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Research report

Early maternal separation: Neurobehavioral consequences in mother rats

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HIGHLIGHTS

- Postpartum separations from their pups alter maternal behavior.
- Mothers separated from their pups show memory impairment for both short and long-term memory.
- Periodic mother-litter postpartum separation increases c-Fos expression in the CeA.

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ABSTRACT

Repeated separation of dams from their pups during the postpartum period may evoke emotional stress in the dam. In the present study we investigated whether prolonged maternal separation is stressful for rat dams by studying different behavioral and central responses known to be affected by stress. After delivery, female Wistar rats were subjected to either animal facility rearing (AFR) conditions or daily 4.5 h of mother–litter separation from postpartum day (PPD) 1–21. Maternal care (pup retrieval) was evaluated at PPD 3. After weaning on PPD 21, anxiety (elevated plus maze) and depression-like behaviors (forced swimming test) were assessed in the dams. Memory abilities (one-trial step down inhibitory avoidance) were tested either 1 h (short-term memory) or 24 h (long-term memory) after training session. Finally, c-Fos expression was examined in the central nucleus of the amygdala. The results revealed that pup retrieval efficiency at PPD 3 was significantly impaired by maternal separation. AFR dams retrieved their pups sooner and engaged in more pup-directed activities (nest building and carrying pups). Separation from pups increased the number of entries in open arms of the plus maze and decreased latency times in the inhibitory avoidance test for both short and long-term memory in the dams. There were no differences in depression-related behavior as assessed using the forced swimming test. Furthermore, maternal separation yielded high c-Fos expression in the central nucleus of the amygdala. Together, these data indicate that repeated maternal separation in the early postpartum period reduces maternal care and impairs the retention memory, providing further evidence for the detrimental neurobehavioral effects of maternal separation in dams.

1. Introduction

Transition to motherhood induces a number of extraordinary physiological, neuroendocrine and behavioral modifications in female mammals. Maternal hypothalamic-pituitary-adrenal (HPA) axis responses to stressors are markedly attenuated through pregnancy and lactation, which may contribute to preventing adverse effects of stress on the mother and offspring [1]. Despite this dampening of the stress response during pregnancy and postpartum, stress during these periods can have long-lasting effects on the outcome of the offspring in both humans and rodents. The postpartum period is a critical time in an adult female's life, during which environmental manipulations may have a unique impact on HPA axis and neurological function [2].

In laboratory rats, the chronic effects of postnatal manipulation of the infant–mother relationship have been studied experimentally for more than 50 years. Among these manipulations, early maternal separation is an animal model widely used as a stressor to study the effects on offspring behavior and physiology. Although results are not consistent within the maternal separation paradigm,

Abbreviations: AFR, animal facility rearing; CeA, central nucleus of the amygdala; EPM, elevated plus maze; FST, forced swimming test; HPA, hypothalamic–pituitary–adrenal; PB, phosphate buffer; PPD, postpartum day.

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in general, adult offspring that were exposed to maternal separation in infancy displayed as adults enhanced stress responsiveness, increased anxiety, helplessness and anhedonia, sensorimotor gating deficits and increased propensity for the intake of addictive drugs (for review see [3–5]).

The parent-infant relationship is a dynamic and reciprocal relationship: both the mother and the pup are equipped with innate motivation toward each other and therefore stimuli derived from one member regulate the behavior and physiology of the other and vice versa. Considering that a mother–pup pair forms a dyad, separation of pup from dam may affect both members of the dyad [6,7].

While most of the studies in which the paradigm of maternal separation is used have focused their attention on the effects on offspring, few have focused on the effects on maternal behavior and physiology.

Although limited, recent work has begun to document the effect of maternal separation stress on dams. Studies suggest that maternal separation can induce short-term (examining post-weaning behavioral profiles) as well as long-lasting changes in the dams. Repeated prolonged separation had enduring impact on the motor responses of dams to a novel environment, to conditioning and to morphine [8,9]. In addition, repeated long-term postpartum separation from their pups induced depression or anxiety-like behavior, which represents postpartum maternal depression in animal models [10–12]. It may also induce neurochemical alterations related to depressive disorders [13]. However, controversies can be found in the literature: it has been reported that brief maternal separations from pups may be stressful for rat mothers and increase anxiety-like behaviors, whereas prolonged separations are not [14]. So, further studies are needed to elucidate the effects of early mother-pup bond disruptions on emotional behaviors in dams that still remain largely unknown.

