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Previous and recent maternal experiences modulate pups' incentive value relative to a male without affecting maternal behavior in postpartum estrous rats

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ABSTRACT

This study extends the behavioral analysis of the postpartum estrus (PPE) which represents a unique period in the female rat's lifetime when maternal and sexual motivations co-exist. The aim of this study was to explore how previous and recent maternal experiences influence the maternal responses to pups when confronted with a male in a preference test or when they are presented independently in the home cage. To achieve this objective, we firstly compared the maternal behavior in the home cage and the preference for pups or a male in a Y-maze of primiparous and multiparous females approximately twelve hours after delivery. No differences were observed in the active and passive components of the maternal behavior of primiparous and multiparous rats; however second-time mothers made more efforts to gain access to the pups and tended to spend more time with them in the Y-maze than maternally inexperienced dams. In a second experiment, we assessed the influence of recent maternal experience with pups on PPE females' behavior by comparing pups vs. male preference and maternal behavior of females that had experienced continuous or limited (approximately two hours) interaction with their litters after parturition was completed. PPE rats subjected to reduced interaction with their pups preferred the male, while females continuously exposed to pups chose them over the male. This change in females' preference was not accompanied by significant alterations of maternal performance in the home cage, although anogenital licking tended to decrease in females with limited mother-litter interaction. Together, the results of these experiments indicate that previous and recent maternal experiences influence the motivational responses of PPE females, and that these effects are more evident when both motivations compete.

1. Introduction

The motivational state of individuals regulates their behavioral responses to stimuli with biological relevance. For instance, maternal animals search physical contact with pups and learn operant tasks to gain access to them (for revision see Olazábal et al., 2013; Pereira and Morrell, 2011; Stolzenberg and Numan, 2011), while sexually active females vigorously seek males' proximity (Beach, 1976; Erskine, 1989; Paredes and Vazquez, 1999; Stolzenberg and Numan, 2011). These social motivations are regulated by endocrine and experiential factors, which modify the willingness of females to engage in maternal (for revision Lonstein et al., 2015; Olazábal et al., 2013; Rosenblatt and Lehrman, 1963) and sexual (Hliňák and Madlafousek, 1983; Pfaus et al., 2012) responses. Interestingly, maternal and sexual motivations co-exist during the postpartum estrus (PPE), a brief period when females express maternal behavior toward pups and sexual behavior to a male (Agrati et al., 2011; Carrillo-Martínez et al., 2011; Gilbert, 1984; Gilbert et al., 1980). Thus, this period offers an excellent opportunity to explore how factors that modulate social motivations influence the response of females to both incentives.

PPE females are maternally and sexually motivated; however, in a preference test using pups and a male as incentive stimuli, primiparous (Prim) rats do not express a clear preference, while multiparous (Mult) females choose the pups and make an effort to gain access to them (Agrati et al., 2008a), indicating that previous maternal experience enhances the incentive value of pups relative to that of the male. In the same line, several studies have shown that

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previous reproductive experience increases maternal responsiveness to pups in future exposures (for extensive revision, see Bridges, 2016). For instance, cycling and late pregnant Prim females have reduced sensitization latency and exhibit improved maternal behavior when compared with virgins (Bridges, 1975, 1977; Fleming and Sarker, 1990; Orpen and Fleming, 1987; Orpen et al., 1987). Furthermore, the maternal behavior of Mult dams, compared with that of Prim rats, is more resistant to the deleterious effects of olfactory denervation (Schwartz and Rowe, 1976).

As maternal motivation regulates the seeking of, as well as the interaction with pups, the behavior of mothers on tests specifically designed to assess maternal motivation – such as unconditioned preference tasks – might predict the execution of direct caretaking activities (for revision see, Ferreira et al., 2012; Lonstein and Fleming, 2001; Pereira et al., 2008). Thus, although controversial, most results suggest that high motivation is associated with better maternal behavior performance (Lee et al., 1999a; Lonstein et al., 1999; Pereira and Ferreira, 2006; Pereira and Morrell, 2011). On these bases, it could be hypothesized that during the PPE, the greater incentive value of pups – relative to that of the male – for Mult rats, compared with Prim ones, will also be reflected in their direct care-taking activities toward pups in the home cage. A putative effect of a previous reproductive experience on females' maternal performance could be more evident on the delivery day than later on the postpartum, because PPE dams have spent few hours interacting with pups and therefore lack the reinforcing effects of mother-infant interaction on maternal motivation and execution (Magnusson and Fleming, 1995; Morgan et al., 1992).

