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Maternal influences on the sexual behavior and reproductive success of the female rat

Nicole M. Cameron *, Eric W. Fish, Michael J. Meaney

Sackler Program for Developmental Psychobiology and Epigenetics at McGill University, Montréal, Canada

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ABSTRACT

In many species, including humans, there is evidence for parental effects on within-sex variations in reproductive behavior. In the present studies we found that variations in postnatal maternal care were associated with individual differences in female sexual behavior in the rat. Females born to and reared by dams that showed enhanced pup licking/grooming (i.e., High LG mothers) over the first week postpartum showed significantly reduced sexual receptivity and alterations in the pacing of male mounting (i.e., longer inter-intromission intervals) observed in a paced mating test. There were minimal effects on the sexual behavior of the male offspring. The female offspring of High LG mothers showed a reduced lordosis rating, a decreased mount:intromission ratio, received fewer ejaculations and were less likely to achieve pregnancy following mating in the paced mating context. The data suggest maternal influences on the sexual development of the female rat that are functionally relevant for reproductive success. Together with previous studies these findings imply that maternal care can 'program' reproductive strategies in the female rat.

Early life experience influences adult sexual behavior in the rat. Prolonged maternal separation during the early postnatal period leads to altered reproductive behavior in males (Rhees et al., 2001). Experimental manipulations that decrease maternal anogenital licking (AGL) reduce penile reflexes in adult males by altering the morphology of motoneurons in the spinal nucleus of the bulbocavernosus (Lenz and Sengelaub, 2006; Moore, 1992b). Reduced pup AGL impairs male sexual behavior, increasing the latency to ejaculate and decreasing mounting efficiency. Neonatal handling, which increases pup licking in the dam (Lee and Williams, 1975; Liu et al., 1997), increases the frequency of anovulatory estrous cycles in adult females (Gomes et al., 2005) and decreases sexual receptivity (Gomes et al., 2006). These findings suggest maternal effects on sexual development in the rat.

Natural variations in maternal care in the rat are an important source of individual differences in neuroendocrine development (Cameron et al., 2005; Meaney, 2001). Such variations in pup licking/grooming LG stably influence estrogen receptor α (ERα) expression in the female offspring in brain regions that regulate reproductive behaviors (Cameron et al., submitted for publication; Champagne et al., 2003b, 2006). Thus, females reared by Low LG mothers are more sexually receptive to males than are the female offspring of High LG mothers (Cameron et al., 2005, 2006). The results of cross-fostering studies reveal evidence for direct effects of postnatal maternal care on both ERα expression (Champagne et al., 2003b) as well as reproductive behaviors, including both maternal behavior (Francis et al., 1999) and sexual receptivity (Cameron et al., submitted for publication). Indeed, such effects include even rudimentary features of sexual development such as the timing of puberty, which occurs at a significantly younger age in the female offspring of Low compared to High LG mothers.

We (Cameron et al., submitted for publication) have reported that the female offspring of High and Low LG dams differed in sexual receptivity, measured using a lordosis rating (Hardy and DeBold, 1972), as well as in the pacing of sexual activity. Female rats tested for sexual behavior in a context (e.g., a pacing chamber, (Erskine et al., 2004)) that permits withdrawal from the male show active pacing of male mounting. Females show a preference for conditions that permit such pacing and the resultant timing of male mounting is functionally important for successful mating (Edwards and Pfeifle, 1983). When tested in the pacing chamber, the female offspring of High LG mothers showed both a reduced lordosis rating and a significantly longer intromission interval (Cameron et al., submitted). In the current study, we examined the functional importance of such differences in sexual behavior using the pacing chamber in which the female was provided the opportunity to mate with two sexually active males; one the offspring of a High LG mother, the other from a Low LG dam. The results of both field studies (Calhoun, 1962) and those in semi-natural environments (McClintock and Adler, 1978; McClintock and Anisko, 1982) suggest that sexually receptive female rats normally copulate with multiple males. Thus, we considered the testing condition that involves multiple males as the most relevant context in which to

* Corresponding author. Douglas Mental Health University Institute 6875 boul. LaSalle, Montréal, Québec, Canada H4H 1R3. Fax: +1 514 888 4081.
E-mail address: nicole.cameron@mail.mcgill.ca (N.M. Cameron).