Although anxiolysis and blunted stress responses of the HPA axis are observed in the majority of mothers, a significant percentage display increased vulnerability to mood disorders, such as postpartum depression (5–25%) postpartum anxiety (5–12%)[15,16]. Despite the high incidence of these disorders, their etiology has not been fully elucidated. However biochemical factors and psychological stress during the postpartum period could trigger them. If left untreated, they can become chronic and subsequently affect the health of the mother and the offspring, as well as bond development [17,18].

Brain areas affected by contact with pups to induce anxiety reduction in mothers have been not explored in detail. The amygdala is a complex brain structure, important for emotional processing, that receives highly processed sensory information from all modalities through its lateral and basolateral nuclei. In turn, these nuclei project to the central nucleus of the amygdala (CeA), which then projects to a variety of hypothalamic and brain stem target areas which directly mediate specific signs of fear and anxiety. The activity of the CeA contributes to the expression of fear-related behaviors [19–21]. Since high expression of c-Fos is an indicator of high activity of these cells, an increase in c-Fos expression in the CeA suggests increased fear behavior.

Considering the importance of a normal interaction between mother and pups during early postnatal life and their relationship to the development of psychopathology for mothers and offspring, this work presents an approach to the neurobiological impact on mothers produced by prolonged separation from their offspring.

2. Materials and methods

2.1. Animals

Virgin adult female Wistar rats weighing 230 ± 10 g (n = 20) were used in these experiments. Pregnancy was induced by fertile copula with an experienced male. Pregnant animals were housed under controlled temperature (20 ± 2 °C) and lighting (0700–1900 h) conditions and supplied with food and water ad libitum. The maternal rats were randomly assigned to one of two rearing conditions: animal facility reared (control group) and separated from their offspring (separated group) (n = 10 in each group). The day of delivery was designated postpartum day 0. Litters were culled to 10 pups (5–6 males, 4–5 females) on the day after birth.

2.2. Maternal separation

In the separated group, rat mothers were separated from their pups for 4.5 h once a day from postpartum day 1 (PPD 1) (separation cycles were completed between 0900 and 1330 h), and separation continued for 21 consecutive days when weaning occurred. In the control group, the rat pups were cared for by their mothers continuously for the same duration (except during cage changes twice a week for standard husbandry maintenance).

Each separation was carried out by removing the mothers from the home cage and placing them in an adjacent cage in the same room. After 4.5 h the mother was returned to the home cage. During each period of separation, the pups were kept together. Control mothers were kept with their pups until weaning, on PPD 21.

2.3. Behavioral tests

2.3.1. Pup retrieval test

Pup retrieval test allows observing some of the principal maternal behaviors.

Retrieval behavior was assessed during early postpartum on day 3 after delivery. In the control group the dam was separated from the litter for less than 3 min and was kept in a holding cage. The pups were scattered into one corner of the cage opposite to the nest site. Then the mother was returned to the cage. In animals subjected to separation from their pups, this procedure was performed immediately before the mother was returned to the breeding box after the separation period.

The occurrences of the following behaviors were scored: pup-retrieval (latencies to retrieve pups back to the nest and number of pups successfully retrieved), latency to the first pup-contact (latency to sniff or contact the first pup); frequencies of rearing and self-grooming (behaviors not directed to the pups). Additionally nest building (defined as manipulation of nesting material with mouth or forepaws toward the nest site) was observed. The nest quality was rated on a 2-point scale ranging from 0 (when no nest was built), 1 (no organized nest in the opposite site to the original location was built) and 2 (when a full nest in the original site was built).

The test ended after 10 min, or when the female had retrieved all the pups. At the end of the test, pups that were not picked up by their mother were returned to the nest site by the observer.

2.3.2. Elevated plus maze (EPM)

To measure anxiety-like behavior, after weaning (PPD 21) the mothers were tested in the elevated plus maze (EPM). The apparatus consisted of a plus-shaped maze placed 50 cm above the floor with two opposite open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm with 40 cm walls). Behavioral testing was conducted in a quiet room. The rats were habituated to the testing room for at least 40 min prior to testing to eliminate the stressor effects of the new environment. At the beginning of the test, each rat was placed onto the central area (10 cm × 10 cm) of the maze facing a closed arm and was allowed to explore the maze freely. During the 5 min exposure, the number of entries and the time spent in each arm were video recorded, and the recordings were then analyzed. An entry was defined as the placing of two forepaws into the arm. Two indices were calculated for examining anxiety: the percentage of time spent on the open arms to the total time spent in all arms (% open arm time) and the percentage of open arm entries to total arm entries (% open arm entries). Total number of arms entries was also analyzed as a measure of general activity. Between each session the maze was wiped clean with ethanol 50%.

