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Maternal Programming of Sexual Attractivity in Female Long Evans Rats

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1. Introduction

Early-life environments and experiences, both pre and post natal, shape the development of numerous biological processes in humans and other mammals (Fish, et al., 2004; Boyce, et al., 2006; Evans and Schamberg, 2009; Lyons, et al., 2010). Diverse disciplines such as social epidemiology and behavioral neuroscience have documented that challenging and/or adverse experiences that occur early in life can influence developmental processes to impact an individual throughout its lifetime. These effects include changes in reproductive behavior and corresponding biology, both of which have been documented in insects, reptiles, amphibians, fish, birds, rodents, and primates (Danforth, 1991; Bass, 1992; Tollrian, 1995; Denver, 1997; Rhen and Crews, 2002). In humans, childhood sexual abuse is strongly associated with early pubertal maturation (Wierson, et al., 1993; Turner, et al., 1999; Zabin, et al., 2005). In rats, lower levels of parental investment markedly change reproductive phenotypes, including precocial puberty (Moore, 1984; Uriarte, et al., 2007; Cameron, et al., 2008a). Despite these well-established effects of early experience on adult physiology and behavior, the means by which these early-experiences are biologically embedded and persist long term is not well understood. The present experiments investigate the impact of variations in early maternal care on physiological and behavioral measures of sexual attractivity and behavior in female laboratory rats.

Life History Theory postulates that humans have evolved to be responsive to early childhood environments that subsequently bias children to adopt different behavioral and reproductive strategies (Belsky, 1991; Coall and Chisholm, 2003; Crews, 2003; Boyce, 2005). Environments may *directly* influence child development or, alternatively, changes may occur through perturbations in parental care and investment that arise in response to varying ecological demands. For example, girls raised with increased exposure to paternal absence are at significantly elevated risk for early sexual activity and adolescent pregnancy (Surbey, 1990; Ellis, et al., 2003). Early sexual activity has been shown to have several

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deleterious effects that include: i) having more and older sexual partners throughout the teen years ii) increased risk of sexually transmitted disease and iii) increased risk of adolescent pregnancy (Coker, et al., 1994; Sandfort, et al., 2008). Thus, the acceleration and maturation of the hypothalamo-pituitary-gonadal (HPG) sex axis in young girls has profound consequences that persist throughout the life course.

Research employing animal models has focused on the role of early maternal care on the regulation and programming of sexual development in offspring, allowing cause-effect relationships between early care quality and adult phenotypes to be established. In rats, for example, developing offspring are very sensitive to the quality of parental care received during the early postnatal period. Maternal licking and grooming (LG) of offspring during the first week of life has been shown to developmentally program the hypothalamo-pituitary-adrenal (HPA) axis of Long Evans rats, later impacting adult stress-reactivity profiles (Francis, et al., 1999b). Specifically, offspring that experience Low maternal LG as pups are more stress reactive, and less exploratory than their High LG counterparts as adults (Francis, et al., 1999b; Fish, et al., 2004; Weaver, et al., 2004). Recent findings (Moore, 1995; Cameron, et al., 2005; Uriarte, et al., 2007) suggest that a similar process occurs for developmental programming of the HPG axis in rats, subsequently influencing adult sexual behaviors.

Disruptions to early postpartum maternal care in rats can perturb neuroendocrine and behavioral processes in the offspring that influence sexual activity and reproduction. For example, female rats subjected to handling as pups exhibit less copulatory behavior and increased anovulatory estrous cycles compared to non-handled controls when assessed later in life (Gomes, et al., 2005; Gomes, et al., 2006; Rainecki, et al., 2008). Similar to the influence of maternal care on the developing HPA axis, Cameron (2005, 2008, 2010) reports that variations in maternal care received during the early postnatal period can also significantly influence aspects of female sexual behaviors later in life. Offspring of Low LG females are reported to reach puberty at an earlier age relative to High LG females. Low LG females exhibit a higher lordosis rating, a lower (but not significant) inter-intrusion interval and have a higher incidence of pregnancy compared to High LG animals. With the exception of pregnancy rate and pubertal onset, these are measures of copulatory behaviors, behaviors that result in the successful transfer of male gametes to the female (Blaustein, 2008). Whereas paracopulatory behaviors, behaviors performed by female rats to elicit mounting behavior from males, are subject to early-life regulation have been explored using a paced mating paradigm (Cameron, et al., 2008b). In the present series of studies, we sought to determine if female sexual behaviors, specifically, paracopulatory behaviors potentially lead to differences in male preference based on maternal history.

