Uterine Positions and Schedules of Urination: Correlates of Differential Maternal Anogenital Stimulation

MERTICE M. CLARK
SUZANNE BONE
BENNETT G. GALEF, JR.
Department of Psychology
McMaster University
Hamilton, Ontario Canada

Lactating Mongolian gerbils, like lactating Norway rats, reliably lick some pups in their litters more than they lick others. Male gerbil pups are licked more by their dams than are their sisters and some males and some females within each litter are licked more often than are their sibs of the same sex. In the present article, we explore the characteristics of same-sex littermates that are correlated with elicitation of extreme amounts of maternal anogenital licking. We found that both the male pup and the female pup in a litter of gerbils that received the most maternal anogenital licking: (1) released greater quantities of urine and (2) exhibited longer latencies to begin to urinate in response to artificial anogenital stimulation than did the male pup and the female pup in a litter that received the least amount of maternal anogenital licking. We also found that foster mothers rearing Caesarean-delivered litters spent more time licking the anogenital areas of: (1) those male pups that, as fetuses, had occupied uterine locations adjacent to relatively few females and (2) those female pups that, as fetuses, had occupied uterine locations adjacent to relatively many males. We discuss implications of these findings for understanding of how maternal behavior may mediate hormonal effects on the development of young gerbils.

Maternal rats provide both more frequent and longer bouts of anogenital licking to their male than to their female young (Birke & Sadler, 1987; Moore & Morrelli, 1979; Richmond & Sachs, 1984). Differences in the stimulation afforded rat dams by their male and female pups have been identified that may be

Reprint requests should be sent to (until 21 August 1989) Mertice M. Clark, Department of Psychology, Campus Box 345, University of Colorado, Boulder, CO 80309, U.S.A.; (after 21 August 1989) Mertice M. Clark, Department of Psychology, McMaster University, Hamilton, Ontario, L8S 4K1, Canada

Received for publication 15 August 1988
Revised for publication 31 October 1988
Accepted at Wiley 28 December 1988

Developmental Psychobiology, 22(4):389–400 (1989)

CCC 0012-1630/89/040389-12$04.00

responsible for some of the variance in the amount of maternal attention directed to rat pups of each sex. First, male rat pups produce more attractive urine than do their sisters (Moore, 1981). Second, male rat pups require more perineal stimulation before beginning to urinate and males release larger quantities of urine in response to artificial perineal stimulation than do female rat pups (Clark & Galef, 1989). Such findings are consistent with the view that odors carried in the urine of male rat pups orient licking by their dams towards male pups rather than their sisters (Moore, 1981) and that the schedules of urine release exhibited by male rat pups are more likely to maintain a bout of maternal anogenital licking than the schedules of urine release shown by their female sibs (Clark & Galef, 1989).

In the present series of studies, we first established that male gerbil pups, like male rat pups (Moore & Morelli, 1979), are the recipients of more maternal, anogenital licking than are their female sibs. We then demonstrated that, in response to artificial, perineal stimulation, male gerbil pups, like male rat pups (Clark & Galef, 1989), deliver urine on a schedule that would tend to induce more prolonged bouts of licking by their respective dams than the schedule of urine production exhibited by female gerbil pups in response to similar stimulation. Having established differences in the interactions of male and female gerbil pups with their dams similar to those previously reported in rats, we then explored factors influencing the amount of maternal, anogenital licking received by gerbil littermates of the same sex.

Experiment 1

The first experiment was undertaken to establish that in gerbils (*Meriones unguiculatus*), as in rats (*Rattus norvegicus*), male and female pups both receive different amounts of anogenital stimulation from their dams and respond differently to artificial perineal stimulation. The present experiment is reported below as two studies: Study 1 was concerned with observation of distribution of maternal, anogenital licking within litters of young gerbils and Study 2 with responses of gerbil pups to artificial stimulation of the perineal area.