2.3.3. Forced Swimming test

In order to evaluate the degree of depressed-like state in the maternal rats, a forced swimming test (FST) was performed. On PPD 22, a pre-test session was conducted for 12 min to eliminate the acute stress of water and to adapt the animals to the water. Twenty-four hours after the pre-test, the maternal rats were tested for 5 min. The animals were placed individually into a glass cylinder 20 cm in diameter and 40 cm in height, filled with water to a height of 30 cm. The temperature of the water was adjusted to 25 ± 2 °C. All the test sessions were videotaped by a camera positioned in front of the water tanks and subjected to analysis later on. During the test session, the climbing time, swimming time, attempts to escape, fecal boli and immobility time were analyzed. Climbing was defined as when the rat was in active vertical motion with its forelegs above the water level. Swimming was measured when animals were making mild swimming movements, more than those necessary to merely keep the head above water. Immobility was defined to occur when no additional activity was observed other than the actions needed to keep the rat's head above the water. After the swimming sessions, the rats were removed from the tank, carefully dried with towels and returned to their home cages. Water in the tank was changed after each animal.

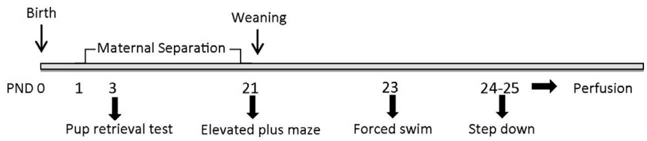

Fig. 1. Schematic time line representation summarizing the experimental procedures in this study.

2.3.4. Step down inhibitory avoidance task

Memory was evaluated by the step-down inhibitory avoidance task. On PPD 25, the maternal rats were trained in a step-down avoidance task. The inhibitory avoidance apparatus consisted of an acrylic box (50 cm × 25 cm × 30 cm). A grid floor made of parallel bronze rods (0.5 cm diameter) 1 cm apart was connected to a shock generator. An escape platform (20 cm × 25 cm) 2 cm above the floor was located at the left extreme of the grid. During the training session, the animals received a 0.35 mA scramble foot shock for 2 s immediately upon stepping down the platform. Memory retention was assessed 1 h (short-term memory-STM) and 24 h (long-term memory LTM) after training. Test sessions consisted of measuring the latency it took each animal to step down from the platform and place their four paws on the grids. Any latency > 300 s was counted as 300 s.

2.4. c-Fos immunohistochemistry

Immediately after the step down test, all rats were anesthetized with a lethal dose of chloral hydrate (0.54 g/Kg ip) and then perfused transcardially with ice-cold 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) containing 2% picric acid. Brains were rapidly removed from the skulls and post-fixed in the same fixative overnight at 4 °C. They were then transferred to 20% sucrose in 0.1 M PB until settled. Coronal sections at a thickness of 40 μm were cut on a freezing microtome and collected in conservative solution (glycerol–etilenglicol), maintained at −20 °C until the immunohistochemistry procedure.

Immunohistochemistry was carried out by the free-floating method. After endogenous peroxidase blocking (10% methanol and 10% H_2O_2 for 30 min) and incubation with albumin 5%, sections were then incubated overnight with the primary antibody (anti Fos, 1:1000 dilution, Ab-5, Oncogen Science) in PBS containing 0.3% Triton X-100, 1% normal serum. After the primary immunoreactions, the sections were incubated with a biotinylated goat anti-rabbit secondary antibody (1:200 dilution, Vector Laboratories) in PBS containing 1% normal serum for 2 h at room temperature. Immuno signals were amplified with Avidin-Biotin-Peroxidase complex (ABC Elite kit, Vector Laboratories) using 3.3′ diaminobenzidine (DAB-Sigma) as chromogen. After staining, sections were mounted on glass slides using Albrecht gelatine (1.5% gelatine/80% alcohol), air dried, cleared in xylene and coverslipped using DPX mounting medium (Fluka, Buchs, Switzerland). As a control, the primary antibody was substituted with normal serum. No corresponding nucleus or cytoplasm was immunostained in the control. Sections were examined under a light microscope (Olympus) and images were captured using a high-resolution digital camera.

Quantification of positive cells for immunohistochemistry was assessed using Image-J software. At least five sections per individual were analyzed.

Time line representation of experimental design is presented in Fig. 1.

2.5. Statistical analysis

Statistical comparisons were performed with the use of the Student *t* test. When variances were not homogeneous, the behavioral data were analyzed by means of non-parametric Kruskal–Wallis test, except for nesting behavior score which was analyzed by Chi-squared test.