The aim of the following study was to investigate the role of maternal care in the developmental regulation of physiological and behavioral measures implicated in female paracopulatory behavior. Specifically, we hypothesized that sexually-experienced male rats, when allowed to choose between High or Low LG females in estrus will prefer to copulate preferentially with Low females. We hypothesized that Low female rats will exhibit increased paracopulatory and copulatory behaviors during estrus relative to High LG female rats, behaviorally rendering them more attractive to males when compared to High LG females. Attractivity, a term coined by Frank Beach (Beach, 1976), is defined by the stimulus value of the female in evoking male sexual behavior. As progesterone treatment potentiates the effects of estradiol on female sexual behaviors (specifically paracopulatory behaviors) we also assessed whether or not estradiol-induced progesterone receptor immunoreactivity (PR-ir) in the ventromedial nucleus of the hypothalamus (VMH) paralleled behavioral differences observed.

2. Methods and materials

2.1. Animals and Housing

Male Long Evans rats used in the study were purchased from Charles River Breeding Laboratories (Wilmington, MA) and pair housed in polypropylene cages ($27.8 \times 17.5 \times 13.0$ cm). Female rats ($n=48$) used in the study were born in our colony which was generated using Long Evans rats originally purchased from Charles River. For all animals, temperature was kept constant at 20 ± 2 °C and relative humidity was maintained $50 \pm 5\%$. Rats were maintained on a 12-h light–dark cycle (lights on 0700 h to 1900 h) and allowed access to food (Purina Rat Chow, Purina Mills, St. Louis, Missouri) and tap water *ad libitum*. Female offspring underwent behavioral testing between PND 85–90. Housing and care of the rats were carried out in accordance with the standards and practices of the UC Berkeley Animal Care and Use Committee.

2.2. Observations of Maternal Behavior

Female rats were bred and permitted to give birth. Day of birth was marked as postnatal day (PND) 0. Maternal observations were performed beginning on PND 1 and continued until PND 8 (Liu, et al., 1997; Francis, et al., 1999a; Champagne, et al., 2003a). Each litter was observed for five hours a day at the following times; 0600 h – 0800 h, 1200 h – 1300 h and 1800 h – 2000 h. During each observation session, litters were observed and behaviors recorded every two minutes (in sum, each litter was observed 150 times per day for eight days). Behaviors recorded included: mother on/off the nest and maternal licking behaviors directed at self or at pups. A maternal care distribution curve was generated by calculating the frequency with which pup-directed maternal licking was observed. Maternal licking was expressed as a percentage of the total number of observations performed for each litter. The mean ($\pm$ SD) frequency of maternal licking was calculated for the cohort and High or Low maternal designations were made relative to this mean. High and Low licking litters were assessed as those falling one SD above or below the mean, respectively. Animals were weaned on PND 22, and pair housed with same sex littermates.