Methods

Subjects and Rearing Conditions

Subjects were litters born to 48, nulliparous, female Mongolian gerbils (*Meriones unguiculatus*) born in the McMaster vivarium to stock acquired from Tumblebrook Farm (Brookfield, MA). At 90 to 100 days of age, each nulliparous female was introduced into the cage of an adult male of proven fertility. These breeding pairs were housed in polypropylene cages (35 × 30 × 15 cm), lidded with hardware cloth (1.27 cm), and carpeted with a thin layer of wood-chip bedding (Betta-chip, Northwestern Products Corp., Warrensburg, NY).

Each breeding pair was examined daily and, when a female was visibly pregnant (e.g., during her last week of pregnancy), her mate was removed from

her cage. Cages containing pregnant females were examined daily (1000 hr) to determine dates of parturition. On the day of birth of a litter (Day 1), each litter was culled to two male and two female pups and each pup in a litter was toe-clipped for permanent, individual identification.

The colony was maintained on ad lib Purina Laboratory Rodent Chow and water in a single temperature-controlled colony room illuminated from 0700 to 1900 hr.

Procedure

Study 1: Observation of maternal, anogenital licking (22 litters). Before beginning to observe a litter, the experimenter numbered the pups in that litter with a black, water-soluable, felt-tip pen (Markette Thinrite marker, Eberhard Faber, Canada), the markings from which disappeared within 48 hr. Across test sessions, the number assigned to each pup varied unpredictably, so an observer could record data while unaware of the sex or identity of individual pups. Upon completion of a period of observation, the identities of individual pups were established by reference to each pup's clipped toes.

Maternal, anogenital licking was observed in each dam's homecage for 20 min on each of Days 5, 9, 13, 17, and 21 postpartum. The observer recorded the amount of time the dam spent licking the perineal region of each numbered pup. At the end of each 20-min session of observation, the permanent toe-clip associated with the temporary number assigned each pup was determined by the observer, so that individual pups could be followed longitudinally.

Study 2: Pup response to artificial, perineal stimulation (26 litters). Using the procedures described in detail in Clark and Galef (1989), both the latency of pups to begin to urinate in response to artificial stimulation of the perineal area and the amount of urine produced by pups in response to a fixed period of stimulation were determined on each of Days 5, 9, 13, 17, and 21 postpartum. In brief, a litter was removed from its dam and each pup was placed in a separate 7.5 × 7.5 × 7.5 cm cubicle floating in a constant-temperature bath maintained at 30°C. Twenty min later, the bladder of each pup was voided of urine by the experimenter. To void a pup of urine, it was held ventral-side up for 10 sec and its anogenital area was then stroked with a piece of blotting paper for 30 sec, at a rate of 3 strokes/sec. Each pup was then returned to its cubicle and 1 hr later its bladder was again voided of urine by an experimenter using a piece of blotting paper (.7g) weighed to the nearest mg. The experimenter recorded both the latency of each pup to begin to urinate (any urine produced was readily observable as a dark stain on the blotting paper) and, at the end of the 30-sec period of stimulation, the weight of urine released by the pup.

Statistical Analysis

To control for litter effects, litter mean scores, rather than data from individual subjects, were used in all statistical analyses. Data from both studies were analyzed using a two-factor ANOVA, with age at testing as a repeated measure.

Results

Study 1

The main results of Study 1 are presented in Figure 1, which shows the mean amount of maternal, anogenital licking received by male and female gerbil pups during each of the five, 20-min sessions of observation. As can be seen in Figure 1, male pups received more anogenital licking than did their female sibs. A MANOVA indicated that differences in anogenital licking due both to pup sex ($F[1,21] = 26.10$, $p < .001$) and to pup age at testing were significant ($F[4,84] = 4.02$, $p < .005$). Differences in dams' licking of male and female pups of different ages resulted in a significant interaction between pup sex and pup age at testing ($F[4,84] = 3.69$, $p = .008$).

