

# Pup age and aggressive behavior in lactating rats

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## Abstract

High levels of aggressive behaviors against intruders in the nest area are displayed by female rats during the first 10 days after delivery, declining thereafter to very low levels, even though lactation continues. Cross-fostering experiments were undertaken to test the hypothesis that pup age may affect aggression in lactating rats. The behavior of females on the 8th day after delivery when raising fostered 8-day-old pups was compared to that of females on the 8th postpartum day raising older pups (18 days old) for the last 5 days, and females on the 18th day after delivery raising fostered 18-day-old pups were compared to females in the same postpartum period nursing younger pups (8 days of age at the time of the maternal aggression test) for 5 days. Pup retrieval activity and plasma prolactin level were also analyzed. Females on the 8th postpartum day nursing 18-day-old pups were less aggressive than females in the same postpartum period, but with 8-day-old pups. Likewise, females on the 18th postpartum day nursing younger pups were more aggressive and presented higher levels of prolactin than females nursing older pups. Thus, pup development can alter the natural decline of maternal aggressive behavior.

## Key words

- Maternal aggression
- Mother-infant interaction
- Cross-fostering
- Prolactin

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## Introduction

Maternal behavior in mammals is one of most stable and highly motivated species-specific behaviors (1,2). The aggressive behavior of lactating females is part of the parental care (3-5), which includes several other protective activities (6). While much work has focused on the study of maternal caretaker behaviors, less is known about the nature and etiology of aggression during the postpartum period.

The marked hormonal changes at the end of pregnancy and during the early postpar-

tum period have been considered to be the endogenous substrates that may influence the onset of maternal behaviors (for a review, see 7). However, the continuous exposure of virgin and ovariectomized female rats without hormonal treatment, as well as males, to pups can elicit maternal behavior (8,9).

On the other hand, the onset of maternal aggressive behavior in rodents seems to require both the hormonal changes occurring at the end of pregnancy and the presence of the pups (10-12). Previous work showed that prolactin is related to the emergence of post-

partum aggression in mice and hamsters (13,14). In rats, gonadal hormones influence the onset of maternal aggression (10,15,16). Indeed, in nonpregnant ovariectomized rats treated with estradiol and progesterone (17,18) as well as in lactating female rats (19), the presence of the pups and their somatosensory stimulation on the mother are necessary to induce aggressive behavior. Moreover, the number of pups increases the propensity of the mother to attack the intruder (5). In mice, suckling can induce aggressive behavior in virgin females (11,20); in rats, however, suckling alone did not provide sufficient stimulation to elicit maternal aggression (19,21).

During the first 10 days after delivery, female rats vigorously attack intruders in the nest area, and thereafter this behavior declines to very low levels, even though lactation continues (22,23). The factors that elicit it appear to be different from those that maintain aggressive behavior during the first 10 days after delivery (for a review, see 7). The natural decline of maternal aggressive behavior after the 10th postpartum day is less understood than its onset. Previous work (24) addressed this issue and showed no relationship between the physical development of the pups and the decline of aggressiveness.

The aim of the present research was to study the effects of age, and therefore of developmental stage and behavior of the pups, on the aggressive behavior of lactating female rats. The presence of the pups is necessary for the aggressive behavior of the dam, since their separation decreases this behavior (19). As the pups grow old, they depend less on maternal care in terms of feeding, although suckling *per se* does not seem to elicit maternal aggression (21) or protection. On the other hand, dams are less stimulated by older pups than younger ones due to their increased independence. Cross-fostering experiments were undertaken to test the hypothesis that pup age could affect

maternal aggression. We planned to test the aggressive behavior of the lactating female 5 days after the cross-fostering procedure. We expected that the longer period of contact between the mother and the fostered pups, as compared to the study of Albert and Walsh (24), that involved only a 2-day interval, would affect maternal aggression. The behavior of females was tested on the 8th postpartum day with 18-day-old pups. Likewise, females on the 18th day after delivery with 8-day-old pups were tested against a male intruder. We hypothesized that the interaction for 5 days with younger pups would increase the aggressive behavior of females on the 18th postpartum day and, conversely, the interaction with older pups would decrease the aggressiveness of females on the 8th day after delivery. We chose to test the aggressive behavior on the 8th and on the 18th day in order to leave the fostered pups with the dams during a naturally high (from the 3rd to the 8th day) or low (from the 13th to the 18th postpartum day) aggressiveness period. In addition, we also tested the effects of this treatment on the hormonal levels of the dams. Although previous work (25) showed no significant reduction in maternal aggressive behavior (only a tendency) after hypophysectomy, which would discount a possibly role of prolactin on this behavior, we planned to measure plasma prolactin levels during a high- and a low-aggression period. Since plasma prolactin levels decline in the second half of the postpartum period (26,27), we aimed to confirm whether younger pups (8 days old) would affect the levels of dams on the 18th day after delivery.

## Material and Methods

### Animals and treatments

Lactating female Wistar rats from the stock of the Federal University of Rio Grande do Sul weighing  $240 \pm 20$  g were used in the experiment. The animals were maintained

on a light/dark (12/12 h) cycle with lights off at 16:00 h and the behavior recordings (aggressive and retrieval) were performed during the dark phase from 16:00 to 18:30 h. The temperature was kept at 22°C and the animals had free access to food and water.

