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Deconstructing Early Life Experiences: Distinguishing the Contributions of Prenatal and Postnatal Factors to Adult Male Sexual Behavior in the Rat

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Abstract

In rodents, a pup's experiences in utero and during postnatal development shape its sexual behavior as an adult and how it is perceived by potential mates. We show that the male rat's sexuality is primarily influenced by the postnatal sex ratio of its litter, but not by the litter's prenatal intrauterine sex ratio or the behavior of its mother. Pups from litters with differing prenatal sex ratios were divided into litters with differing postnatal sex ratios. We found that males raised in a female-biased litter exhibited less mounting than males raised in either a male-biased litter or one with an equal sex ratio, and were less attractive to sexually receptive females, eliciting fewer soliciting behaviors, such as hop-darts, and fewer lordosis behaviors. However, the number of intromissions and ejaculations did not differ across groups, which suggests that males from female-biased litters mate as efficiently as males raised in other sex ratios, but do not require as many mounts to do so. The reported differences in sexual behavior did not vary with the quality of maternal behavior or with sexual experience in adulthood.

Keywords

bias, maternal, mounting, intromission, proceptivity, prenatal, postnatal, sex

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Does family play a role in shaping the adult phenotype? Although family demographics and sexual orientation in humans (such as homosexuality and bisexuality) is well studied (Blanchard, 1997; Blanchard, Zucker, Bradley, & Hume, 1995; James, 2005; Rust et al., 2000; Zucker et al., 1997), to our knowledge, the effect of the gender complement of an individual's siblings on the sexual behavior of that individual later in life has not been studied. Both early prenatal environment and postnatal rearing conditions contribute to sexual behavior in adulthood in nonhuman primates (Goldfoot, Wallen, Neff, McBrair, & Goy, 1984; Goy, Wallen, & Goldfoot 1974; Wallen, Goldfoot, & Goy, 1981). However, it is difficult to interpret the results of these studies, determine the relative effects of specific developmental periods and associated stimuli, and link them with observed differences in adult behavior. Controlling for the cumulative nature of multiple factors during development is a challenge, requiring a well-established animal model that can be experimentally manipulated to distinguish between the component elements.

Sexual behavior in male rats occurs in copulatory bouts, each involving a sequence of mounts and intromissions, and

ending with ejaculation. Mating efficiency, defined in this article as the ratio of the number of ejaculations to the sum of the number of mounts and the number of intromissions, differs among male rats. The female rat regulates the sequence and timing of copulatory bouts, determining when the male can mount and intromit, and signaling her readiness to mate with characteristic hop-darts, a series of hopping and darting movements (McClintock & Adler, 1978). Sexuality, then, includes both the behavior of the individual and how potential mates perceive that individual (Beach, 1979; Crews, 1992).

These adult sexual behaviors have their beginnings in an individual's experiences early in life. In many small mammals, testosterone secreted by the male fetus during embryonic development influences the sexual development of the fetus's closest siblings in the uterus, ultimately influencing their

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physiology and behavior in adulthood (Ryan & Vandenberg, 2002). Following birth, a mother's behavior toward her pups also influences the development of behaviors later in life (Champagne & Curley, 2010; Meaney, 2001; Moore, 1992, 1995). For example, pups born to mothers who exhibit high levels of licking and grooming of their offspring are less reactive to stress as adults than pups born to mothers who exhibit low levels of licking and grooming, as maternal licking of a pup increases serotonin tone and the number of glucocorticoid receptors in the pup's hippocampus (Meaney, 2001). Maternal licking of pups is particularly important in the phenotypic expression of sex-typical adult characteristics, and mother rats spend more time licking the anogenital region of their male pups than their female pups (Meaney, 2001; Moore, 1995, 2007; Uriarte, Breigeiron, Benetti, Rosa, & Lucion, 2007).