Study 2

Figure 2 presents data describing the mean latencies to urinate (Panel A) and mean amounts of urine produced (Panel B) by male and female pups on each day of testing. As can be seen in Figure 2, and as statistical analyses confirmed, male pups took longer to begin to urinate ($F[1,25] = 17.12$, $p < .001$) and produced larger amounts of urine ($F[1,25] = 18.94$, $p < .001$) than did their sisters. With increasing age, latencies of both males and females to urinate decreased ($F[4,100] = 17.92$, $p < .001$), while the quantity of urine produced by both males and females increased ($F[4,100] = 15.15$, $p < .001$).

Discussion

The results of Experiment 1 essentially repeat in Mongolian gerbils, observations previously reported in Norway rats: Male gerbil pups received more

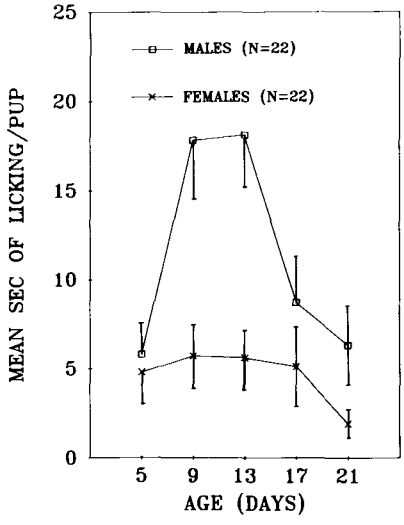

Fig. 1. Mean time spent by dams anogenital-licking male and female pups during 20-min observation periods. Flags = ±1 SEM.

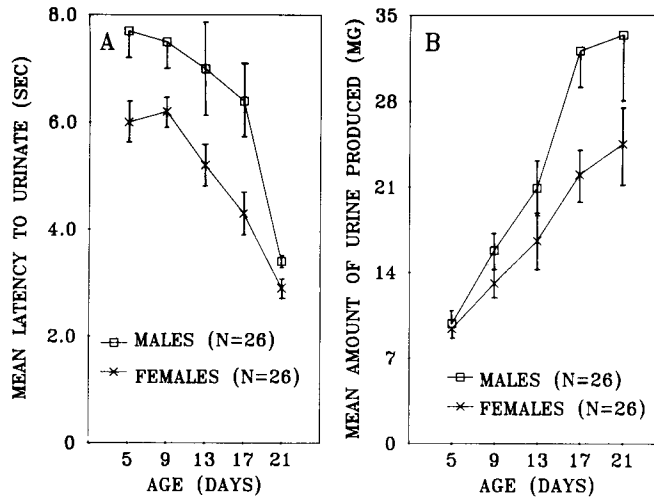

Fig. 2. Mean latency to urinate (Panel A) and mean amount of urine produced (Panel B) by male and female pups during 20 sec of stimulation. Flags = ±1 SEM.

anogenital licking than did their female sibs and, in response to an artificial perineal stimulus, male gerbil pups both took longer to begin to urinate and produced more urine than did their female sibs.

Although the main purpose of Experiment 1 was to provide a context within which to study in Mongolian gerbils factors resulting in differential elicitation of maternal, anogenital licking within sexes, the present findings are in themselves of some interest.

Mongolian gerbils (*Meriones unguiculatus*) and Norway rats (*Rattus norvegicus*) are members of different subfamilies (the *Cricetidae* and *Muridae*) of the suborder of Myomorph rodents. While there exists, so far as we know, little information on comparative, behavioral development of mammals at the level of detail reported here, the present results suggest that some compelling communalities in mother-young interaction may exist across species. It would, for example, be interesting to know whether greater anogenital stimulation of males is characteristic of Myomorph rodents, of rodents generally, or of mammals as a class. As the taxonomic breadth of a behavioral trait increases, so does the probability that the trait is functionally significant. The present data suggest that preferential anogenital stimulation of males may be widely distributed, at least in Old World rodents.

