



The maintenance and termination of maternal behavior in rabbits: Involvement of suckling and progesterone



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HIGHLIGHTS

- Weaning occurred on lactation day 29, but only in concurrently pregnant rabbits.
- Nipple-removed not pregnant does showed maternal-like behavior for 40 days.
- Concurrence with pregnancy reduced nursing behavior to 26 days.
- Progesterone (P) injections did not induce weaning in lactating-only does.
- Daily P injections reduced milk output in late lactation.

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ABSTRACT

Rabbits mated at postpartum estrus become concurrently pregnant and lactating. They wean the first litter shortly before delivering the second one. Lactating-only rabbits do not spontaneously wean their young. These differences suggest that: a) suckling contributes to the long-term maintenance of maternal responsiveness and b) pregnancy-associated factors are essential to promote weaning. To explore if suckling stimulation interacts with the pregnancy condition we compared the behavior of intact and thelectomized (thelx; nipple-removed) rabbits, mated or not at postpartum estrus (Experiment 1). All lactating-only rabbits still showed nursing behavior by postpartum day 44; only 71% of thelx not pregnant does displayed maternal responsiveness for 31 days and none by postpartum day 44. If mated at postpartum estrus maternal responsiveness was observed only in 25% of pregnant–lactating does on postpartum day 29 and in 8% of thelx–pregnant rabbits on postpartum day 26. As in pregnant–lactating does progesterone (P) is present across ca. 3 weeks and then declines, but it is absent in lactating-only rabbits, in Experiment 2 we explored the effect of injecting P to lactating-only animals on their maternal responsiveness and milk output. P injections (20 mg/day) were given across lactation days 1–30 or 1–23. Neither treatment modified maternal behavior: nearly all females entered the nest box, crouched over the litter and suckled it for ca. 3 min, as did oil-injected nursing rabbits. In contrast, both P treatments accentuated the decline in milk output, with respect to oil-treated does. Results suggest that suckling promotes the long-term maintenance of maternal behavior while pregnancy-associated factors (not P) are essential to trigger weaning.

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1. Introduction

The investigation of maternal behavior in mammals has largely concentrated on the processes that promote its onset; comparatively few works have explored the ways through which maternal behavior is maintained and ended for reviews see: [1,2]. In most studied species the intense relation between mother and young that began at parturition gradually wanes across lactation and eventually leads to the final separation of both parties. The process of weaning involves two components: i) a behavioral one, i.e., the mother's and the progeny's

“willingness” to interact with each other and ii) a somatic one, i.e., milk production. To what extent are lactation and maternal behavior linked? How is the mother's behavior modulated by the growth of the young? We know that rodent mothers reduce the number of nursing bouts per day by actively avoiding the suckling attempts of the progressively older litter rats [3], mice [4], hamsters [5]. Yet, milk production in rats increases across the first two weeks of lactation, remains at a plateau across days 15–20, and decreases thereafter [6]. However, the actual intake of milk by the litter declines earlier (between days 15 and 20), i.e., not as a consequence of a diminished milk production but because the young start ingesting solid food. A reduced frequency of suckling stimulation, plus the activation of a beta-adrenergic tone that reduces milk ejection [7,8] by “old” litters, combine to halt milk production. Thus, in lactating rats both components of weaning, i.e., maternal behavior

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and milk output, are linked together. These components, however, can be dissociated in virgin “sensitized” rats daily exposed to continuously growing foster young. Although unable to nurse sensitized virgins reduce their maternal responsiveness and increase their rejection of the pups as they become older. In contrast, if either lactating mothers or sensitized virgins are continuously provided with “young” litters (aged 4–8 days) they retain their high maternal responsiveness for four weeks [3]. These results indicate that, in both lactating and “sensitized” rats, stimuli provided by “old” pups promote the cessation of maternal behavior while stimuli from “young” litters encourage its maintenance. Yet, if mated at postpartum estrus, rats initiate a second pregnancy concurrently with their first lactation. Under these conditions maternal behavior towards the (first) litter decreases much more steeply than in lactating-only animals [9]. These findings indicate that factors associated with pregnancy also contribute to weaning in rats.