2.3 Male Mate Preference Task

All testing was performed during the beginning of the dark phase of the light-dark cycle (between 1900 h – 0200 h) and digitally recorded. All animals were examined in a counterbalanced manner with an equal number of animals from both maternal conditions being tested at each time point. The testing apparatus consisted of three transparent plastic Plexiglas chambers ($21 \times 15 \times 10$ inches) connected by two cylinders ($8'' \times 4''$ dia.) in a linear manner. The outer chambers housed High and Low LG tethered female rats in estrus ($n=11$ pairs of High/Low females) that were behaviorally screened prior to the task. Females were screened by pairing with an established stud male. Females who did not exhibit lordosis upon male stimulation were excluded from testing. The middle chamber was neutral. A vasectomized male rat was placed in the neutral chamber. Female rats were given 10 minutes to acclimate, while males were given 5 minutes prior to testing with access to females prohibited. They were otherwise left undisturbed. After the short acclimation period, males were given 15 minutes to explore, inspect, investigate and “select” a female. Male behaviors scored included the following: i) time spent in proximity to High or Low female rat ii) frequency of mounts iii) intromission frequency iv) inter-mount interval v) inter-intromission interval and vi) ejaculations.

2.4 Female Sexual Behaviors

Similar to the Male Mate Preference Task, High and Low LG adult female rats in estrus (behaviorally confirmed) were tethered to the outer-chambers of the testing apparatus and all

behaviors were digitally recorded. Females were acclimated to the tethers for at least ten minutes before the onset of the task, and were tethered behind the forelimbs using an 8" nylon harness. The location of the female rats was counterbalanced, with High and Low females being equally represented in the left and right chambers among trials. During the 15 minute test, the following proceptive and receptive behaviors were scored: i) frequency of ear-wiggling ii) frequency of hopping/darting and iii) lordosis quotient (lordosis responses/ mounts and intromissions).

2.5 Male Olfactory Preference Task

To assess if male mate selection was due to olfactory cues emitted rather than female behaviors, a simple olfactory preference task was performed. Using the three-chambered apparatus described above, soiled bedding from High and Low LG estrus female rats was placed in the outer test chambers. Males were placed in the neutral chamber, allowed to acclimate for five minutes and then were given equal access to the two outer chambers. The placement of the bedding was counterbalanced in the outer chambers for each trial to minimize lateralization effects. Total time spent in the High LG, Low LG and neutral chambers was recorded.

2.6 Hormone Treatment and Perfusion

Upon completion of behavioral testing, 20 female rats (n = 10 High and 10 Low) were bilaterally ovariectomized and allowed to recover for seven days. After recovery, females were injected (s.c.) with 10 ug of estradiol benzoate (EB) in 0.1 ml sesame oil vehicle followed 48 hours later by a 500 ug (s.c.) injection of progesterone in 0.1 ml vehicle. This hormone treatment regimen is sufficient to facilitate the expression of sexual behavior in female rats as well as induce progesterone receptor immunoreactivity in brain regions critical to sexual behavior (Boling and Blandau, 1939; Blaustein, 1989). Four hours after the final hormone treatment, rats were screened for behavioral estrus and subsequently administered a lethal dose of sodium pentobarbital (50 mg/ml). Rats were perfused using 0.9% saline followed by 4% fresh paraformaldehyde in 0.1M PBS. Brains were removed, post-fixed in 4% paraformaldehyde in 0.1M PBS for three hours and subsequently placed in 30% sucrose/0.1 M PBS solution overnight at 4°C until saturated. Forty μ m sections were then cut on a cryostat and saturated in anti-freeze cryoprotectant at -20°C until immunohistochemistry (IHC) was performed to label progesterone receptors (PR).