The effect of recent physical interaction with pups on maternal responsiveness, especially during the delivery day, is well documented. Parturient female rats that are not allowed to interact with pups after parturition are not maternal when exposed to pups several days later. However, allowing the dams to interact with their pups for 30 min after birth sustains maternal memory over 10 days (Fleming and Sarker, 1990; Orpen and Fleming, 1987), and a two hour-interaction period exerts a stronger effect and maintains maternal responsiveness up to 25 days (Bridges, 1975; Fleming and Sarker, 1990; Jakubowski and Terkel, 1986). These studies indicate that increasing the period of mother-infant interaction when firstly exposed to pups enhances maternal sensitivity. In addition, physical interaction with pups is required to induce conditioning place preference to an environment associated with pups (Fleming et al., 1994) and instrumental bar-pressing response to gain access to them (Lee et al., 1999a). It has been proposed that the reinforcing effect of mother-litter interaction not only allows pups to act as unconditioned stimuli in the formation of place preference or operant conditioning, but also mediates the strengthening of maternal motivation during the postpartum period (Magnusson and Fleming, 1995; Morgan et al., 1992; Olazábal et al., 2013; Orpen et al., 1987; Pereira and Morrell, 2011).

If maternal experience after giving birth reinforces maternal motivation, it could be hypothesized that reducing the period of mother-infant interaction after parturition will decrease the incentive value of pups relative to a male for PPE females, increasing the preference for males in this model.

In summary, this study is aimed to delve the influence of previous and recent maternal experiences on the behavior of females during PPE by assessing: (1) whether differences between Prim and Mult females regarding the incentive value of pups relative to a male are reflected on the execution of direct caretaking activities in the home cage, and (2) whether reducing mother-infant interaction after parturition modifies the incentive value of pups relative to a male and the maternal behavior of females. As PPE females are maternally and sexually motivated, sexual behavior with a male was registered to evaluate whether changes in maternal motivation affect sexual proceptive responses.

2. Methods

2.1. Animals

Female and male rats (*Rattus norvegicus*, Wistar strain, 0–180 days old) were used. All animals were housed in a temperature and humidity-controlled environment (21 ± 1 °C and 50–70%, respectively) under a 12 h light-dark cycle (lights on at 0500 h). Breeding was achieved by placing a receptive female rat with a sexually active male. Mult females were obtained by re-mating females with previous single experience of parturition and lactation in their first estrus after weaning their litter. Pregnant females were individually housed on gestational day 21, and starting at day 22, the presence of pups was checked every hour. Approximately two hours after parturition was completed, litters were culled to eight individuals (four of each sex) (except for females in the Limited experience group in Experiment II, where pups were removed). Animal care and experimental procedures were in accordance with Uruguayan law (Law No. 18 611) for the care and use of laboratory animals and the experimental protocol was approved by the Ethical Committee on Animal Care and Protocols of the "Facultad de Ciencias" (No. 240011-000656-1 6).

2.2. Pups vs. male preference test

Females' preference between pups and a male was assessed in an acrylic transparent three point star-shaped maze, consisting of three equal-sized chambers (25 cm wide × 30 cm long × 18 cm high) at the end of each arm (10 cm wide × 60 cm long × 10 cm high). Two of the chambers contained either eight pups (pup chamber), or a sexually active male (male chamber), while the third chamber remained empty (neutral chamber) (Agrati et al., 2008a). Each chamber had an acrylic transparent division with air holes allowing the females to see, hear and smell the stimuli without physical contact.

The tests were conducted approximately 12 h after parturition, at least two hours after lights off, to ensure maximal sexual activity (Carrillo-Martínez et al., 2011; Connor and Davis, 1980; Gilbert, 1984; Gilbert et al., 1980). Rats were placed in the center of the maze and allowed to entirely explore the empty maze for a 15-min habituation period. Thereafter, the stimuli were placed in the chambers and the cumulative time the females spent in each chamber was recorded during a 20-min testing period. The number of visits and the time spent in each compartment were recorded when the female placed the whole body or the head plus the forepaws inside the chamber. Additionally, we measured the number of attempts to gain access to each stimulus (bouts of biting and scratching the division) performed by the females. The entire maze was cleaned with a 50% ethanol/water solution and dried thoroughly between tests. Across females, within each experimental group, the locations of the stimuli were randomly counterbalanced and the stimulus males were never used more than once on the same day.

Measures of preference included (1) the percentage of animals in each group investing in a particular chamber at least 50% of the total time spent in the three compartments, with the additional caveat that this time had to be at least 25% greater than that spent in either of the two remaining chambers, and (2) the time spent in each chamber (Agrati et al., 2008a; Pereira and Ferreira, 2006).

2.3. Maternal behavior test

In Experiment I, the whole litter was removed from the home cage and re-introduced 15 min later in the opposite corner to the female's nest. The number of the following maternal behavior components was continuously recorded during 30 min: retrieval of the

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