Immunohistochemical data were analyzed using a linear mixed effect model, which is a modified analysis of variance/covariance (ANOVA/ANCOVA) that allows for the analysis of dependencies in the data. Separation and amygdala were modeled as factors and amygdala sections were randomized.

In all comparisons, *p* values of less than 0.05 were considered to indicate statistical significance. Data were presented as mean ± standard error.

All experimental procedures were performed according to International Guidelines on Care and Use of Laboratory Animals with protocols approved by the National University of Córdoba.

3. Results

3.1. Effect of maternal separation on maternal behavior

The analysis of the pup retrieval test revealed that at PPD 3, dams that were subjected to prolonged separation of 4.5 h daily from PPD 1–21 took approximately 250% more time to retrieve their entire litter compared to dams that remained with their pups 458.57 ± 70 s vs. 218.5 ± 61.42 s, $h = 7.48$ $p = 0.004$). There were no significant differences in the latency to the first contact (Fig. 2 A and B).

The nest building behavior of dams on day 3 after delivery revealed that Separated dams reconstructed the nest anywhere outside the original site within the cage than control dams (X^2 13.33, $p = 0.001$). Analysis of non pup-directed behaviors showed that there were significant differences between groups on the number of rearing 2.12 ± 2.3 times vs. 4.3 ± 1.3 ($t = -2.98$, $p = 0.01$), (Fig. 2C). Nevertheless there were no significant differences between groups on the number of grooming behavior.

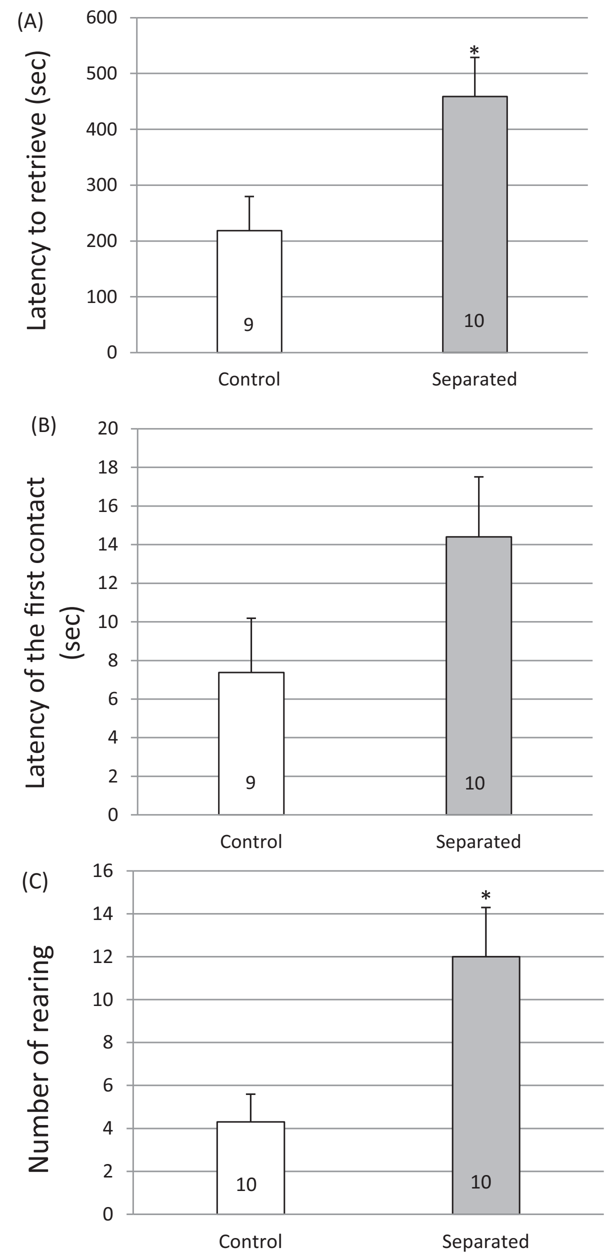

Fig. 2. Maternal behavior assessed using pup retrieval test at PP day 3, during a 10-min. Latency to retrieve pups to nest site (A), latency to first contact to their pups when mother it is put into the cage (B), number of rearing (C) in dams that were never separated from their pups (control),or separated from their pups (separated). Results are expressed as mean ± SEM. The number of animals per group is included inside each bar. Separated dams significantly different (*) $p < 0.05$ from Control dams.