2.7 Progesterone Receptor Immunocytochemistry and Image Analysis

For the PR IHC, sections were first washed (6X) in .01M PBS for ten min each wash. Sections were then incubated in 0.5% hydrogen peroxide (10 min) in order to reduce endogenous peroxidase activity. The hydrogen peroxide rinse was followed by three (10 min) PBS washes and incubated in 20% normal goat serum with 0.3% PBT in order to reduce non-specific staining. Sections were then incubated overnight in a rabbit polyclonal primary antiserum generated against the DNA binding domain of human progesterone receptor (1:500, A0098, DAKO, Carpinteria, CA) in 0.3% PBT for 72 hours. Residual primary antibody was removed by three (10 min) washes in 0.3% PBT. Sections were then incubated in a biotinylated goat anti-rabbit secondary antibody (1:200, T0411, Vector, Burlingame, CA) for one hour, followed by three (10 min) PBT washes. Next, sections were incubated in avidin-biotin complex (Vectastain Elite, Vector Laboratories, Burlingame, CA) for one hour followed by three (10 min.) PBS washes. Sections were exposed to diaminobenzine (DAB, SK-4100, Vector Laboratories, Burlingame, CA) for five minutes. Sections were immediately washed in three (10 min.) washes in PBS to stop the reaction. Sections were mounted onto gelatin-coated slides and cover slips were applied using Permount mounting medium (ProSciTech, Australia).

PR-immunoreactive cells in the VMH were assessed using three matched sections of the VMH (Plate 56, 59, 60, Paxinos and Watson, 6th ed.) for each animal. Sections were analyzed by capturing images under 20X magnification with a Zeiss Axio Imager M1 with an Axio Cam Mrm TV2/cc 0.63x Camera. Cells were counted using NIH Image J to label cells with a pixel density darker than 130 (where 0 =black and 255 white). Where cells were clustered and difficult to individually discriminate, images of the masked region were captured at 40x magnification and analyzed in the same manner using Image J. The numbers of immunoreactive cells were quantified by observers blind to the experimental conditions. A total of six hemi sections were assessed per animal (three matched sections/animal), averaged for a total count and expressed as the mean $\pm$ SEM. Sections were counted by two independent investigators to ensure reliable and valid quantification of immunoreactive cells.

2.8 Data Analysis

All behavioral measures were analyzed by a one-way analysis of variance (ANOVA) or a Student t-test. Prior to analysis, a D'Agostino-Pearson omnibus test for normality was conducted. If behaviors failed normality ($p < .05$), a Mann-Whitney t-test was used in order to account for non-Gaussian distributions. The following data was determined to be non-parametric: intromission frequency, inter-intromission interval, and male olfactory preference. All other data was parametric. Densitometric measurements of PR-ir were determined by Student's t-test. Results were considered statistically significant when $p < 0.05$.

3. Results

3.1 Maternal Observations

Dams naturally differed in frequency of licking/grooming and arched-back nursing over the first six–eight days postpartum. A frequency distribution of maternal licking across all litters was created as previously described (Francis, et al., 1999b). A maternal care score was generated by calculating the frequency of maternal licking observed relative to the total number of observations performed over the entire observation period. Percent licking in this cohort ranged from 4.00 – 12.33 % with a mean licking score of 6.84% across all litters. Female rats reared in litters that fell ± 1 SD away from the mean were used in the remainder of the study. In sum, five High and seven Low LG litters were generated out of a total of 30 litters.

3.2 Male Mate Preference Task

Male rat sexual preference for female rats reared under a High or Low LG maternal conditions was tested. Males spent significantly more time in proximity to Low LG females compared to High LG females ($p < 0.01$). Indeed, males spent nearly twice the amount of time with Low LG females relative to High LG females (6 min 52 s vs. 3 min 28 s, respectively) (Figure 1A). Males also exhibited greater mounting ($p < 0.05$) and intromission frequencies ($p < 0.05$) with Low LG females compared to High LG females (Figures 1B, and C) as well as exhibiting a shorter inter-mount interval ($p < 0.01$; Figure 1D). Whereas male behavior did not differ in inter-intromission intervals ($p > 0.05$; Figure 1E), a general trend of shorter latency between intromissions exists towards Low LG females. Furthermore, across all trials ($n=11$ /group) ejaculations occurred exclusively with Low females ($n= 3$ total ejaculations) ($p < 0.05$; Figure 1F).

