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Maternal and affective behaviors of lactating rats reared in overlapping litters

Natalia Uriarte [a,c,*], María Victoria Fernández [a,c], Daniella Agrati [b,c], María José Zuluaga [b,c], Marcela Ferreño [b,c], Annabel Ferreira [b,c]

[a] Laboratorio de Neurociencias, Sección Biomatemática, Facultad de Ciencias, Universidad de la República, Uruguay
[b] Sección Fisiología y Nutrición, Facultad de Ciencias, Universidad de la República, Uruguay
[c] Laboratorio de Experimentación Animal, Facultad de Ciencias, Universidad de la República, Uruguay

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ABSTRACT

Postpartum mating in rats gives rise to complex family units consisting of the mother and two overlapping litters. As a consequence, newborn pups of the second litter, since the moment they are born, acquire experience not only from interaction with the mother and age-matched littermates but also from interaction with older siblings. Newborn pups reared in overlapping litters (OLs) receive a different pattern of maternal stimulation compared to those reared in single litters (SL: one litter of same aged pups), as the mothers reduce some maternal behavior components and juvenile pups from the first litter develop maternal behavior. Since there is strong evidence showing that variations in maternal behavior are transmitted throughout generations, we hypothesized that the altered pattern of maternal stimulation received by OL reared females would modify their behavior during motherhood. To test this hypothesis maternal behavior, maternal aggression and experimental anxiety of dams reared under OL and SL conditions during the first postpartum week were compared. No differences were found between the groups in their maternal behavior and aggression. This result may be explained by the maternal behavior of the juveniles that could compensate for the deficits in the caregiving behaviors received by OL litters. However, a subtle temporal reorganization of the licking behavior was found in OL reared mothers, together with an increased anxiety-related behavior in the plus maze test. These results suggest dissociation in the effects provoked by early environmental alterations on different behavioral systems, and more importantly, that independently of their early family composition, both groups can cope effectively with the changing demands of the pups.

1. Introduction

In mammals maternal behavior is essential for the survival of the offspring and represents the most relevant source of sensory stimulation for altricial pups, sculpting the development of their nervous system (Suchecki et al., 1993; Anisman et al., 1998; Caldji et al., 1998). The long term physiological and behavioral consequences that can arise from disruptions of this early maternal environment have been studied extensively employing experimental interventions like the neonatal handling and maternal separation procedures (Weinberg et al., 1978; Pryce et al., 2001; Cirulli et al., 2003; Benetti et al., 2007). However, the ethological relevance of the information provided by these models has been questioned based on their artificial nature (Wurbel, 2001; Macri et al., 2004; McLeod et al., 2007; Zuluaga et al., 2014).

On the other hand, several studies have shown that natural variations in maternal behavior provoke long-term changes in the behavioral and neuroendocrine responses of the pups (Caldji et al., 1998; Champagne et al., 2003; Uriarte et al., 2007). In this line of thought, we validated a new model to study the effects of variations in early social environment on the individuals' development under an ethologically relevant context: the overlapping of litters (OLs) (Uriarte et al., 2008, 2009). OL occurs in natural conditions when, following the successful mating during the postpartum estrus, two successive litters coexist within the maternal burrow (Calhoun, 1963; Gilbert et al., 1983). As a consequence, mother rats will raise pups from different ages with different sensory traits, physiological demands, and behavioral capabilities at the same time.

Being reared in this complex early environment profoundly affects the neurobehavioral phenotype of adult rats. Thus, compared to animals reared in single litters (SLs), OL reared females

* Corresponding author at: Laboratorio de Neurociencias, Sección Biomatemática, Facultad de Ciencias, Universidad de la República, Iguá 4225, CP 11400 Montevideo, Uruguay. Tel.: +598 25258618x7151; fax: +598 25258617.
E-mail address: natiuria@gmail.com (N. Uriarte).

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show a decrease in their behavioral inhibition assessed in the open field test, a blunted corticosterone secretion in response to restraint stress and a reduction in their sexual receptiveness during proestrus (Uriarte et al., 2009). These behavioral changes may be due to the altered maternal care received, since during the overlapping of the two litters mothers modify their maternal behavior when compared to dams taking care of a single litter. Although OL mothers adapt their behavior according to the characteristics and needs of the two different-aged litters and direct most of their attention to neonates, pups from the second litter receive significantly less licking from the mother than pups raised in a single litter (Uriarte et al., 2008).

Taking into account these results where mothers of OL alter their maternal behavior, and previous studies demonstrating that variations in maternal behavior can be transmitted throughout generations (Francis et al., 1999; Champagne and Meaney, 2001; Gonzalez et al., 2001; Fleming et al., 2002), we hypothesized that females reared in OL would modify their own maternal behavior when adults.