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examine the functional importance of the maternal effect on sexual behavior in the female offspring. Moreover, this testing condition also provided the opportunity to examine possible partner-preferences. Interestingly, group mating under laboratory conditions reveals individual differences in partner-preference (McClintock and Anisko, 1982). While little is known about the factors that determine partner-preference in the female rat, apart from influences such as strain (Austin and Dewsbury, 1986; Coria-Avila et al., 2006, 2004), the female pacing of copulation is associated with partner-preferences (Coria-Avila et al., 2006; Paredes and Alonso, 1997; Paredes and Vazquez, 1999). Partner preference experiments that allow females to simultaneously mate with two males (Lovell et al., 2006) or four males (Ferreira-Nuno et al., 2005) provide evidence for a preferred mate, defined as the male with which the female spent the most time during testing. Such preferences appear stable over the testing period (French et al., 1972).

In the studies reported here we investigate the functional significance of maternal effects on sexual behavior in the female offspring and on female partner preference. Effects of natural variations in maternal care on the male's behavior were first observed during training with a stimulus female. The behaviors of receptive females and experienced male offspring from High and Low LG dams were then observed during mating using the pacing chamber. The results further reveal maternal effects on the sexual behavior of female offspring and suggest that these effects are potentially important for reproductive success.

Methods

Animals

Long–Evans hooded rats derived from animals obtained from Charles River Canada (St. Constant, QC) and born in our colony at the Douglas Mental Health University Institute were used in these experiments. Maternal behavior was examined as previously described (Champagne et al., 2003a,b; Myers et al., 1989). Dams were housed in a maternal observation room with a 10:14 light cycle (lights on at 09:00 h). The behavior of each dam was observed for five 75-minute daily observation periods for the first 6 days postpartum with three periods during the light (10:00, 13:00, and 17:00 h) and two during the dark (07:00 and 20:00 h) phase of the cycle. Within each observation session, the occurrence of pup LG of each mother toward any one of her pups was scored every 3 min. The pup LG scores over the entire period were calculated for each lactating female in the cohort (~40–60 dams). Several years of such observations reveal that the frequency of pup LG is normally distributed and varies little across successive cohorts (Champagne et al., 2003a). Dams with mean pup LG scores 1 SD above or below the mean were defined as High or Low LG mothers respectively.

Pups were weaned at Day 21, transferred into a colony room, and pair-housed in same-sex, same-litter groups in Plexiglas cages with food and water available *ad libitum*. The colony room was under a reversed light cycle (lights on from 20:00 to 08:00 h.). Adult female (275–325 g) or male (350–400 g) offspring of High and Low LG mothers were used in these experiments. Females were previously trained in a pacing chamber made of Plexiglas (37.5×75×30 cm) divided into three sections by two barriers. Each barrier had two holes (radius=2.5 cm) small enough to permit the female, but not the male to pass (Erskine, 1987). The stage of the estrous cycle was determined by daily vaginal smears taken between 9:00 and 10:00 h. Testing was carried out on proestrus between 12.00 and 15.00 h in a room under red light conditions. All experiments were performed according to guidelines developed by the Canadian Council on Animal Care using protocols approved by the McGill University Animal Care Committee.

Experiment 1: Female partner-preference in contact

Male training

Naïve male offspring of High and Low LG mothers were habituated to the Plexiglas pacing chamber (37.5×75×30 cm) over six, once daily, 5-minute sessions. Following habituation, the males were tested for sexual behavior towards an ovariectomized, stimulus female rat treated with estradiol benzoate (10 μg) and progesterone (500 μg) 48 and 4 h prior to testing. Each session began by placing the male in the chamber, followed 5 min later by the introduction of the stimulus female. The interaction was recorded using a standard video recorder. The females were not permitted the opportunity to pace mating in the training sessions. The session lasted for 30 min after the first mount or a total of 30 min if no mounting occurred, although certain sessions were extended beyond 30 min to include a complete post-ejaculatory interval. Five sessions were conducted at 5 to 7 day intervals and scored for the following sexual behaviors: frequency of mounts, intromission, and ejaculations, percent of efficiency (mount/intromission) (Moore, 1992b) and the number of mounts and intromissions required to achieve the first ejaculation. Subsets of the males (High: n=5/15; Low: n=7/15) that achieved at least two ejaculations on repeated tests were used as stimulus males in the partner-preference test.

