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Urine Marking and Maternal Aggression of Wild Female Mice in Relation to Anogenital Distance at Birth

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PALANZA, P., S. PARMIGIANI AND F. S. VOM SAAL. *Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth.* PHYSIOL BEHAV 58(4) 000–000, 1995.—A series of experiments were conducted with wild house mice to verify the effect of intrauterine position on females' anogenital distance at birth (AGD) and to examine the relationships between a female's AGD, used as a bioassay of androgen exposure during fetal life, and her social behavior and reproductive success in adulthood. Experiment 1 showed that cesarean-delivered females that developed in utero between two males (2 M females) have significantly longer AGD's than females positioned between two females (0 M females). We then categorized naturally delivered females shortly after birth as having a long, medium or short AGD. In adulthood, these females were tested for their behavior towards unfamiliar pups, their rate of urine-marking in response to a variety of social stimuli, postpartum aggression and success in protecting their litters in response to male and female intruders. Adult females with different AGD's at birth did not differ either in their behavior toward pups or in their rate of urine marking. Conversely, males housed across a wire mesh partition from a long-AGD female deposited a higher number of urine marks than those exposed to a short-AGD female. When tested after delivering a litter, long-AGD females displayed more tail-rattling (a component of agonistic behavior) towards intruders of both sexes in comparison to short-AGD females. These results are consistent with the hypothesis that females with a long AGD are exposed to higher levels of testosterone during fetal life than females with a short AGD. Although not related to AGD, other measures of maternal aggression were affected by postpartum day, sex of intruders and a female's infanticidal potential as virgin.

Urine marking Wild female mice Anogenital distance Maternal aggression

IN THE house mouse, prenatal exposure to steroid hormones is a potent source of variability for many aspects of a female's anatomy, physiology and behavior (30). The position of a fetus within the uterus, in relation to the sex of its adjacent littermates, influences its exposure to gonadal hormones and therefore its anatomical and psychosexual development, including aggressive behavior. A female mouse fetus located between two males (2 M) receives the greatest testosterone exposure, while an individual between two females (0 M) receives the least; 1 M fetuses, between one male and one female, receive intermediate levels of testosterone and estradiol.

Previous experiments on CF-1 mice have shown that, relative to 0 M females, 2 M females have greater anogenital distances at birth (AGD), are less attractive and arousing to males, have longer estrus cycles, are less active, weigh more, and produce less potent puberty-inhibiting pheromones (reviewed in 29). 2 M female CF-1 mice are more aggressive toward other females than are 0 M females (30). However, in the study of aggression in mice, little attention has been paid to female agonistic behavior and its potential role in the regulation of social organization. Recent studies have shown that females compete among themselves for the opportunity to reproduce and can play an important role in determining social dynamics throughout aggression and infanticide (14,34).

Contrary to findings with domestic stocks of mice, most wild female mice exhibit infanticide as virgins, and this behavior is related to intrasexual competition for resources (9,12). Aggression by females when nursing young (i.e., maternal aggression) may influence the capacity for litters to be successfully defended against potentially infanticidal conspecifics that intrude into the

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nest area. Aggressive behavior by females thus appears to contribute to spatial distribution and reproductive success (14,34). It follows that factors affecting the development of a female's aggressive behavior toward conspecifics may be of importance in determining her reproductive fitness as well as shaping social structure.

The purpose of the first study was to determine (using a stock of wild mice trapped in Canada) if anogenital ditstance at birth correlates with intrauterine position, which correlates with prenatal exposure to testosterone (30,33). In the remainder of our studies, the relationship between AGD at birth and a female's behavior in adulthood was assessed; we categorized females as having a long or short AGD at birth and then in adulthood, examined their behavior towards unfamiliar pups, their rate of urine marking of a novel environment in a variety of social situations, postpartum aggression (after delivering a litter) toward male and female intruders, and success at protecting their young during intruder tests.

EXPERIMENTAL METHODS AND RESULTS

Animals

Mice were trapped near Calgary, Alberta, Canada and maintained as an outbred colony at the University of Texas-Austin and, subsequently, at the University of Missouri-Columbia. Animal rooms were maintained at 23° on a 12L:12D cycle, with lights on at 1000 h. Water and food were available ad lib.

EXPERIMENT 1

RELATIONSHIP OF ANOGENITAL DISTANCE AT BIRTH TO INTRAUTERINE POSITION

For production of animals from known intrauterine positions, 273 young–adult virgin females were paired with young–adult sexually naive males. On the day that a female delivered a litter, the pups were removed and euthanized to eliminate lactation-induced diapause and the prolongation of pregnancy. In this stock, if parturition occurs during the early portion of the light phase of the light:dark cycle (prior to about 1400 h on this light:dark cycle), postpartum estrus begins around the end of the light phase. However, if parturition occurs later during the day, postpartum estrus reliably occurs the following night. This finding, based on preliminary studies, determined when a female was assumed to mate and thus determined when the subsequent litter (based on mating at postpartum estrus) was removed from the mother by cesarean delivery. We have found that females of this, as well as other (12), wild stocks cannot be examined for the presence of a copulatory plug without inducing abortion (due to stress; 32). This technique thus allowed us to reliably determine when to deliver the litter (on day 18 of pregnancy, with day 0 = mating) without handling the female. Cesarean delivery of pups more than 12 h prior to normal parturition results in a high rate of mortality.