Experiment 2

The results of Experiment 1 established that male gerbil pups, like male rat pups, receive more anogenital stimulation than do their female sibs. In the course of conducting Experiment 1, we noticed that not only were male pups licked more than their sisters, but also that, within litters, across days, some individual males and some individual females were the recipients of far more maternal attention than were their siblings of the same sex. Because we had found that both

relatively long latencies to initiate urination and the production of relatively large amounts of urine in response to perineal stimulation were characteristics associated with males, the sex receiving the greater amount of maternal attention, we hypothesized that these characteristics might also be associated with those individuals within a sex that received disproportionate maternal stimulation.

Methods

Subjects and Rearing Conditions

Fourteen nulliparous, female Mongolian gerbils that gave birth to litters containing three or more pups of each sex and their young served as subjects in the present experiment. Both breeding of dams and rearing and maintenance of young were carried out as described in Methods of Experiment 1, except that each litter in the present experiment was culled to three male and three female pups on the day of parturition. In four litters, two or more pups of one sex died before reaching 16 days of age. This left us with 10 litters containing at least two male pups, 12 litters containing at least two female pups, and 10 litters containing at least two male and two female pups to use as subjects in the present experiment.

Procedure

Maternal, anogenital licking was observed on Days 1, 3, 5, 7, 9, 11, 13, and 15 postpartum and both the latency of each pup to urinate and the amount of urine it produced in response to artificial, perineal stimulation was determined on alternate days from Day 2 to Day 16 postpartum. The procedures used to determine the amount of maternal, anogenital licking and the parameters of response to artificial stimulation of the perineal area were, respectively, those describe in Procedures of Studies 1 and 2 of Experiment 1.

Data Analysis

We first determined the identity of the male pup and the female pup within each of our 14 litters that received either the greatest or the least number of seconds of anogenital licking during the 8 days of observation. We then compared the responses to artificial, perineal stimulation of the male and the female within each litter that received the greatest and least amounts of maternal, anogenital stimulation.

Results and Discussion

The main results of Experiment 3 are presented in Figures 3 and 4, which show, respectively, (Panel A) the mean latencies to urinate and (Panel B) the mean amounts of urine produced in response to artificial, perineal stimulation by that male pup (Fig. 3) and that female pup (Fig. 4) in each litter that received the most and the least amount of anogenital stimulation from their respective dams.

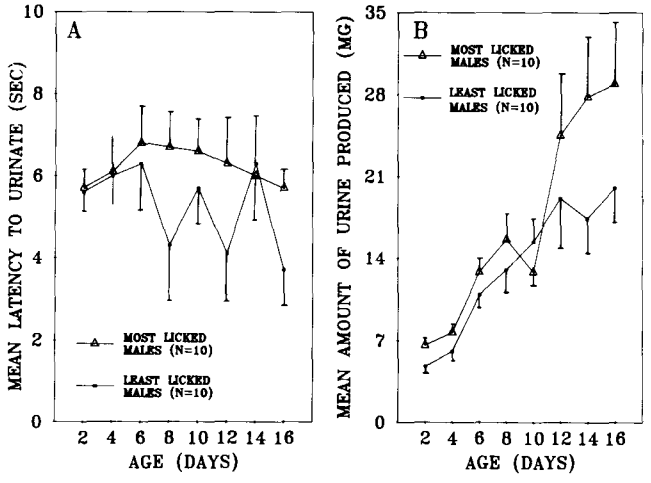

Fig. 3. Mean latency to urinate (Panel A) and mean amount of urine produced (Panel B) by male pups receiving the most and least amounts of anogenital-licking by dams. Flags = ±1 SEM.