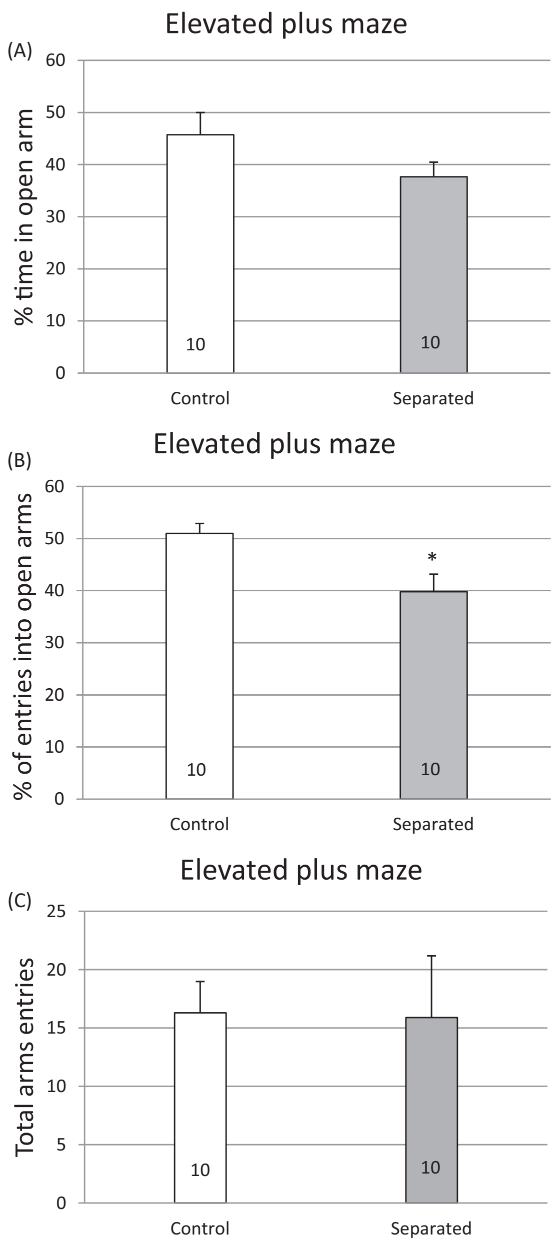

Fig. 3. Anxiety-like behaviors assessed using EPM at PP day 21. Percentage time spent in the open arms (A), percentage of entries made to open arms (B) and total number of arms entries (C) in dams that were never separated from their pups (control) or separated from their pups (separated). Results are expressed as mean ± SEM. The number of animals per group is included inside each bar. Separated dams significantly different (*) $p < 0.05$ from Control dams.

3.2. Maternal separation effects on anxiety-related behavior

At PPD 21, dams were tested for anxiety-like behavior on the EPM. There was a significant difference in percent of open arm entries. Separated mothers made less entries than controls, 39.7 vs. 50.98 ($h = 4.86$, $p = 0.02$). No statistical differences were observed in the percent of time spent on the open arms, neither on total arms entries (Fig. 3).

3.3. Effects of maternal separation on depressive-related behavior

Using the forced swimming test at PPD 23, dams were scored for depression-like behaviors such as time spent immobile, swimming, climbing, and attempts to escape. In our experiment, mothers that had been repeatedly separated from their pups did not show statistical differences in any of the behaviors analyzed in the FST compared with the control group (Table 1).