There is evidence that the prenatal sex ratio of the litter influences circulating levels of testosterone in the pregnant female (Cameron et al., 2008; Clark, Crews, & Galef, 1991, 1993), and that the postnatal sex ratio of the litter determines the amount of maternal licking of offspring (Moore & Morelli, 1979). However, little is known about how the prenatal and postnatal developmental periods, and the environmental factors in each period, differ and interact. The effect of progressive developmental changes on behavioral outcomes has been studied in precocial birds, in which the difference in timing of developmental periods is a major factor in differentiating filial imprinting versus sexual imprinting (Bateson, 1991). We conducted our study to determine the relative influence of early prenatal experiences in utero (indicated by pup sex ratio at birth), versus postnatal experiences due to litter sex ratio, on male sexuality in adulthood. By manipulating the postnatal sex ratio of the litter and controlling for the prenatal sex ratio, we found that manipulating the litter following birth influenced sexual behavior and attractiveness of the litter's male pups in adulthood. We also found that the amount of time the mother spent crouched over her litter, and not the amount of time she spent licking her pups, was an additional factor that influenced adult male sexual behavior.

Materials and Method

Subjects and housing

The mothers in this study were 88 primiparous female Sprague-Dawley rats (60–90 days old) born at the University of Toronto at Mississauga from an original stock of rats obtained from Charles River Farms (St. Constant, Quebec, Canada). The animals were individually housed in a clear Plexiglas cage (44 cm long × 22 cm wide × 30 cm high) containing wood-shaving bedding and provided with ad libitum access to food (Purina Lab Diet 5012; Ralston Purina, St Louis, MO) and water. Lighting was maintained on a 12-hr light/dark cycle: 12 hr of light beginning at 8:00 a.m., followed by 12 hr of dark beginning at 8:00 p.m. Room

temperature was held constant at 24 °C, and humidity was held constant at 40%. Anogenital distance (AGD) was not measured at birth, nor at any other time during the course of this study. AGD is sexually dimorphic, with males having a larger AGD than females in various rodents (Ryan & Vandenberg, 2002). In females, the AGD is used as a proxy for inferring intrauterine position, as females that develop in the uterus between two males have a larger AGD than females that develop in the uterus between two females. This effect is largely restricted to females, as androgens diffusing from a male fetus affect a neighboring female fetus, but not a male fetus, because males produce testicular androgens (Ryan & Vandenberg, 2003).

Groups

Females were mated with a breeder male for 1 week. At birth, designated postnatal day zero (PND 0), the sex of the pups was determined, and the ratio of the litter was recorded as one of the following: (a) an equal (E) litter, containing equal numbers of males and females or no more than one individual more of one sex than the other (e.g., 6 males and 7 females in one litter); (b) a male-biased (MB) litter, containing at least 50% more males than females (e.g., 9 males and 5 females); or (c) a female-biased (FB) litter, containing at least 50% more females than males (e.g., 5 males and 9 females).

After identifying the sex ratio of each litter at birth (which reflects the prenatal sex ratio of the litter), we randomly assigned the mothers (dams) and their litters to one of three postnatal sex-ratio categories by culling each litter (to 8 pups) to produce one of three compositions. Each reconstituted litter was then defined as (a) an equal (E) litter, containing equal numbers of male pups and female pups (i.e., 4 males and 4 females); (b) a male-biased (MB) litter, containing 3 times as many males as females (i.e., 6 males and 2 females); or (c) a female-biased (FB) litter, containing 3 times as many females as males (i.e., 2 males and 6 females). In cases in which the dam gave birth to an insufficient number of males or females to complete the necessary postnatal sex ratio, foster pups from other litters born on the same day were added to the litter to fulfill the ratio requirement. The dam and the litter were then placed back in the transparent maternal observation cage, as described in the section on maternal-behavior testing.

Figure 1 depicts our experimental design and illustrates how prenatal and postnatal litter sex ratios were controlled. The nine experimental groups comprised different combinations of prenatal and postnatal conditions (total number of litters = 88): (a) MB prenatal/MB postnatal (MB/MB; $n = 11$), (b) MB prenatal/E postnatal (MB/E; $n = 10$), (c) MB prenatal/FB postnatal (MB/FB; $n = 9$), (d) E prenatal/MB postnatal (E/MB; $n = 10$), (e) E prenatal/E postnatal (E/E; $n = 10$), (f) E prenatal/FB postnatal (E/FB; $n = 11$), (g) FB prenatal/MB postnatal (FB/MB; $n = 10$), (h) FB prenatal/E postnatal (FB/E; $n = 6$), and (i) FB prenatal/FB postnatal (FB/FB; $n = 11$).