Females were tested against a male intruder on the 8th and 18th day after delivery. In all groups, pups were cross-fostered 5 days before the behavioral test (3rd and 13th postpartum day). The number of pups per dam was set at 7.

Females were divided into 4 groups: group F8+P8 (N = 14), females tested on the 8th day after delivery raising 8-day-old pups; group F8+P18 (N = 13), females tested on the 8th day after delivery raising 18-day-old pups; group F18+P18 (N = 10), females tested on the 18th day after delivery raising 18-day-old pups, and group F18+P8 (N = 19), females tested on the 18th day after delivery raising 8-day-old pups. The cross-fostering procedures adopted were the same as in previous studies (3,28). By observing the dams and pups for some hours after cross-fostering and on the next day, we confirmed that the females did not reject them. All cross-fostered pups were grouped in the nest and they did not show any evident sign of physical suffering.

### **Behavior recording**

Females were first mated with males and, after being considered pregnant by visual and manual inspection, they were housed individually in 50 x 45 x 25-cm Plexiglas home-cages with wood shavings for bedding and nest building. On the 8th or the 18th day after delivery, the behaviors of the female in the presence of an intruder male were videotaped for 10 min beginning immediately after placing the intruder into the home-cage where the female was housed with her cross-fostered pups. The intruders were young males smaller than the females and were used only once. In this study and in other

studies from this laboratory (3,29,30) using the same rat strain, no pup was attacked by the intruder. The behaviors of the females towards the intruder recorded during the maternal aggression tests have been described elsewhere (3,31), and can be briefly summarized as sniffing, lateral attack, frontal attack and biting.

In order to avoid a possible interference of aggressive behavior with the retrieval activity, we measured it 24 h after the maternal aggression test. In the retrieval test, the female was removed from the home-cage and the pups were distributed around in the cage. The latency (s) to retrieve the 1st and the 7th pup was recorded only in the groups in which pups were 9 days old and raised by dams on the 9th or 19th postpartum day (groups F8+P8 and F18+P8).

### **Prolactin radioimmunoassay**

Immediately after the retrieval test, on the 9th and 19th day after delivery and about 30 min after the beginning of the dark phase of the cycle, females of all groups were killed by decapitation and blood was collected from the trunk. Samples were centrifuged for 10 min at 3000 rpm; the plasma was separated and stored frozen and the concentration of prolactin was determined by radioimmunoassay according to the method described by Niswender et al. (32). Anti-rat PRL-S9 was the specific prolactin antibody used and PRL-RP3 was the standard preparation adopted. The precipitation antibody (2nd antibody) was produced in the laboratory of Dr. Celso R. Franci (Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil), and all assays were performed in this laboratory.

### **Statistical analysis**

In order to test the effects of pup age on the behavior of the dams, comparisons were performed between dams during the same

postpartum period. The mean ( $\pm$  SEM) frequency of behaviors during the 10-min recording sessions was compared between the 2 groups of dams on the 8th (F8+P8 compared to F8+P18) and 18th postpartum day (F18+P18 compared to F18+P8) using the Student *t*-test. The mean ( $\pm$  SEM) concentration of plasma prolactin was also compared between the same groups using the Student *t*-test. The median latency (s) to retrieve the pups was compared between groups F8+P8 and F18+P8 using the Mann-Whitney U-test. In all comparisons,  $P < 0.05$  was considered statistically significant.

Figure 1 - Mean ( $\pm$  SEM) frequency of sniffing, lateral attack, frontal attack and biting the intruder male by the lactating female with cross-fostered pups during 10-min recording sessions. F8+P8 (female on the 8th postpartum day raising 8-day-old pups); F8+P18 (female on the 8th postpartum day raising 18-day-old pups); F18+P18 (female on the 18th postpartum day raising 18-day-old pups); F18+P8 (female on the 18th postpartum day raising 8-day-old pups). N = Number of animals in each group. \* $P < 0.05$ , F8+P18 compared to group F8+P8; + $P < 0.05$ , F18+P8 compared to group F18+P18 (Student *t*-test).

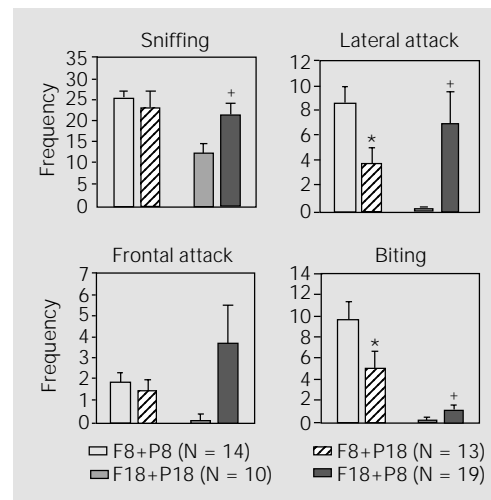
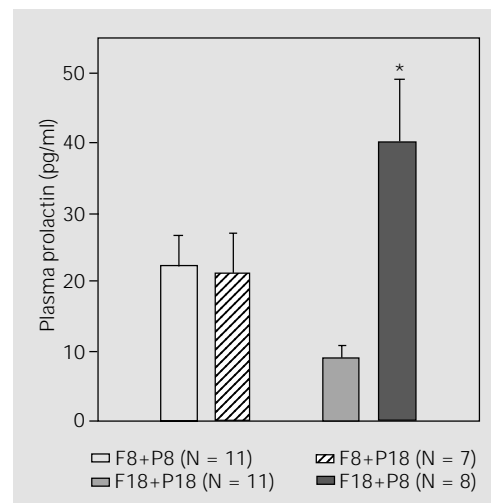


