. Numan M. Introduction: the Parental Brain. *The Parental Brain: Mechanisms, Development, and Evolution*. Oxford Academic; 2020.
59. Numan M, Stolzenberg DS. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Front Neuroendocrinol*. 2009;30(1):46-64.
60. Eklund MB, Johansson LM, Uvnas-Moberg K, Arborelius L. Differential effects of repeated long and brief maternal separation on behaviour and neuroendocrine parameters in Wistar dams. *Behav Brain Res*. 2009;203(1):69-75.
61. Marmendal M, Roman E, Eriksson CJ, Nylander I, Fahlke C. Maternal separation alters maternal care, but has minor effects on behavior and brain opioid peptides in adult offspring. *Dev Psychobiol*. 2004;45(3):140-152.
62. Own LS, Patel PD. Maternal behavior and offspring resiliency to maternal separation in C57Bl/6 mice. *Horm Behav*. 2013;63(3):411-417.
63. Pryce CR, Bettschen D, Feldon J. Comparison of the effects of early handling and early deprivation on maternal care in the rat. *Dev Psychobiol*. 2001;38(4):239-251.
64. Stamatakis A, Kalpachidou T, Raftogianni A, et al. 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*. 2015;52:212-228.
65. Zimmerberg B, Kim JH, Davidson AN, Rosenthal AJ. Early deprivation alters the vocalization behavior of neonates directing maternal attention in a rat model of child neglect. *Ann N Y Acad Sci*. 2003;1008:308-313.
66. Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997;277(5332):1659-1662.
67. Smotherman WP. Mother-infant interaction and the modulation of pituitary-adrenal activity in rat pups after early stimulation. *Dev Psychobiol*. 1983;16(3):169-176.

68. Stolzenberg DS, Hernandez-D'Anna KL, Bosch OJ, Lonstein JS. Maternal Behavior from a Neuroendocrine Perspective. *Oxford Research Encyclopedia of Neuroscience*. Oxford University Press; 2019: 1-64.
69. Bayerl DS, Kaczmarek V, Jurek B, et al. Antagonism of V1b receptors promotes maternal motivation to retrieve pups in the MPOA and impairs pup-directed behavior during maternal defense in the mpBNST of lactating rats. *Horm Behav*. 2016;79:18-27.
70. Jacobson CD, Terkel J, Gorski RA, Sawyer CH. Effects of small medial preoptic area lesions on maternal behavior: retrieving and nest building in the rat. *Brain Res*. 1980;194(2):471-478.
71. Gammie SC. Current models and future directions for understanding the neural circuitries of maternal behaviors in rodents. *Behav Cogn Neurosci Rev*. 2005;4(2):119-135.
72. Everett N, Baracz S, Cornish J. Oxytocin treatment in the prelimbic cortex reduces relapse to methamphetamine-seeking and is associated with reduced activity in the rostral nucleus accumbens core. *Pharmacol Biochem Behav*. 2019;183:64-71.
73. Neumann I, Ludwig M, Engelmann M, Pittman QJ, Landgraf R. Simultaneous microdialysis in blood and brain: oxytocin and vasopressin release in response to central and peripheral osmotic stimulation and suckling in the rat. *Neuroendocrinology*. 1993a; 58(6):637-645.
74. Neumann I, Russell JA, Landgraf R. Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: a microdialysis study. *Neuroscience*. 1993b; 53(1):65-75.
75. Ferris CF, Kulkarni P, Sullivan JM Jr, Harder JA, Messenger TL, Febo M. Pup suckling is more rewarding than cocaine: evidence from functional magnetic resonance imaging and three-dimensional computational analysis. *J Neurosci*. 2005;25(1):149-156.
76. Numan M, Stolzenberg DS, Dellevigne AA, Correnti CM, Numan MJ. Temporary inactivation of ventral tegmental area neurons with either muscimol or baclofen reversibly disrupts maternal behavior in rats through different underlying mechanisms. *Behav Neurosci*. 2009; 123(4):740-751.
77. Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc Natl Acad Sci USA*. 2001;98(22):12736-12741.
78. Rae M, Zanos P, Georgiou P, Chivers P, Bailey A, Camarini R. Environmental enrichment enhances conditioned place preference to ethanol via an oxytocinergic-dependent mechanism in male mice. *Neuropharmacology*. 2018;138:267-274.
79. Zanos P, Georgiou P, Wright SR, et al. The oxytocin analogue carbetocin prevents emotional impairment and stress-induced reinstatement of opioid-seeking in morphine-abstinent mice. *Neuropsychopharmacology*. 2014;39(4):855-865.
80. Lonstein JS. Regulation of anxiety during the postpartum period. *Front Neuroendocrinol*. 2007;28(2–3):115-141.
81. Neumann ID. Brain mechanisms underlying emotional alterations in the peripartum period in rats. *Depress Anxiety*. 2003;17(3):111-121.
82. Bousalham R. MAternal separation affects mothers' affective and reproductive behaviors as well as second offspring's emotionality. *J Behav Brain Sci*. 2013;3:409-414.
83. Maghami S, Zardooz H, Khodagholi F, et al. Maternal separation blunted spatial memory formation independent of peripheral and hippocampal insulin content in young adult male rats. *PLoS One*. 2018; 13(10):e0204731.
84. von Mucke-Heim IA, Urbina-Trevino L, Bordes J, Ries C, Schmidt MV, Deussing JM. Introducing a depression-like syndrome for translational neuropsychiatry: a plea for taxonomical validity and improved comparability between humans and mice. *Mol Psychiatry*. 2022;28(1):329-340.
85. Ji H, Su W, Zhou R, et al. Intranasal oxytocin administration improves depression-like behaviors in adult rats that experienced neonatal maternal deprivation. *Behav Pharmacol*. 2016;27(8):689-696.
86. Khodagholi F, Maleki A, Motamedi F, Mousavi MA, Rafiei S, Moslemi M. Oxytocin prevents the development of 3-NP-induced anxiety and depression in male and female rats: possible interaction of OXTR and mGluR2. *Cell Mol Neurobiol*. 2022;42(4):1105-1123.
87. Kalyani M, Callahan P, Janik JM, Shi H. Effects of pup separation on stress response in postpartum female rats. *Int J Mol Sci*. 2017;18(7):1370.
88. Leuner B, Mirescu C, Noiman L, Gould E. Maternal experience inhibits the production of immature neurons in the hippocampus during the postpartum period through elevations in adrenal steroids. *Hippocampus*. 2007;17(6):434-442.
89. Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. *Am J Physiol Endocrinol Metab*. 2006;291(5):E965-E973.

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