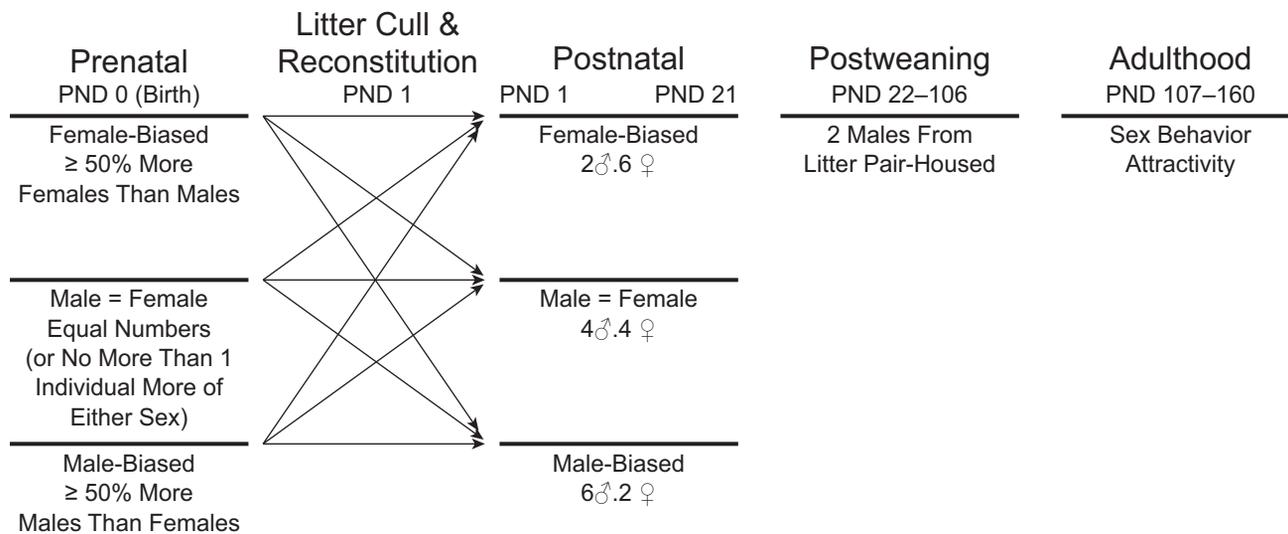


Fig. 1. Diagram of the experimental design used to determine the effects of prenatal and postnatal sex ratios on adult sexuality. The top row indicates the four experimental stages: prenatal-intrauterine (three conditions at postnatal day zero, PND 0, or birth), postnatal (three conditions, from PND 1 to PND 21), postweaning, and adulthood. The three prenatal litter conditions were as follows: female-biased, equal numbers of males and females, and male-biased. On PND 1, prenatal litters were culled to create three postnatal litter compositions: female-biased (2 males and 6 females), equal (4 males and 4 females), and male-biased (6 males and 2 females). Arrows indicate the nine combinations of prenatal and postnatal conditions. At weaning, animals were housed in pairs with another animal in the same group until adulthood and testing. One male from each litter was tested for sexual behavior in adulthood.

Maternal-behavior testing

To assess the effect that maternal behavior directed toward the pups has on the offspring's sexual behavior in adulthood, we observed each dam's behavior in a transparent maternal observation cage (51 cm long × 40.5 cm wide × 21 cm high), which also served as the home cage for the dam and her litter following birth (see Lonstein & Fleming, 2002, for further details). As our research focus was on the influence of the mother's behavior on the behavior of her offspring in adulthood, we did not record either interactions between the pups or the pups' responses to the dam. As the unit of measure was the litter, focal animal observations were conducted. In order to determine maternal behavior toward individual pups, we applied food coloring (Club House, McCormick Canada, London, Ontario, Canada) on the backs and sides of the pups each day (just prior to maternal testing) with a paintbrush. The 2 male pups of interest were marked with blue dye, 2 female pups were marked with red dye, and the remaining pups in the litter were marked with yellow dye.