Figure 2 - Mean ( $\pm$  SEM) level of plasma prolactin of lactating females on the 9th and 19th postpartum day (one day after the aggressive behavior test) with cross-fostered pups. See Figure 1 for group identification. N = Number of animals in each group. \* $P < 0.05$  compared to group F18+P18 (Student *t*-test).



## Results

Figure 1 shows that on the 8th postpartum day, the frequency of lateral attack and biting in group F8+P18 was lower than in group F8+P8. On the 18th postpartum day, the frequency of sniffing, lateral attack and biting was higher in group F18+P8 than in group F18+P18.

Both the latencies to retrieve the first and the last pup of group F18+P8 (12 (4/37) and 50 (40/152) median and interquartile range, respectively) were significantly different from group F8+P8 (71.5 (14/900) and 235.5 (95/900), respectively). However, comparisons of the effect of pup age on dam behavior could not be performed because the 19-day-old pups (age at the day of the retrieval test) were already very mobile and therefore the retrieval test would be meaningless. Nevertheless, results showed that females on the 19th day after delivery were able to retrieve the pups very quickly.

Figure 2 shows that on the 19th postpartum day the plasma prolactin concentration in group F18+P8 (N = 8) was higher than in group F18+P18 (N = 11). On the 9th postpartum day (day of the test), the 2 groups of mothers (F8+P8, N = 11 and F8+P18, N = 7) did not present any difference.

## Discussion

Results showed that females on the 8th postpartum day which had been nursing older pups for the previous 5 days were less aggressive than females in the same postpartum period, but with 8-day-old pups. Likewise, females on the 18th postpartum day nursing younger pups (8 days old at the time of the maternal aggression test) for the previous 5 days were more aggressive than females nursing 18-day-old pups. On the other hand, plasma prolactin concentration in females on the 19th postpartum day nursing younger pups (9 days old at the time of the test) for 6 days was higher than in females

during the same lactation period, but raising 19-day-old pups.

The results of the present study suggest that pup age may affect the maternal aggressive behavior. Younger pups fostered for 5 days by females that are in a naturally low aggressive period (after postpartum day 13) may increase the aggressive behavior of the mother, and older pups may decrease maternal aggression.

Previous work (24) showed no difference in maternal aggressive behavior of Long-Evans lactating females on the 11th and 19th postpartum day nursing 4-day-old pups. However, in the previous study, 2-day-old pups were cross-fostered and the aggressive behavior test was performed 2 days later, while in the present study they remained 5 days with the cross-fostered dam. We may speculate that the duration of fostering (5 days in our experiment), and therefore its cumulative effect, could be an important variable to change the behavior of the dams.

The behavior of the pups could explain the increased aggressiveness. Pups from 3 to 8 days of age remain in the nest most of the time and are more dependent on the mother. At 13 to 18 days of age, pups have already opened their eyes and show spontaneous locomotion in the environment (33-35). It may be suggested that the degree of independence of the pups from the mother may be the cause of the reversed effects on maternal aggression. Maternal aggressive behavior is an interaction between the female and a male intruder, but seems to depend on the mother-infant relationship.

The increased level of plasma prolactin in females on the 19th postpartum day raising younger pups (9 days old) during the previous 6 days compared to females during

the same postpartum period raising older pups could be explained by the higher frequency of nipple stimulation. However, females on the 9th postpartum day raising older pups (19 days old) during the previous 6 days showed no difference compared to females during the same postpartum period raising younger pups. Results also showed that the increased plasma prolactin level observed in females 19 days postpartum, raising 9-day-old pups, was correlated with an increased aggressive behavior. On the other hand, females 8 days postpartum raising 18-day-old pups showed a decrement of maternal aggression, but no change in plasma prolactin measured one day after the aggressive behavior test. Previous studies on rats showed that during the first days after delivery dams spent most of their time nursing the pups, while, during the third postpartum week, there was a marked reduction in time spent in the litter chamber (36,37). Plasma prolactin levels were lower during the second half of the lactating period than during the first (26,27). We may infer that the increased plasma prolactin levels 19 days postpartum in dams raising 9-day-old pups may be due to increased nursing activity, although we did not measure pup body weight.

We may conclude that the higher the mother-infant physical contact, the greater the aggressiveness of the dam. The increased survival independence of the pups in terms of feeding and environmental exploration may decrease the mother's protective behaviors.

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