As can be seen in Figures 3 and 4, the relative amount of maternal anogenital stimulation received by a pup was related both to its latency to urinate and to the amount of urine it produced in response to artificial, perineal stimulation. On average, the male in each litter that received the most maternal stimulation had both a longer latency to urinate ($F[1, 9] = 6.99$, $p = .027$) and released a larger quantity of urine ($F[1, 9] = 11.13$, $p = .009$) than the least maternally-stimulated male. Similarly, the female pup in each litter licked most by its dam required more stimulation by the experimenter before starting to urinate ($F[1, 11] = 7.95$, $p =$

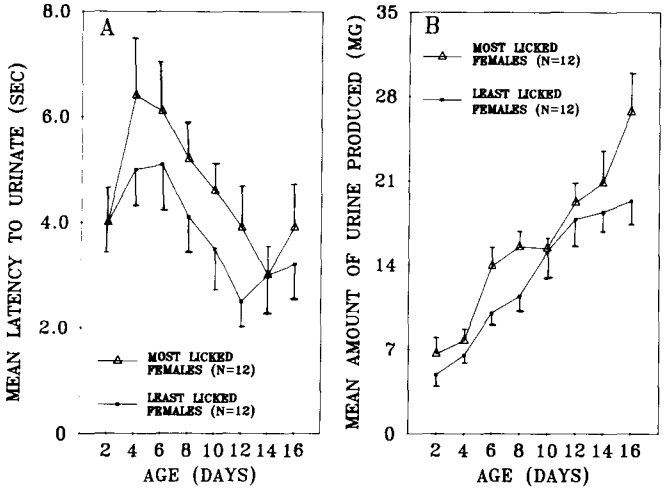

Fig. 4. Mean latency to urinate (Panel A) and mean amount of urine produced (Panel B) by female pups receiving the most and least amounts of anogenital-licking by dams. Flags = ±1 SEM.

.017) and produced a larger volume of urine ($F[1,11] = 10.88$, $p = .007$) than did the female pup in each litter receiving the least amount of anogenital stimulation from its dam.

As in Experiment 1, male pups in the present experiment received more maternal anogenital licking ($F[1,13] = 7.33$, $p = .018$) and, in response to artificial stimulation, both exhibited longer latencies to urinate ($F[1,13] = 37.5$, $p < .001$) and produced larger quantities of urine ($F[1,13] = 9.17$, $p = .01$) than did their female sibs.

The results of the present experiment indicate that preferential anogenital licking within a sex, like preferential anogenital licking between sexes, is related both to differences in the latencies of individuals to urinate and differences in the amounts of urine produced by individuals. Those male and female pups that required relatively little stimulation before urinating and that released relatively small volumes of urine were recipients of relatively little maternal, anogenital stimulation.

Experiment 3

The results of Experiment 2 demonstrated that some male and some female pups within a litter received disproportionate amounts of anogenital licking from their dams. In other words, mothers treated some female pups more like males than their sisters and some male pups more like females than their brothers. Those males treated by their dams like females responded to artificial stimulation of their perineal region in a more feminine fashion than other males. Those females treated by their dams like males responded to perineal stimulation in a more masculine fashion than other females. Thus, the results of Experiment 2 are consistent with the view that differences in the amount of anogenital licking that pups received reflected the fact that both some males and some females were more masculinized during development than others of their sex.

The uterine position of female rodents relative to males has been shown previously to affect many morphological and behavioral traits, particularly those traits influenced by early exposure to androgen (Clark & Galef, 1988; Clemens, Gladue & Coniglio, 1978; Gandelman, vom Saal & Reinisch, 1977; Hauser & Gandelman, 1983; Meisel & Ward, 1981; vom Saal, 1981; vom Saal & Bronson, 1980; vom Saal & Moyer, 1985). Those females that are located *in utero* adjacent to either one or two males (1M or 2M females, respectively) tend to be masculinized relative to their sisters that are located *in utero* next to no males (OM females).

In the present experiment, we asked whether those female gerbil pups that matured *in utero* adjacent to relatively few males (and, therefore, were exposed to less androgen and were less masculinized during fetal life than their sisters) received less maternal, anogenital licking than did those females that matured *in utero* adjacent to relatively more males and were, therefore, more masculinized than their sisters. We also examined male pups to determine whether those males that matured *in utero* adjacent to relatively more females were less likely to receive maternal anogenital stimulation than were their brothers that developed *in utero* adjacent to relatively few females.