Fifteen-minute retrieval tests were completed every 2 days between PND 2 and PND 12. Retrieval tests were conducted using a computer-event recorder (IBM PC-XT, IBM, Thousand Oaks, CA) and the BEST (Behavioral Evaluation Strategy and Taxonomy) software (Sharpe & Koperwas, 1999) to determine the frequency and duration of the following behaviors: (a) hovering (the dam sat over the pups while she engaged in some other behavior), (b) low crouching (the dam remained motionless over the pups with her back slightly arched), (c) high crouching (the dam was situated rigidly over the pups with a high arched back), (d) retrieving a male pup after the pups had been reintroduced to the cage (the dam picked up a

blue male with her mouth and carried the pup to the nest quadrant), (e) body licking a male pup (the dam licked a blue male pup everywhere, excluding the anogenital region), (f) anogenital licking a male pup (the dam licked the anogenital region of a blue male pup), and (g) mouthing a male pup any time after the initial pup retrieval (the dam picked up a blue male pup using her mouth and then transferred it from one area of the nest to another).

Weaning

On PND 21, all pups were separated from the dam. The 2 blue males from each litter were pair-housed. These males were housed in the same way and in the same conditions as the mother rat had been prior to parturition.

Sexual-behavior testing of adult males

Housing. Between PND 99 and PND 106, all males were placed in a reverse 12-hr light/dark cycle, that is, 12 hr of dark beginning at 8:00 a.m., followed by 12 hr of light beginning at 8:00 p.m. This cycle was used as a preparation for testing of sexual behavior, as this type of behavior occurs more readily during the dark portion of the light/dark cycle (Agmo, 1997). Animals were maintained in the reversed light cycle throughout the testing period, which started at least a week after they had been first placed into that cycle, starting on PND 120, and through PND 160.

Apparatus. Sexual behavior was tested in a bilevel Plexiglas chamber that was 72 cm long by 62 cm high by 17 cm wide.

The upper and lower platforms measured 41 cm by 15 cm by 2.5 cm, and were separated by an area measuring 13.5 cm in length by 8 cm in width. Two removable trays with wire mesh were located on each platform. Ramps measuring 15 cm in length by 7 cm in width (top ramp) and 13.5 cm in length by 7 cm in width (bottom ramp), as well as landings, were located at the ends of the platforms.

Stimulus female rats. Ninety-eight virgin females (60 days old) were ovariectomized (OVX) under anesthesia (isoflurane; Baxter Corporation, Mississauga, Ontario, Canada) using a single medial dorsal incision. All stimulus females were raised in mixed litters of unknown sex ratios. After a 1-week recovery period, females were housed in pairs and placed in the same reverse-light-cycle conditions as the experimental males, as described in the Housing section. Sexual receptivity in the OVX females was induced via subcutaneous injections of estradiol benzoate (10 μ g in 0.1 ml of sesame oil) 48 hr prior to testing and injections of progesterone (500 μ g in 0.1 ml of sesame oil) 3 to 4 hr prior to testing. This routine replicated the natural estrous cycle in rats. The OVX females were habituated to the bilevel chambers for 15 min each day for 3 days and received seven trials of sexual experience with stud males in the bilevel chambers at 4-day intervals to ensure that they were sexually experienced. Females were randomly paired with the experimental males during sexual-behavior testing.

Handling and habituation. Experimental males were habituated to the bilevel chamber for 15 min and handled for 5 min each day for the 4 consecutive days prior to their first sexual encounter with the females. Habituation to the testing apparatus and procedures is important because a novel environment disrupts sexual behavior in naive male rats (Pfaus & Wilkins, 1995). The bilevel chamber was cleaned with 70% alcohol between habituation periods.