3.3 Female Sexual Behaviors

Low LG females exhibited a significantly higher lordosis quotient (number of lordosis responses/number of mounts; $p < 0.05$) compared to High females (Figure 2A). High and Low LG females also differed significantly in paracopulatory behaviors. Low females engaged in more hopping and darting ($p < 0.01$) compared to High LG females, whereas ear wiggling was not significantly different ($p > 0.05$) (Figures 2B and 2C). Collectively, Low females appear to exhibit more paracopulatory and copulatory behaviors compared to High females.

3.4 Male Olfactory Preference Task

When placed in the neutral arena of a three-chambered testing apparatus and allowed to explore olfactory cues (soiled bedding) from High and Low LG females, males did not demonstrate a preference for odors from females from either maternal condition ($p > 0.05$). Males did, however, spend significantly more time in the chambers containing female olfactory cues relative to the neutral center chamber ($p < 0.0001$; Figure 3).

3.5 Progesterone Receptor Immunoreactivity

Estradiol-induced PR was quantified in the VMH for both Low and High LG females. Consistent with the behavioral data, the number of cells expressing PR-ir in the VMH was significantly higher in estrogen-primed Low LG females compared to High LG females ($p < 0.05$; Figure 4).

4. Discussion

The findings presented suggest that the quality of maternal care received early in life may differentially program physiological and behavioral measures implicated in rat female sexual function in adulthood. Specifically, female rats raised in a litter that received low levels of maternal care, when tested later as adults, demonstrated higher levels of paracopulatory and copulatory behaviors when compared to females raised in a litter that received high levels of maternal care. Interestingly, females reared under conditions of varying maternal care were also differentially 'attractive' to novel male rats. Males, when allowed to select between High or Low LG female rats in estrus, chose primarily to mount, intromit, and ejaculate with Low LG females. This was not due to differences in pheromonal/odor cues emitted by the Low LG females as males spent the same amount of time exploring olfactory cues from High and Low LG animals. Differences between High and Low LG female rats were not limited to behavior, but also extended to neurophysiology. In female rats, the ventromedial nucleus of the hypothalamus (VMH) has been studied extensively for its role in sexual behavior. Interestingly, Low LG females, as adults, had greater estrogen-induced PR-ir in the VMH compared to High LG females. In accordance with the literature, these results confirm that early life maternal care may influence female sexual behavior; these findings further demonstrate that differences in female sexual behavior can drive male mate preference through behavioral and not pheromonal signaling.

4.1 Neuroendocrine underpinnings of female sexual behavior

Our results suggest that male preferences for Low LG females is guided, in part, by the pattern of female sexual behavior (Figures 1a–f and 2a–c) rather than changes in pheromonal cues emitted by females (Figure 3). During proestrus, female copulatory and paracopulatory behaviors are dependent on estrogen and progesterone stimulation of the neural circuits driving sexual behavior (Boling and Blandau, 1939; Blaustein, 2002). The present findings suggest that differences in maternal care manifest in adulthood as changes

in the activity of this hormone-dependent circuitry (Figure 4a) and consequent changes in paracopulatory and copulatory behaviors that drive male partner preference.

Activation of VMH progesterin receptors by estrogens influences many neuroendocrine processes including pre-ovulatory gonadotropin secretion and female sexual behavior (Rubin and Barfield, 1983; Olster and Blaustein, 1988). One mechanism by which maternal care may be influencing the immunoreactivity of PR in the VMH is by influencing the pathway leading to PR induction by estradiol. Several co-regulators of PR induction involved in female sexual behavior may be regulated by differential maternal care in the VMH. Potential targets include steroid receptor co-activator 1 (SRC-1), SRC-2, and cAMP binding protein, all of which have been shown to play a modulatory role in PR and estrogen receptor (ER) induction involved in female sexual behavior (Molenda, et al., 2002; Molenda-Figueira, et al., 2006). Similarly, estrogen receptor beta (ER β), like estrogen receptor alpha (ER α), can influence the expression of female sexual behavior by conferring estrogen sensitivity and PR induction (Helena, et al., 2009). Whereas, ER β has not been shown to influence PR-ir in the VMH, this receptor has been shown to act in the locus coeruleus to mediate sexual behavior.