Maternal behavior consists of a complex collection of activities, which can dynamically change over time and also adapt to the context. Thus, pup care taking activities fluctuate across the light/dark cycle and through the course of the postpartum period (Smith et al., 1976; Leon et al., 1984; Pereira et al., 2008; Jensen Peña and Champagne, 2013). Interestingly, it has been shown that interference of early mother–pup interactions alter the temporal characteristics of maternal behavior rather than the overall frequency of its components (Leon et al., 1984; Macri et al., 2004), highlighting the importance of analyzing the temporal dimension of this behavior.

Maternal behavior can be assessed by different methods that provide valuable and complementary information. While the continuous observation of maternal behavior following a brief period of separation from the litter is more suitable for analyzing active motivational components in a short period of time (Pereira and Ferreira, 2006), the periodic observation (spot-checks) of the mother's undisturbed behavior allows a deeper analysis of its temporal dynamic characteristics (Lonstein and Fleming, 2002; Champagne et al., 2007).

Motherhood does not only imply the development of care taking activities directed to the pups, but also involves changes in the affective responses to other environmental stimuli or situations, such as the development of maternal aggression and maternal anxiolysis (Ferreira et al., 2002). Lactating females vigorously attack intruders in their home cages (Erskine et al., 1978; Ferreira and Hansen, 1986; Mayer and Rosenblatt, 1987) and exhibit a reduction of their anxiety-related behaviors in several experimental models (Fleming and Luebke, 1981; Hard and Hansen, 1985; Ferreira et al., 1989, 2002; Bitran et al., 1991; Lonstein et al., 19982). These behavioral adaptations complement direct pup-caring behavior, as they provide additional protection to the altricial pups from potential predators or dangerous situations (Pereira et al., 2005).

Based on the previous evidence exposed, this study aims to analyze the maternal behavior, maternal aggression and anxiety-related behavior of lactating female rats reared in OL during the light/dark periods of the photoperiodic cycle in the first week postpartum.

2. Materials and methods

2.1. Animals

Male and female rats (*Rattus norvegicus*, Wistar strain, from 0 to 120 days old) were used for this study. The animals were housed under controlled temperature (22 ± 1 °C) and humidity (65%) in a 12 h light–dark cycle (lights on at 0500 h) with free access to food and water. Animal care and experimental procedures were in accordance with Uruguayan law (N°18 611) for the care and use of laboratory animals. Experimental protocol was approved by the local Ethical Committee on Animal Care and Protocols.

2.2. Experimental groups and general procedure

To assess the changes in maternal and affective behaviors of postpartum females raised in OL, two groups were used:

Single litter (SL) reared dams, $n = 9$: lactating female rats reared in single litters by multiparous mothers.
Overlapping litter (OL) reared dams, $n = 8$: lactating female rats reared in overlapping litters (a 10-day cohabitation period with their mothers and two juvenile siblings from the first litter).

To synchronize parturitions, adult females reared in SL or OL were monitored for estrous cycle and mated overnight during the proestrous phase. One week prior to expected parturitions, females were housed individually. On postpartum day (PPD) 0, litters were counted, sexed, and culled to eight pups (with usually 4 or at least 3 pups of each sex). Lactating females were left undisturbed and their maternal behavior was recorded by a spot-check observational procedure in the breeding room from PPD1 to PPD7. During the light phase of PPD8 the animals were transported to an adjacent testing room with the same temperature and photoperiod conditions of the breeding room and left to acclimatize at least one hour before starting the tests. Afterwards, a 30-min maternal behavior test was performed followed by a 10-min aggression test in the females' nest-cage. Finally, one hour after the beginning of the dark phase the dams were tested in the elevated plus maze for 5 min.

2.3. Breeding procedure to obtain experimental animals

To obtain adult females reared in single or overlapping litters the following procedures were carried out:

Single litters

Females reared in single litters were obtained from multiparous mothers in their second parturition, as the overlapping litter females were reared by mothers who were in their second lactation. Approximately seven days before delivery (gestational day 16, GD16), pregnant primiparous females were individually housed and, starting at GD20, the presence of pups was checked twice a day. On the day of birth litters were culled to eight individuals (four of each sex) (postpartum day (PPD) 0). Litters remained with their mothers until weaning on PPD21.

Overlapping litters

On GD16, pregnant nulliparous females were housed until parturition alongside the same males they had previously mated with, in order to copulate at postpartum estrus. After the postpartum estrus, vaginal smears were performed to check for the presence of spermatozoids to confirm successful mating; and the males were removed. On GD21 of the second pregnancy the juveniles from the first litter, except one male and one female, were weaned. These two juveniles remained in the maternal cage for 10 days after the birth of the second litter. Starting on GD22 of the second pregnancy the presence of pups was checked twice a day. Newborns were weighed and culled in the same manner as single litter animals in both parturitions. Once the 10-day-period of cohabitation with the juveniles ended, the mother and the second litter were left undisturbed until weaning on PPD21. The OL reared females were obtained from these second litters.