Rabbits show a different pattern of maternal behavior as their only contact with the young is during nursing, which occurs just once per day, inside the maternal nest, with circadian periodicity [10–12], and lasts 3–5 min [13–16]. These *behavioral* characteristics remain unchanged throughout 30 days of lactation despite changes in three parameters: a) milk output (which increases across the first 15 lactation days and gradually declines thereafter [14,17,18]; b) the feeding habits of the continuously growing young: they rely entirely on milk for the first week of lactation, start nibbling on the nest material (i.e., straw or hay) and the fecal pellets deposited by the mother at each nursing bout across weeks 2–3, and begin ingesting solid food pellets by week 4 [19]; c) the characteristics of suckling stimulation of the litter, i.e., while young kits promote milk ejection older ones inhibit it [20]. Consequently, in rabbits, the *behavioral* and *somatic* aspects of weaning are dissociated from each other, unlike what occurs in rats. Lactating-only rabbits do not spontaneously end their maternal behavior, for at least 40 days: in the laboratory they continue to enter the nest box to interact with the litter even after milk output has markedly declined [18,19,21]. This observation suggests that suckling may contribute to the long-term maintenance of maternal behavior in rabbits. Indeed, nipple-removed (thelectomized; thelx) does show maternal responsiveness towards the (foster) litter, despite their incapacity to nurse. This behavior, however, has been evaluated only across the first week postpartum [22]. Thus, to determine the contribution of suckling to the long-term maintenance of maternal responsiveness we compared the behavior towards the litter of lactating and thelx rabbits across several weeks postpartum (Experiment 1). If mated at postpartum estrus rabbits become concurrently pregnant and lactating and, under such condition, they *do cease* interacting with their first litter inside the nest box a few days before the second litter is born [18,19,21]. This indicates that factor(s) associated with pregnancy play a key role in ending maternal responsiveness in rabbits. To explore if an interaction exists between factors that promote the maintenance of maternal behavior (presumably suckling) and those that end it (pregnancy-associated ones) we also explored, in Experiment 1, two groups of rabbits mated at postpartum estrus: pregnant-lactating and pregnant-thelx.

As Experiment 1 revealed a critical role of pregnancy-associated factors for the inhibition of maternal behavior, in lactating and in thelx does, in Experiment 2 we investigated if progesterone (P) was one of such inhibitory factors. P is absent in lactating-only rabbits [14] but, in concurrently pregnant-lactating does, P increases across lactation and declines from around day 25 onwards [23]. These changes in P levels *during lactation*, observed in does mated at postpartum estrus, coincide with: a) their engagement in nest-building during late pregnancy [23]; b) the decline in food intake that takes place a few days before parturition [23]; c) the days on which they wean the first litter [18,19], and d) the steep decline in milk production that occurs before weaning [17–19,23]. From these results we hypothesized that *the decrease in P concentration* (rather than the mere presence of P) halts maternal behavior *and* reduces milk production. To this end, in Experiment 2 we

compared the effect of giving daily, uninterrupted injections of P to lactating does vs the effect of *withdrawing* this hormone after three weeks on maternal behavior and milk output across postpartum days 1–30.

2. Materials and methods

2.1. Animals

Adult white New Zealand virgin female rabbits (3.5–4.5 kg body weight) bred in our colony were used. They were kept in individual wire mesh cages (52 cm long × 42 cm wide × 41 cm high) inside the rabbit colony under controlled light (14L:10D; lights off at 2100 h) and natural temperature (13–25 °C) conditions. Every day rabbits were given 350 g of Purina rabbit pellets and water ad libitum. Females were mated with sexually active bucks inside a round (1 m in diameter) wire mesh arena. The day of mating was considered day 0 of pregnancy. Throughout this work animal care and surgical procedures (see below) complied with the Law for the Protection of Animals (Mexico).

2.2. Behavioral measurements

On pregnancy day 21 a transparent acrylic nest box (50 cm long × 30 cm wide × 32 cm high) with a rectangular (20 cm × 22 cm) opening on one side was introduced into the female's cage to allow the construction of the maternal nest, as previously described [24]. Starting on pregnancy day 30, females were spot-checked across the day to determine the approximate time of delivery. At parturition mothers were left undisturbed for 5–8 h, after which the pups were removed (litter size was adjusted to 8 pups) and kept away from the doe, inside a box containing paper shavings, under a mild heat source. On postpartum day 1 the pups were weighed before introducing them into the nest box to record the following parameters, as described earlier [14,22]: a) duration of nursing, which is the time elapsed between the mother's entrance into the nest box and her exit after suckling the litter and b) milk output (see below). Each behavioral test lasted from the time pups were introduced into the nest box until the mother jumped out of it, or for 1 h if a mother refused to enter the nest box. As nursing inside the nest box is the only maternal behavior displayed by rabbits (in the laboratory) from parturition onwards [14] its occurrence was taken as an indicator of “maternal responsiveness”. After completion of the test pups were weighed again: the difference in their body weight (with respect to the one shown before nursing) indicated milk output. The litter was then returned to the container with paper shavings and left undisturbed for 24 h. This procedure was repeated daily throughout the experiments.

2.3. Statistical analysis

In Experiment 1 to compare between two groups (i.e., lactating-only vs pregnant-lactating; thelx-unmated vs thelx-pregnant) the proportion of females that showed maternal responsiveness a chi-square test for repeated measures was performed, using all the values in each group [25,26]. An additional comparison was made between lactating-only vs pregnant-lactating animals, using the same test, but considering only the values of days 26–31, as this was the period during which maternal responsiveness declined in concurrently pregnant-lactating does. In Experiment 2 to determine the effect of P injections on milk output we used a repeated measures General Linear Model test among the three experimental groups. This was followed by Tukey's post-hoc test to determine significant differences between two groups.

2.4. Experiment 1: maternal responsiveness of intact and thelectomized (thelx) rabbits mated or not at post-partum estrus

2.4.1. Surgery

Does were deeply anesthetized with ketamine (25 mg/kg, i.m.; Laboratoires Virbac, Carros, France) and xylazine (8 mg/kg, i.m.; Bayer

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