Female sexual behaviors

Adult female offspring of High and Low LG mothers were placed individually into the central compartment of the pacing chamber with two sexually experienced males (one High LG and one Low LG) placed in the left and right compartments of the pacing chamber. To avoid side preferences, the High and Low males were counterbalanced between the left and right sides of the chamber across individual subjects. The females interacted with the males for 45 min or until a maximum of 15 vagino-cervical stimulations (VCS: intromissions and ejaculations). The female could move easily between the three sections of the chamber by using the holes in the walls of the pacing chamber to either interact with one of the males on either side or remain alone in the central compartment of the chamber.

The latency to each intromission (including ejaculations) or mount without-intromission (mount) received by the female was recorded for each mating session. We also scored paracopulatory behaviors including hopping, darting and ear wiggling performed by the female (Blaustein and Erskine, 2002). The percentage of lordosis in response to a mount (lordosis quotient, LQ) and the mean intensity of the lordosis posture (lordosis rating, LR; (Hardy and DeBold, 1972)) of the female were calculated using a 4-point scale (0 for no lordosis; 3 for maximum dorsiflexion of the back). The mean inter-intermission-interval (III) measured in seconds between intromissions or ejaculations was derived from a temporal analysis of the mating session. The percent of exits made by the female from either of the male compartments following a mount, intromission or ejaculation and the length of time to return to a male compartment (latency after mount, intromission or ejaculation) were calculated (Frye and Erskine, 1990). The mean number of mounts, intromissions, ejaculations, the number of aggressive-like behaviors (boxing, pinning, pouncing) and III performed by each male were scored and the percent of efficiency (mean intromissions and ejaculations/mounts) was calculated.

Reproductive success

The females were returned to their home-cages in the colony room after testing, housed individually, and allowed to give birth. Pregnancy rate and number of pups were measured. Male reproductive success was assessed by observing percent of High and Low LG males that first successfully ejaculated with every High and Low LG female tested in the partner-preference study.

Experiment 2: Female partner-preference in no-contact

To examine non-sexual attractants (odors, visual cues or non-sexual behaviors) we exposed a group of High and Low LG virgin females to either High or Low LG caged males or their soiled bedding. All females were previously trained in the pacing chamber with the High and Low LG males as described above. On the day of proestrus, half of the females were allowed to explore the three sections of the chamber for 20 min during which time two males (one High and one Low LG offspring) were caged, and placed randomly in the left and right sections. For the second group of females, soiled bedding from High or Low males were placed in the left and right sections. The time spent by the female exploring each male or the male bedding and the number of times they entered the right and left compartments were recorded.

Statistical analysis

Statistical analysis of the male sexual behavior during training session used repeated-measures ANOVA (maternal phenotype X test session). In normally distributed sexual behaviors such as intromission and mount, statistical analysis involved either two-way ANOVA's (female maternal phenotype X male maternal phenotype: for female sexual behavior) followed by a Tukey's test as post-hoc analysis or t-tests in the case of simple effects between two groups. A Mann–Whitney U test was used to analyze lordosis quotient and rating, ejaculation and III between groups and a Wilcox test was used to analyze within group effect. For ease and clarity of graphical presentation, data for the female groups were collapsed across males when there was no significant interaction between maternal phenotype of male X maternal phenotype of the female (i.e., when the behavior of the female was not influenced by the phenotype of the male). The pregnancy for the females, the difference in percent frequency of efficiency, and the probability of being the first male to reach ejaculation in the partner-preference test were compared using the Likelihood-Ratio Chi-Square test.

Results

Male sexual behaviors

There was little evidence for differences in male sexual behavior between High and Low LG naïve male offspring during the five training sessions conducted with a sexually receptive, stimulus female (Table 1). There was, however, a trend for an effect of maternal phenotype on the mean number of mounts displayed by the males [F(1,25)=4.07, p=0.06]. Note that although the number of mounts observed during training sessions 3 and 4 in High and Low males appear to differ, there was no significant main effect of maternal