Methods

Subjects and Rearing Conditions

Twenty-six litters of Mongolian gerbils, Caesarean delivered from nulliparous females taken from the McMaster colony, served as subjects. Twenty-six additional females from the same source served as foster mothers to Caesarean-delivered young.

Procedure

Twenty-four days after a female had been observed to copulate, she was anesthetized by ether inhalation, her abdomen opened, her uterus externalized, and her fetuses removed. Both the sex and the uterine location (vom Saal, 1981) of each fetus was recorded as it was removed. Each infant was also toe-clipped for permanent identification and each was then placed in one cubicle of an ice-cube tray floating in a constant-temperature bath maintained at 30°C. Once all pups had been removed from a dam, she was euthanized by anesthetic overdose.

Following delivery, each pup was left floating in its cubicle in the constant temperature bath for 3–4 hr. Each pup was then rubbed with a piece of cotton batting that had first been rubbed on the ventral gland of the female that was to foster rear that pup. Immediately after rubbing, each pup was placed with its foster mother. Foster mothers had vaginally delivered a litter within 24 hr of the time of Caesarean delivery of the litter each was to foster rear.

On each of Days 5, 9, 13, 17, and 21 postpartum, each foster mother and litter were observed for 20 min. During each 20-min observation period, the amount of maternal, anogenital licking received by each pup was recorded. as described in Procedure of Study 1 of Experiment 1.

Statistical Analysis

Evidence that uterine location of a pup, relative to fetuses of the opposite sex, affected a foster dam's anogenital licking was available from examination of litters that, on postdelivery Day 21, contained pups of the same sex that had developed in more than one type of uterine location (for females; 0M, 1M, or 2M; for males; 0F, 1F, or 2F). Litters containing males in two types of uterine location were examined to determine the percentage of litters in which (1) males in 0F locations received more maternal, anogenital licking than did males in either 1F or 2F locations and (2) males in 1F and 2F locations received more maternal anogenital licking than did males in 0F locations. Litters containing females, were examined to determine the percentage of litters in which (1) females in 2M locations were licked more frquently than females in either 1M or 0M locations and (2) females in 1M or 2M locations were licked more than females in 0M locations. If litters contained more than one pup in a particular type of uterine location, means, rather than individual data points, were used for comparisons.

Results

Of the 26 litters examined in the present experiment, 16 contained males in more than one uterine location and 15 contained females in more than one uterine

TABLE 1. Percentage of Litters in which Female Pups that had been Adjacent to more Males in Utero *Received more Maternal Anogenital Licking than Female Pups that had been Adjacent to Fewer Males* in Utero.

Number of Litters	Comparison A		B	Percent of Litters A > B
1	2M females	vs.	0M females	100.0
4	2M females	vs.	1M females	75.0
2*	2M females	vs.	0M and 1M females	100.0
8	1M females	vs.	0M females	87.5

* In those litters in which there were pups in all three uterine locations, 2M females were compared with 0M and 1M females combined to insure independence of all comparisons.

location. The main results of Experiment 3 are presented in Tables 1 and 2, which show, respectively, for males and for females: (Table 1) the percentage of litters in which females developing in uterine locations adjacent to more males were licked more frequently than females developing in uterine locations adjacent to fewer males and (Table 2) the percentage of litters in which males developing in uterine locations adjacent to more females were licked more frequently than males developing in uterine locations adjacent to fewer females.

Examination of the data presented in Tables 1 and 2 reveals that uterine location of a pup, relative to fetuses of the opposite sex, affected the amount of anogenital licking received by a pup. As can be seen in Table 1, in 13 of the 15 litters that contained females in more than one type of uterine location, females that had developed *in utero* adjacent to more males were licked more than females that developed in uterine locations adjacent to fewer females (sign test, $p < .01$). In 13 of 16 litters, males that had developed in uterine positions adjacent to fewer females received more anogenital licking from their dams than did males that had developed in uterine locations adjacent to more females (sign test, $p < .02$). The results of Experiments 3 are, thus, consistent with the hypothesis that prenatal

TABLE 2. Percentage of Litters in which Male Pups that had been Adjacent to fewer Females in Utero *Received more Maternal Anogenital Licking than Male Pups that had been Adjacent to More Females* in Utero.