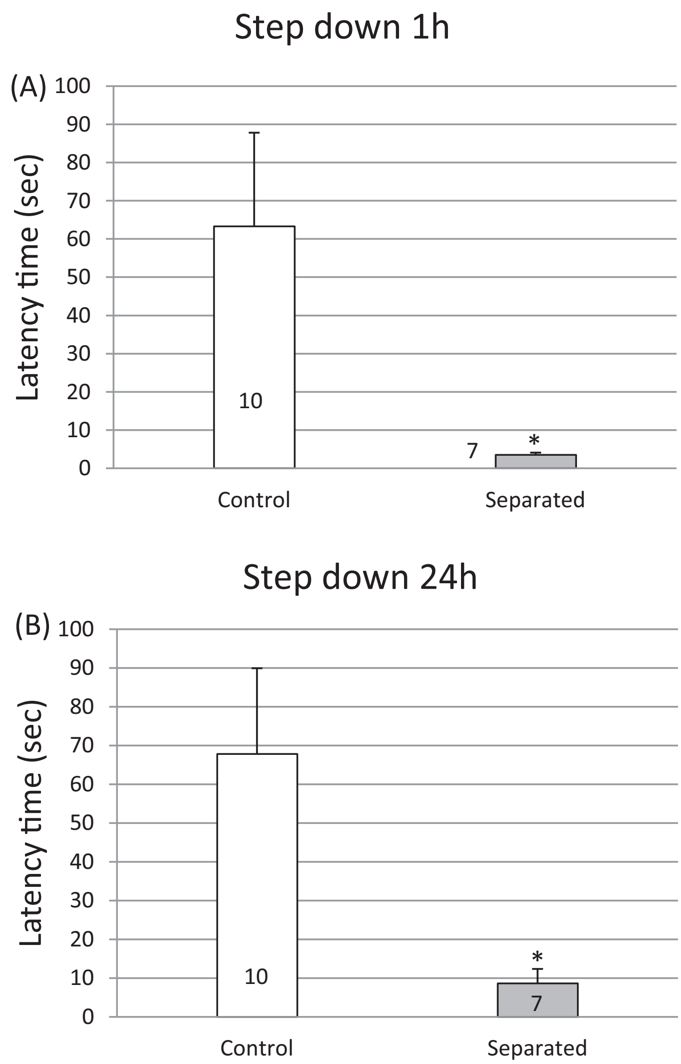

Fig. 4. Latency in stepping down from platform in the inhibitory avoidance test. Step down test at 1 h (C) and 24 h (D) later the training. Groups correspond in dams that were never separated from their pups (control) or separated from their pups (separated). Results are expressed as mean ± SEM. The number of animals per group is included inside each bar. Separated dams significantly different (*) $p < 0.05$ from control dams.

3.4. Maternal separation effects on step-down inhibitory avoidance

At PPD 25 the latency to step down from the platform on day 1 (training) was compared to the latency 1 h and 24 h after the training session. Separated dams showed shorter latencies to step down at both retention times 1 h, 63.27 vs. 3.53 s, ($h = 4.55$, $p = 0.03$) (Fig. 4A), and 24 h 67.83 s vs. 8.65 s ($t = 2.63$, $p = 0.027$) compared to control dams (Fig. 4B).

3.5. Fos expression

The central nucleus of the amygdala was analyzed, considering its role in the expression of fear related behaviors.

The number of Fos-positive cells in the central nucleus of the amygdala was significantly higher in separated dams ($F = 11.21$, $p > 0.01$) (Figs. 5 and 6).

Table 1
Forced swim test.

Treatment	Forced swim test (time in s)				
	Immobility	Swimming	Climbing	Attempts to escape	Number of boli fecal
Separated	212.1 ± 20.19	43.4 ± 9,38	27.8 ± 7.35	0.2 ± 0.2	26
Control	238.1 ± 12.59	23.44 ± 2.29	23.44 ± 6.26	0.9 ± 0.53	43

Note: Values represented in (Mean ± SEM). The number of animals per group was $n = 10$.

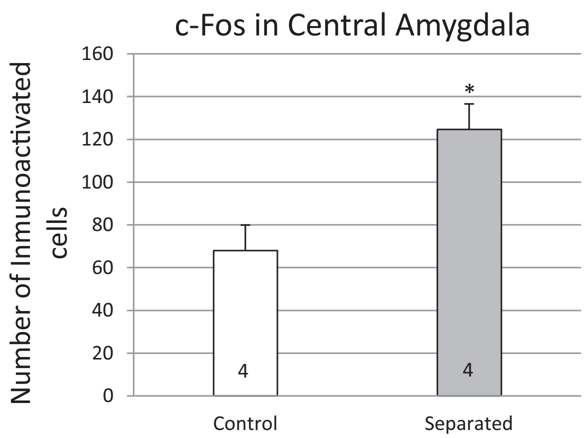

Fig. 5. Effects of maternal separation on c-Fos immunoreactivity in the central nucleus of the Amygdala. Each column represents the mean ± SE of the positive cells counted in nucleus in the different experimental groups: in dams that were never separated from their pups (control) or separated from their pups (separated). The number of animals per group is included inside each bar. Separated dams significantly different (*) $p < 0.05$ from Control dams.

4. Discussion

The results of the present study demonstrate that dams separated for 4.5 h daily for the first three weeks after birth had lower performance in maternal care than dams that were not separated. Separated dams took approximately 250% more time to retrieve their entire litter compared to the control dams, which retrieved their pups sooner, engaged in more pup-directed activities (sniffing and carrying pups) and had a longer latency for self-grooming and rearing than separated dams. A similar result was shown by Maniam and Morris, who reported that separated dams took 140% longer to collect the litter [12]. Although the difference was not significant, the latency time to first contact between separated dams and their pups was longer. This could be considered as a delay in recognizing the offspring and worry about them, possibly provoked by separation.

