Behavioural Brain Research 248 (2013) 25–31

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Early maternal separation: Neurobehavioral consequences in mother rats

Julieta Paola Aguggia [1], Marta Magdalena Suárez [1], María Angélica Rivarola *

Cátedra de Fisiología Animal, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299, X5000JJC Córdoba, Argentina

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.

ARTICLE INFO

Article history:
Received 2 March 2013
Received in revised form 26 March 2013
Accepted 28 March 2013
Available online 6 April 2013

Keywords:
Maternal separation
Maternal behavior
Anxiety
Depression
Learning
Memory
Amygdala

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.

* Corresponding author. Tel.: +54 351 4332100x239.
E-mail address: arivarola@efn.uncor.edu (M.A. Rivarola).
[1] Tel.: +54 351 4332100x239.

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http://dx.doi.org/10.1016/j.bbr.2013.03.040

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.