Behavioral testing. Beginning on approximately PND 120, males were tested every 4 days, receiving a total of 10 trials of sexual experience. On each test day, only females that displayed proceptive-receptive behaviors (i.e. hop-darts, ear wiggling, and lordosis) toward a stud male were used for sexual testing.

Males were placed alone on the lower platform of the bilevel chamber for a 5-min acclimation period and motivation check before the female was introduced into the chamber. After the acclimation period, the female was placed in the chamber on the opposite platform to where the male had moved at that time. Each sexual test lasted for 30 min. A DCR-TRV33 video camera (Sony, Whitby, Ontario, Canada) was used to videotape the sexual test, which was coded at a later time. Once the test period was over, the female was removed from the chamber first, and then the male was removed. The bilevel chambers were cleaned with 70% alcohol between tests. All animals were placed in their home cages and returned to the housing room following testing.

Coding of behaviors. Sexual-behavior tests were coded using an IBM PC-XT computer-event recorder (using BEST software, Educational Consulting, Thousand Oaks, CA). Behavioral measures for both experimental males and stimulus females were analyzed. For males, the duration and frequency of the following behaviors were determined: (a) self-grooming (the male licked his body, including genital region); (b) anogenital sniffing (the male placed his snout on or near the female's anogenital region); (c) aggression (any type of attack against the female, including biting, dragging, punching, or kicking); (d) mounting (the male climbed onto the female's back and clasped her flanks with no thrusting); (e) attempted mounting (the male abandoned a mount, or the female escaped from the grasp of the male before he could properly mount); (f) intromissions (a mount immediately followed by pelvic thrusting); (g) ejaculations (a mount followed by an intromission, but the male remained on the female for a longer period than in a normal mount and convulsed), or the male remained in position and moved slowly, while the female moved away); and (h) level change (the male switched between upper and lower platforms, with a level change consisting of the male placing all four paws on the platform not previously occupied).

The frequency and duration of the following behaviors in stimulus females were collected: (a) hop-dart (small jumps on one spot or a short, quick run, after which the female temporarily froze with her back to the male), (b) solicitation (the female approached the male and after obtaining his attention, ran away, temporarily freezing with her rump oriented to the male and her head turned toward the male), (c) lordosis (the female had an arched back and remained briefly in that position following palpitation of the flanks by the male), (d) defensive action (the female turned onto her back while punching and kicking the male when he approached), (e) aggressive action (the female stood on her hind legs and boxed with the male), and (f) level change (the female switched between the upper and lower platforms, with a level change consisting of the female placing all four paws on the platform not previously occupied).

Statistical analysis

We studied the average frequencies and durations of maternal behaviors across all days by carrying out analyses of variance (ANOVAs). We determined differences in maternal behavior toward male pups via 3 (prenatal sex ratio) \times 3 (postnatal sex ratio) ANOVAs conducted on the averages of all maternal behaviors toward male pups and female pups. All male and female sexual behaviors in Experimental Trials 1, 5, and 10 were examined with 3 (day) \times 3 (prenatal sex ratio) \times 3 (postnatal sex ratio) repeated measures ANOVAs. Analyses were conducted using all subjects, including those that did not exhibit mounting.

To determine whether mothers' maternal behavior or the adult sexual behavior of male offspring differed as a function of whether the litter from which the males were derived was consistent from the prenatal period to the postnatal period (i.e., with the same prenatal and postnatal sex ratios) or was

inconsistent (i.e., different prenatal and postnatal sex ratios), we carried out additional analyses on all relevant maternal and sexual behaviors. We conducted a *t* test to compare consistent groups with inconsistent groups on total maternal behaviors and a 2 (consistent vs. inconsistent) \times 3 (Trial 1 vs. Trial 5 vs. Trial 10) analysis of the sexual behaviors.