The previously mentioned transcription factors may provide proximate mechanisms for the role of maternal care on PR-ir and sexual behavior. However, maternal care has also been explicitly implicated in altering the neuroendocrine circuits involved in regulating gonadotropin-releasing hormone (GnRH), a key peptide involved in regulating the HPG axis. This is believed to be mediated by epigenetic regulation of ER α in the medial preoptic area (MPOA) and increasing ER α immunoreactivity in the anteroventral paraventricular nucleus (AVPV) of the female LE rat (Champagne, et al., 2003b; Champagne, et al., 2006; Cameron, et al., 2008c). Previous studies have shown that Low LG females exhibit higher ER α expression in both the MPOA and AVPV and can increase the likelihood of female paracopulatory behaviors (Champagne, et al., 2003b; Champagne, et al., 2006; Cameron, et al., 2008b). The MPOA is rich in GnRH cell bodies and input to this nucleus from estrogen-responsive cells in the AVPV can influence GnRH and downstream luteinizing hormone (LH) and estradiol. As estradiol enables PR-ir in the VMH, feedback signaling may increase PR-ir in the VMH and related paracopulatory behaviors shown in the present work. In accordance with our findings, Cameron et al. 2008 report that Low LG females exhibit greater GnRH immunoreactivity, higher estradiol and progesterone profiles during proestrus, and a significantly higher amplitude LH surge after ovariectomy and estrogen replacement. Thus, Low LG animals have greater LH, estradiol and progesterone release, likely due, at least in part, to greater ER α expression in the AVPV. In such a fashion, natural differences in copulatory and paracopulatory behaviors may arise that can influence male preference.

High and Low LG animals expressed similar levels of copulatory and paracopulatory behaviors following ovariectomy and hormonal replacement (data not shown, also reported in Cameron 2008). This finding suggests that early life maternal care may have an organizing effect on the developing neuroendocrine systems responsible for female sexual behavior whereas the downstream targets of these sex steroids remains intact. It should be noted, however, that the arcuate nucleus, a neural locus largely implicated in regulating anterior pituitary hormonal release and GnRH, exhibits no differences in PR-ir between groups, indicating that the impact of differential maternal care is selective for progesterone sensitive targets that are specifically involved in female sexual behavior. The means by which early life experience leads to these neuroendocrine changes represents an important area for further inquiry.

4.2 Strengths and Limitations

The results from this study are in agreement with those investigating the effects of neonatal handling on offspring sexual behavior as well as reports using cross-fostering paradigms to study similar phenomena (Moore, 1984; Padoin, et al., 2001; Gomes, et al., 2005; Gomes, et al., 2006; Uriarte, et al., 2007; Cameron, et al., 2008b). However, several caveats should be considered in interpreting the present findings: i. ecological validity in the method of testing female sexual behavior in a constrained environment, ii. vaginal cervical stimulation (VCS) received by females during the male preference task may potentiate any effects observed, and iii. female auditory cues, which may influence male preference behaviors (in conjunction with paracopulatory behaviors), were not recorded.