Number of Litters	Comparison A		B	Percent of Litters A > B
9	0F males	vs.	1F males	67.7
3*	0F males	vs.	1F and 2F males	100.0
4	1F males	vs.	2F males	100.0

* In those litters in which there were pups in all three uterine locations, 0F males were compared with 1F and 2F males combined to insure independence of all comparisons.

exposure of pups to hormonal influences from siblings mediates differences in the amount of maternal, anogenital licking received by pups of the same sex within a litter.

General Discussion

The results of the present series of studies suggest a rather intriguing series of reciprocal interactions between experience and genotype in the development of individual differences in behavior. Apparently, genetic determinants of gender-correlated phenotypic characteristics interact with the uterine environment in which an individual matures to influence its schedule of urination. Schedules of urination influence the amount of maternal, anogenital stimulation an individual receives, and amount of anogenital stimulation influences the expression of phenotypic characteristics (Clark & Galef, 1988; Moore, 1982, 1983).

Although data currently available are not sufficient to demonstrate a causal connection at each link in the chain of events described above, correlations demonstrated, both by us and by others, suggest important causal relationships among genotype, uterine location, infant behavior, maternal care, and adult phenotype. At birth, each juvenile rodent may have already experienced both endogenous and exogenous hormonal stimulation that shapes the maternal care it receives and, thus, the behavioral phenotype that it develops.

Notes

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada to M.M.C. and B.G.G., Jr. from the McMaster University Research Board to B.G.G., Jr.

References

Birke, L. I. A., and Sadler, D. (1987). Differences in maternal behavior of rats and the sociosexual development of the offspring. *Dev. Psychobiol., 20:* 85–99.

Clark, M. M., and Galef, B. G., Jr. (1988). Effects of uterine position on rate of sexual development in female Mongolian gerbils. *Physiol. Behav., 42:* 15–18.

Clark, M. M., and Galef, B. G., Jr. (1989). Male rat pups are more hesitant to urinate in response to anogenital stimulation than are their female sibs. *Dev. Psychobiol., 22:* 81–85.

Clemens, L. G., Gladue, B. A., and Coniglio, L. P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Horm. Behav., 10:* 40–53.

Gandelman, R., vom Saal, F. S., and Reinisch, J. (1977). Contiguity to male fetuses affects morphology and behavior of female rats. *Nature, 266:* 722–724.

Hauser, H., and Gandelman, R. (1983). Contiguity to males in utero affects avoidance responding in adult female mice. *Science, 220:* 437–438.

Meisel, R. L., and Ward, I. L. (1981). Fetal female rats are masculinized by male littermates located caudally in the uterus. *Science, 213:* 239–242.

Moore, C. L. (1981). An olfactory basis for maternal discrimination of sex of offspring in rats (*Rattus norvegicus*). *Anim. Behav., 29:* 383–386.

Moore, C. L. (1982). Maternal behavior of rats is affected by hormonal condition of pups. *J. Comp. Physiol. Psychol., 96:* 123–129.

Moore, C. L., and Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. *J. Comp. Physiol. Psychol., 93:* 677–684.

Moore, C. L. (1983). Maternal contributions to the development of masculine sexual behavior in laboratory rats. *Dev. Psychobiol., 17:* 347–356.

Richmond, G. and Sachs, B. D. (1984). Maternal discrimination of pup sex in rats. *Dev. Psychobiol., 17:* 87–89.

vom Saal, F. S. (1981). Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. *J. Reprod. Fertil., 62:* 397–405.

vom Saal, F. S., and Bronson, F. H. (1980). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science, 208:* 597–599.

vom Saal, F. S., and Moyer, C. L. (1985). Prenatal effects on reproductive capacity during aging in female mice. *Biol. Reprod., 32:* 1116–1126.