Nest-building behavior was impaired in separated dams compared to control dams. Separated dams also showed more exploratory activity, in the form of a higher number of rearings. Returning to the cage to find all her offspring out of the nest appears to create a conflict situation for the dam, resulting in less offspring-directed behavior.

Previous studies indicate that maternal separation increases plasma corticosterone [12]. Other studies have shown that chronic corticosterone administration reduced the quality of maternal care provided to the pups. Dams spent more time off the nest and less time nursing their pups and showed an increase in depressive-like behavior [22]. Similarly, Kurata et al.'s rodent studies found that an early adverse maternal environment, such as separation, decreased maternal active nursing [23]. In line with this, we speculate that repeated prolonged separation may evoke emotional stress in the dams, increasing plasma corticosterone, a mechanism which may alter maternal behavior.

Although we assessed pup-retrieval only on day 3 after birth, and it is possible that other maternal behaviors are also altered, in the present study we add important evidence on how maternal separation during early postpartum affects maternal behavior, impairing care for the offspring. With repeated separations, the dam may learn that it cannot control access to its pups, and therefore the efforts to do so, exhibited in active maternal behaviors such as pup-licking, decline [10]. Future studies could analyze other maternal behaviors, such as huddling and nursing during the period from birth until weaning, for a more complete characterization of the effects of maternal separation on maternal behavior.

In terms of anxiety-like behavior, separated dams had a significant difference in the percentage of open arms entries and spend 20% less time in the open arm although it did not reach statistical significance versus control dams. This suggests that prolonged daily separation of dams from their pups increased anxiety-like behavior. Other studies have shown that prolonged maternal separation from their pups leads to disturbance in maternal care behavior, inducing depression or anxiety-like behavior [10,11].

Although we expected depressive behavior in separated dams in the forced swimming test, no significant differences were found in either of the parameters measured. Previous studies detected an increase in immobility and a significant decrease in swimming,

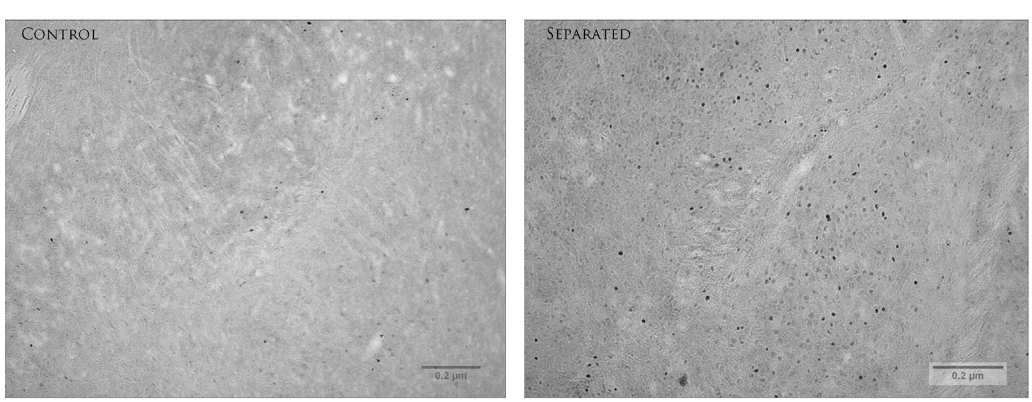

Fig. 6. Representative photomicrographs showing Fos-like immunoreactivity in the CeA.

supporting the idea that maternal separation induces depressive symptoms [2,10,11]. In other studies, FST immobility correlated negatively with pup-licking across all mothers, regardless of separation history, and for both days of the FST. This suggests that depression-like states may contribute to the level of expression of maternal behavior [10].

Literature on the neurobiology of anxiety during postpartum both in humans and in rodents is relatively scant. Human epidemiology suggests that women after parturition are more likely to be anxious or depressed, but breastfeeding reduces anxiety in recent parturients, and enhancing mother–infant contact, with its concomitant release of endogenous anxiolytic neurochemicals, has been suggested to reduce anxiety in new mothers [15,24].