- i. The current findings, unlike previous studies, document differences in High/Low LG female paracopulatory behaviors in addition to the previously noted differences in copulatory behavior. Female sexual behaviors are most commonly studied through the use of a paced mating procedure. This is traditionally assessed using a pacing chamber, in which the female regulates the timing and number of interactions with male conspecifics by approaching and withdrawing. Females pace interactions with males in order facilitate fertility and fecundity (Frye and Erskine, 1990). The current experiments did not utilize a female paced mating paradigm, but rather used a protocol in which female rats were tethered. This limited the control exerted by the female over the 'sexual repertoire' but allowed for the assessment of male mate selection between two female rats. Whereas this task has allowed us to gain some insight into female sexual behavior and male mate selection that could not be assessed using conventional methods, it does constrain the ecological validity of our findings.
- ii. Another caveat lies in the manner in which male rats approached, mounted, intromitted, and ejaculated with High or Low LG females. Female copulatory behaviors are regulated, in part, by VCS in conjunction with steroid hormones. VCS, including male intromissions and ejaculations can, over time, facilitate the expression of lordosis in the absence of estradiol or progesterone (Blaustein, et al., 2009). Furthermore, male copulatory stimulation and non-intromissive copulatory behavior can facilitate female copulatory and paracopulatory behaviors (Blaustein, et al., 2009). Consequently, the order in which male conspecifics "chose" females in our task may have affected female behavior. This point did not likely impact the present findings, as our data indicate that males initially "chose" High and Low LG females with an equal probability.
- iii. Finally, whereas olfactory and visual cues were assessed to determine if these cues influence female attractivity, auditory cues emitted by the female were not investigated. It is possible that Low LG rats attract male conspecifics through this mechanism (White and Barfield, 1989).

4.3 Conclusion

The present findings reveal that early life experiences in female rats influence multiple aspects of later sexual functioning. The quality of maternal care received early in life influences later paracopulatory behaviors, copulatory behaviors and progesterone receptor immunoreactivity in relevant neural loci. By extension, these differences in sexual function result in male rats choosing, almost exclusively, to partner with female rats reared under Low LG maternal conditions. While it remains to be determined if research on sexual behavior in rodents has predictive validity for aspects of human sexual function it is compelling to consider the possibility that similar processes of developmental programming are at play in humans. The findings from the current study suggest that social interventions

targeted at ameliorating conflict and strife in the home and improving parental support could have a powerful impact on the sexual maturity and function of young girls at the level of behavior *and* physiology.

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Figure 1.

Male Mate Preference. (A) Mean ($\pm$ SEM) time spent by male rats in proximity to High or Low LG females. The filled bars represent females reared in High maternal litters and the open bars represents females reared in Low maternal litters. The grey bar represents the neutral chamber where no females were present. Males spent significantly more time investigating Low females relative to Highs ($n=11$). (B) Mean ($\pm$ SEM) mount frequency with High and Low female rats. (C) Mean ($\pm$ SEM) intramission frequency with High and Low female rats. (D) Mean ($\pm$ SEM) inter-mount interval with High and Low female rats. (E) Mean ($\pm$ SEM) inter-intramission interval with High and Low female rats. (F) Total number of ejaculations across all trials ($n=11$) in both High and Low female rats. All observed ejaculations ($n = 3$) occurred with Low LG females. * $p < 0.05$; ** $p < 0.01$

Figure 2. Female Behavior. (A) Mean ($\pm$ SEM) lordosis quotient for High and Low LG females exposed to a novel male rat. (B) Mean ($\pm$ SEM) frequency of hopping and darting in High and Low LG females exposed to a novel male. (C) Mean ($\pm$ SEM) ear wiggle frequency of High and Low females exposed to a novel male. * $p < 0.05$; ** $p < 0.01$.

Figure 3. Male olfactory preference. Mean ($\pm$ SEM) time spent in one of three chambers that contained different olfactory cues. Males spent roughly equal time investigating olfactory cues from High and Low females and significantly less time in the neutral chamber. $**p < 0.01$.

Figure 4.

Effects of early maternal rearing on estrogen-induced progesterone receptor expression in the ventromedial hypothalamus (VMH) of female rats. (A) Low-power photomicrograph depicting representative immunostaining for PR in High and Low females (left, upper panel) and plate 56 of Paxinos and Watson (2007), depicting location of the VMH (left, lower panel). (B) The mean ($\pm$ SEM) number of cells expressing PR immunoreactivity in High and Low LG female rats ($n=7$ /group). * $p<0.05$.