To determine whether maternal behavior is related to sexual behavior, we carried out Pearson correlations relating maternal behavior to adult male sexual behavior. Two major maternal behaviors—total licking (body and anogenital combined) duration and total crouching (high and low combined) duration (averaged across all six maternal behavior tests)—were correlated with male sexual behaviors, including frequencies of mounts, intromissions, and ejaculations at each of the three time points (Trials 1, 5, and 10 of testing). Bivariate correlations using prenatal and postnatal sex ratios as covariates did not change the significant correlations. In addition, for all analyses, *p* values accepted as significant were corrected for multiple testing using the Benjamini and Hochberg false discovery rate (Benjamini & Hochberg, 1995).

Results

Sexual behavior

Sexual behavior in experimental males. We found no significant effect of prenatal sex ratio for any sexual behaviors in males. When all males were included in the analyses, the only

differences we observed were between postnatal-sex-ratio groups, and these effects were primarily on mounting behaviors. There was no difference in the number of males that mounted a female either in the initial test or in subsequent tests. We obtained the same results even when we excluded nonresponsive males from the analysis. Analysis of the total number of mounts (mounts alone, mounts with intromissions, and mounts with ejaculations) revealed a significant effect of postnatal sex ratio, $F(2, 78) = 4.85, p = .010$, with FB males displaying fewer mounts than either E males or MB males (Fig. 2a). However, we found no differences in the frequency of intromissions (Fig. 2b) or the frequency of ejaculations (Fig. 2c) in the three postnatal-sex-ratio groups. Although there were group differences in total male mounting, we found no difference in mating efficiency (the ratio of ejaculations to the sum of mounts plus intromissions) across the postnatal-sex-ratio groups (Fig. 2f). We did find an increase in the frequency of mounts, intromissions, and ejaculations in all groups across the three time periods, with a main effect of number of trials—mounting: $F(2, 156) = 9.076, p = .000$; intromissions: $F(2, 156) = 5.45, p = .005$; and ejaculations: $F(2, 156) = 77, p = .000$. We found no statistically significant differences for any other male sexual behaviors or for any of the adult sexual behaviors as a function of whether the prenatal sex ratio and postnatal sex ratio were consistent or inconsistent.

Sexual behavior of stimulus females. Analysis of the proceptive and receptive behaviors of females toward the males