Studies in rats showed a positive correlation between breastfeeding stimuli by pups and greater prolactin secretion by dams, decreasing HPA reactivity during stress. Physical interaction with pups was sufficient alone to produce a temporary anxiolytic effect in mother rats [25]. Nursing mothers were also found to have less activation of brain regions that respond to stress than virgin rats [1]. Thus, disrupting the interaction between the mother and its pups, as by removal of the litter, results in a great diminishment of physical contact, predisposing to anxiety during postpartum. The underlying mechanisms are mostly unknown but, as in other psychological illnesses, are probably complex with a large number of interacting mechanisms. Nonetheless, alterations in the HPA axis, prolactin and oxytocin signaling, ovarian, placental and neural steroids could contribute to elevated anxiety [15,25,26].

Despite the high comorbidity between anxiety and depression, recent studies using chronic psychosocial stress paradigms result in an increase of anxiety- but not of depression-related behaviors [32]. Other studies reported depression behavior in the forced swimming test but not in the tail suspension test in a mouse model [33]. Similarly, ten days exposure to social stress does not alter the behavior in forced swimming or tail suspension, but leads to an anhedonic phenotype[34]. These antecedents highlight the importance of using more than one behavioral test to typify depression-like behaviors in some animal models.

The FST probably needs further characterization and validation in terms of other components of human depression, including anhedonia, HPA hyperactivity and response to antidepressant treatment. Validation would warrant detailed investigation of the neurobiology of long maternal separation-induced maternal depression-like behavior. It could be argued that repeated separations may cause habituation to stress in mothers, which would be why they did not develop symptoms of depression at the time they were evaluated. For example, Eklund's study found no evidence that mothers separated 4 h a day for 2 weeks in early postpartum were stressed [14]. Further studies should be done to verify depression in separated dams.

Studies employing the expression of the phosphoprotein Fos as a marker of neuronal functional activation have shown that exposure to stress promotes cellular activation in central neuronal structures implicated in the stress response, such as the paraventricular nucleus and the amygdala [27]. The results of our evaluation of c-Fos expression indicated that the central nucleus of the amygdala (CeA) was significantly larger in separated dams.

The amygdala plays an important role in anxiety and fear behavior; it is well known as an integrating center of stress. The amygdala was also implicated in mechanisms of fear memory formation and extinction and in the etiology of depression [28]. The CeA is important in conditioned fear and in modulating affective responses to stress. Maternal separation results in an increase of c-Fos expression in the CeA, which could be correlated with the increase in anxiety-like behaviors in the elevated plus maze in dams separated from their offspring. Smith and Lonstein have analyzed brain areas in which sensory cues from pups affect neural modulation in response to axiogenic experience [29]. Our results add interesting implications for understanding the brain areas affected by maternal separation in relation to anxiety disorders in postpartum.

Regarding the effects of maternal separation on latency in the inhibitory avoidance test, our results indicate that early maternal separation produces a cognitive impairment in mothers, affecting both short-term and long-term memory. These data are consistent with those of Sung et al., who show that mothers separated from their pups 6 h daily for 14 consecutive days show lower latency over control. They also found decreased neurogenesis and increased apoptosis in the hippocampus of mothers separated from their young, concluding that probably the stress of separation affects the circuits involved in the processes of acquisition, consolidation and retrieval of memory of an aversive stimulus [11].

The expression of maternal behaviors is important in protecting females from the detrimental effects of stress on conditioning [30]. In line with this, our results showed high impairment of maternal behavior together with impaired performance in the memory test. Contact with pups and the nurturing and care-giving activities that this elicits are essential for maintaining many behavioral, neural and hormonal adaptations of postpartum females, not only those related with maternal behavior but also those for anxiety reduction and for protection from detrimental effects on processes involved in learning and memory. Sensory stimulation provided by pups resembles that associated with a complex enriched environment [30]. Previous studies from our laboratory have demonstrated that environmental enrichment has a positive effect on cognitive development in laboratory rats [31]. Similarly, environmental enrichment associated with maternal experience and the presence of pups promotes resistance to stress in the learning and memory processes.

The data presented here indicate that the stressful experience caused by separation from pups during postpartum has a negative effect on both short and long-term memory and we hypothesized that it abolishes the beneficial effect of the enriched experience represented by motherhood on these functions.

In conclusion, the stress of maternal separation during the postpartum seems to induce alterations in the dam's pup-retrieval behavior, increases anxiety-like behavior, and has a detrimental effect on cognitive processes which is prevented by the rewarding aspects of physical contact with pups.

Understanding how an undisturbed mother-infant relationship modulates the neurochemical, physiological and behavioral profiles in dams will contribute to a better knowledge of postpartum psychiatric disorders and the detrimental outcome of separations for both the mother and child. However, additional basic and clinical studies are required to further assess the causes of mood and anxiety disorders.

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