Of the initial male and virgin female pairs, approximately two-thirds of the animals successfully produced a first litter and then again, approximately two-thirds of these animals produced a second litter ($n = 130$ litters) which was cesarean delivered for measurement of animals from different intrauterine positions.

Pups were delivered beginning at 1000 h on Day 18 of pregnancy. Pregnant females were killed by CO_2 asphyxiation and cervical dislocation, pups were rapidly removed, and their intrauterine position was recorded. Anogenital distance was measured using an Olympus dissecting microscope with a micrometer lens (accurate to 0.05 mm). Measurements were made without knowledge of intrauterine position by one person to whom pups were passed. Body weight of each pup was also recorded. These pups were placed with foster mothers but were not used for the postnatal studies described here.

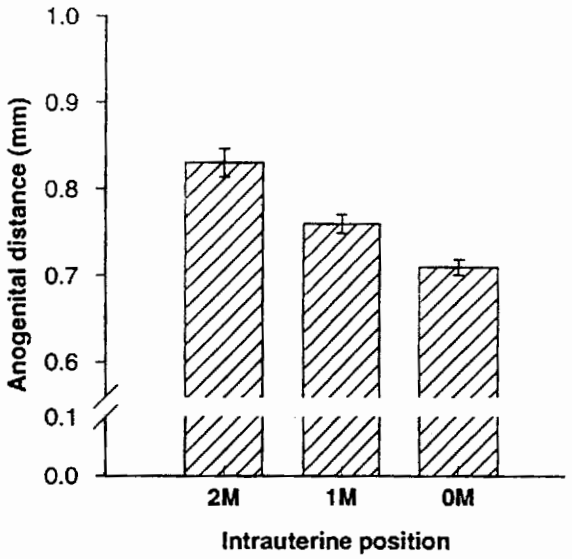

FIG. 1. Mean ± SE Anogenital distance at birth of female pups from different intrauterine position. (2 M vs. 1 M and 0 M, $p < 0.001$; 0 M vs. 1 M, $p = 0.08$).

Anogenital distance and body weight were measured for females categorized as occupying three different intrauterine positions: 0 M (between two female fetuses; $n = 69$); 1 M (between a male and a female fetus; $n = 80$), 2 M (between two male fetuses; $n = 56$). With a litter size of 6–8, which is normal for this stock, on average, there should be one-0 M and one-2 M animal per two litters, based on intrauterine position being a random event. This prediction is consistent with our results. Anogenital distance in males does not vary in relation to intrauterine position (29) and is not reported here.

Data were analyzed using the Statystical Analysis System (SAS), general linear model. Data were analyzed by both ANOVA and analysis of covariance (ANCOVA), with body weight used as the covariate, to determine whether some portion of the variance in AGD might be accounted for by differences in body weight. Planned comparisons were made using the LSmeans test. The null hypothesis was rejected at the 95% confidence level.

Figure 1 shows the mean (+SE) AGD in relation of females' intrauterine position. There was a significant effect ($p < 0.001$) of intrauterine position on AGD. 0 M and 2 M females differed significantly ($p < 0.001$), while 1 M females differed from 2 M females ($P < 0.001$) but not 0 M females ($p = 0.08$). There were no significant differences between the groups in body weight: 2 M = 1.18 ± 0.026 g; 1 M = 1.15 ± 0.015 g; 0 M = 1.16 ± 0.019 g. ANCOVA revealed that body weight accounted for a significant portion of the variance in AGD ($p < 0.01$), but after being adjusted for body weight, mean AGD's for 2 M, 1 M, and 0 M females were virtually identical to the means presented above, showing that differences in AGD occurred as a function of intrauterine position independent of a slight effect of body weight on AGD.

We also examined two additional categories of females: those occupying an intrauterine position at either end of a uterine horn (next to the ovary or cervix) with an adjacent male fetus (1 M-end female) and an adjacent female fetus (0 M-end female).

The mean (±SEM) AGD for 1 M-end females (0.748 ± 0.015 mm; $n = 26$) was not significantly different from the mean AGD for 1 M females (that were located between a male and a female fetus; 0.758 ± 0.011 mm; $n = 80$). Similarly, the mean AGD for 0 M-end females (0.716 ± 0.014 mm; $n = 35$) was not significantly different from the mean for 0 M females (that were located between two female fetuses; 0.729 ± 0.010 mm; $n = 69$). However, body weights for both 0 M-end females (1.26 ± 0.03 g) and 1 M-end females (1.25 ± 0.03 g) were significantly ($p < 0.05$) greater than body weights for 0 M, 1 M or 2 M females, none of which developed at the end of a uterine horn.