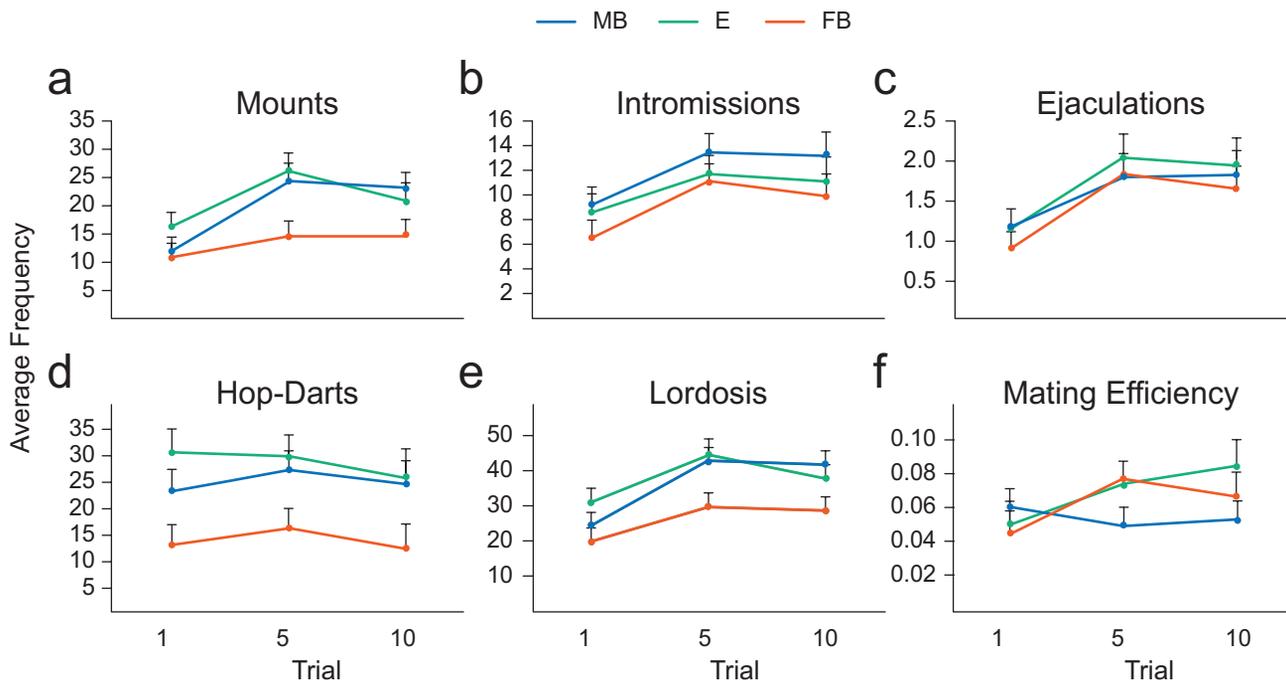


Fig. 2. Mating behavior of male rats (a–c, f) and their stimulus partner females (d–e). The males were reared in litters of three different sex ratios: male-biased (MB; 6 males and 2 females), equal numbers of males and females (E; 4 males and 4 females), and female-biased (FB; 2 males and 6 females). Each panel shows the mean frequency of mating behavior of males or of the ovariectomized, hormonally primed female rats with which they were tested over three trials (1, 5, and 10). Results are shown for (a) mounts, (b) intromissions, (c) ejaculations, (d) hop-darts, (e) lordosis, and (f) mating efficiency (the ratio of ejaculations to the sum of mounts plus intromissions). Error bars indicate 1 SEM.

revealed no significant effect of prenatal sex ratio and no significant interaction between prenatal sex ratio and postnatal sex ratio. However, we observed postnatal-sex-ratio effects on hop-darts and solicitations combined (designated hop-darts), $F(2, 78) = 7.05, p = .002$ (Fig. 2d), and lordoses, $F(2, 78) = 5.47, p = .006$ (Fig. 2e). Post hoc Tukey significance tests showed that females exhibited fewer hop-dart and solicitation behaviors as well as fewer lordoses when paired with FB males than when paired with E males or MB males.

Maternal behavior

To determine whether males and females within a litter received different amounts of maternal licking as a function of prenatal sex ratio and postnatal sex ratio, we conducted a 2 (sex) $\times 3$ (prenatal sex ratio) $\times 3$ (postnatal sex ratio) ANOVA on all licking behaviors (both anogenital and body) directed at all designated male pups and all designated female pups. The duration of licking behaviors was averaged across the six maternal-behavior tests (Days 2, 4, 6, 8, 10, and 12 postpartum). We found a main effect of gender: Male pups were licked more than female pups, $F(1, 73) = 5.80, p = .018$. We also found a main effect of prenatal sex ratio on overall licking, $F(2, 73) = 3.90, p = .024$: Pups in MB litters received the most licking, and pups in E litters received the least amount of licking. There was no effect of postnatal sex ratio on licking. The absence of significant interactions for prenatal sex ratio, postnatal sex ratio, and sex indicates that males were not licked proportionately more than females in any prenatal or postnatal condition. Other maternal behaviors did not differ as a function of prenatal sex ratio or postnatal sex ratio. None of the maternal behaviors differed as a function of whether prenatal sex ratio was consistent or inconsistent with postnatal sex ratio.

Relation of maternal behavior to sexual behavior

We found a significant relation between total time the dam spent crouching over the litter of pups (a combination of both high and low crouching durations) and (a) male mounting frequency in Trial 1, $r = .287, p = .01$; (b) male ejaculation frequency in Trial 1, $r = .377, p = .001$ ($p = .006$ after correction); and (c) male ejaculation frequency in Trial 5, $r = .344, p = .002$ ($p = .018$ after correction). After correcting for false discovery rate, we did not find any significant correlations between maternal licking and any of the male sexual behaviors.

Discussion

Litter composition at birth in mammals usually reflects the sex ratio of the prenatal litter, but this does not mean that the prenatal and postnatal periods do not have specific effects on sexual development. It is also possible that the cumulative developmental process before and after birth has unique

emergent properties on subsequent behavior later in adulthood that are unknown. According to Mayr (1988), such emergence can occur “when two entities are combined at a higher level of integration, not all the properties of the new entity are necessarily a logical or predictable consequence of the properties of the components” (p. 34). Research demonstrates that the intra-uterine sex ratio in rats that influences adult behavior does not take into account the postnatal sex ratio of the litter (Clark, Bone, Bennett, & Galef, 1989; Kinsley, Konen, Miele, Ghiraldi, & Svare, 1986). In addition, studies in rats demonstrating that the sex ratio of the postnatal litter influences maternal behavior do not take into account the prenatal sex ratio of the pregnant mother’s litter (Hard & Larsson, 1968; Moore & Morelli, 1979; Richmond & Sachs, 1984).

In our study, we examined the effects of prenatal and postnatal conditions and sex ratios separately, deconstructing the progression of developmental experiences, and showed that the postnatal sex ratio of a rat litter affects mounting behavior of the litter’s males in adulthood. In contrast to the overall licking behavior of the mother toward the neonates, which was clearly affected by sex ratio of the birthed litter, the sexual behaviors of the pups in adulthood were influenced more by the litter composition (the number of male and female littermates) than by the prenatal litter ratio. Moreover, although we did not observe any significant differences in maternal behavior as a function of postnatal sex ratio, we found that when we correlated maternal behavior with the sexual behavior of male offspring, mothers who had crouched over their litters for longer periods of time during the first 10 postnatal days analyzed had male pups who as adults engaged in more mounting, intromitting, and ejaculating behaviors, especially on their first sexual encounter (i.e., when they were sexually inexperienced). We did not observe any significant correlations between maternal licking and male sexual responses after we statistically corrected our data for multiple testing.

Why more maternal crouching enhances the male sexual behavior of offspring when they mature into adults is not clear. However, it is likely that males that spent more time as pups being nursed and attended to by the mother also received more maternal stimulation, in the form of being moved within the nest, and nuzzling under the mother’s ventrum. The possibility that the effect we observed was related to being licked or groomed by the mother is not likely, as we did not see any significant link between maternal licking and the sexual behavior of adult offspring. Our finding that males raised with more female littermates are less active sexually and show the least increase in sexual activity with more sexual experience points to the importance of the postnatal environment on adult sexual behavior. However, when we considered only ejaculation and mating efficiency as indicators of reproductive success, males raised with more female littermates did not differ significantly in adult sexual behavior from males raised in litters with equal numbers of males and females, or litters with more males than females. One explanation for this finding may be that females find these males less attractive, and solicit

them less than males raised in litters with other sex ratios. As a result, these males mount females less frequently than other males.

The causal direction of the relationship between male sexual behavior and attractiveness cannot be ascertained in this study. It is evident that in rats, litter composition, and particularly being raised with many females, alters the response of the male in adulthood toward females as sexual stimuli. Our results also suggest that these males are less attractive to females. How the various developmental experiences summate remains to be determined. For example, it is possible that such males produce different cues or behaviors as a function of their early experience with many females and few males. A range of factors, such as odor cues (Kannan & Archunan, 1997; Lopez, Olster, & Ettenberg, 1999; Moore, Jordan, & Wong, 1996) and ultrasonic vocalizations in the form of mating calls (Burgdorf et al., 2008), affect the attractiveness of sexual partners. Males reared in a female-biased litter may be less attractive to females because they secrete less or different odor cues, or they may exhibit less sexual interest in females by emitting fewer ultrasonic vocalizations and approach responses. Another hypothesis is that males reared with too many females find them less attractive because of reduced novelty, as there is evidence that novelty can enhance the expression of copulatory behavior in male rats (Pfaus & Wilkins, 1995). We hope that our results will stimulate further research in this area.

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Declaration of Conflicting Interests

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

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