The finding that fetuses at the ends of the uterine horns are heavier that fetuses in more central locations is consistent with prior findings that fetuses at the ends of the uterine horns receive the greatest amount of blood flow (and thus nutrition). Blood enters the loop artery feeding each uterine horn from each end of the loop (5,31). These findings show that proximity to female fetuses does not influence the AGD measure, although comparisons of 2 M and 0 M males show that proximity to female fetuses (and elevated estradiol) has effects on reproductive organs (29). Also, the findings show that differences in AGD due to proximity to male fetuses occur independent of effects on body weight of position with the uterus relative to the ovary or cervix. It is thus inappropriate to present AGD data as a ratio of AGD/body weight (31).

Postnatal Studies

For postnatal studies with female mice categorized on the day of natural birth as having a long, medium or short AGD, we used the female offspring of 70 pairs of wild mice bred at the University of Missouri. Within the first 12 h after natural delivery, pups were weighed, sex was determined, and the anogenital distance (AGD) of each female pup was measured to the nearest 0.05 mm using an Olympus dissecting microscope with an ocular micrometer. The distribution of the AGD measures for females used in this study is shown in Fig. 2.

Female pups were marked by using a toe-clipping pattern to allow individual identification, and then they were returned to their mothers. Litters were culled to six pups by eliminating or adding male pups, but no litter had more than 4 female pups. After weaning at 23 days of age, females were housed with same-sex littermates in polypropylene cages measuring 29 × 18 × 13 cm.

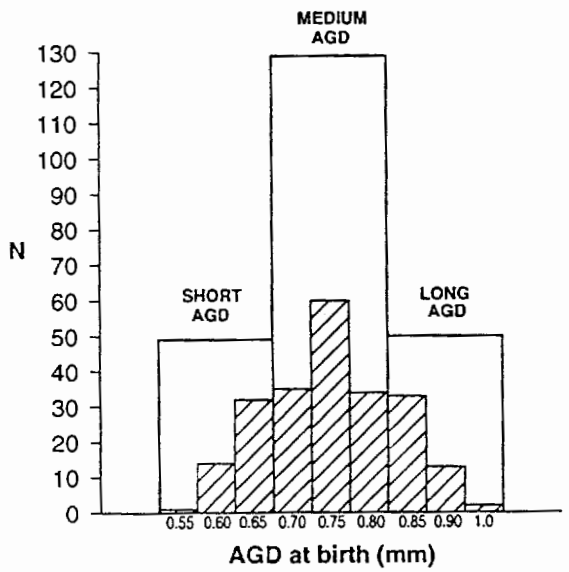

FIG. 2. Frequency and experimental categories of AGD measures of female wild mice within 12 h after delivery.

On the basis of findings from Experiment 1, and also by vom Saal & Bronson (30) and vom Saal, et al. (33), females with different AGD measures were assigned to three experimental categories (Fig. 2).: Short AGD (0.55–0.65; $N = 49$); Medium AGD (0.70–0.80, $N = 129$); Long AGD (0.85–1; $N = 50$).

EXPERIMENT 2

PRELIMINARY TEST FOR BEHAVIOR TOWARD PUPS

At 90–100 days of age all females were housed individually, and 24 h later they were tested for their behavior toward a single 1–3 day pup. The pup was placed into a corner of each animal's home cage with a minimum of disturbance. Females were scored as infanticidal if they attempted to bite the pup at which time the test was terminated; the pup was immediately removed and euthanized by CO_2 asphyxiation. Females that did not attack the pups within 20 min were labelled as noninfanticidal. The unharmed pups were returned to their mothers. Throughout the study we took care to minimize the stress imposed on animals, both adults and infants. The number of pups used for infanticide tests was minimized by confronting adults with a single pup and removing the pup as soon as it was attacked (4).

Adult females with different AGD measures at birth did not differ in their behavior toward a newborn pup: approximately 55% of females exhibited infanticide, regardless of neonatal AGD.

EXPERIMENT 3

URINE MARKING BEHAVIOR

Although females mark their environment at lower rates than males, female urinary marking may also play an important role in communication between female mice as well as in inter-sexual communication. It has recently been suggested that in natural populations of mice, females urine mark to advertise their dominant breeding status to other females; urine-marking appears to be dependent on female social/reproductive status (7).

The present experiment examined whether AGD at birth would relate to rates of urine marking by females exposed to another female or to a male. Moreover, since females from different intrauterine positions differ in their attractiveness to and sexual arousal of males (24,30), the rate of urine marking by males when exposed to females with different AGD was also assessed.

At 100 days of age females were individually housed for 24 h in polypropylene cages (29 × 18 × 13 cm) and then transferred to the test cages. Urine marking tests were conducted for 1 h in clean 30 × 30 × 15 cm cages divided into two equal chambers by a removable 0.6 cm wire-mesh barrier. This testing situation allowed visual, auditory, olfactory and even tactile communication between animals in opposing chambers. The floor of the cages was covered by a large sheet of Whatman No. 2 filter paper during the 1 h test.

Test females were placed into the test cages and assigned to one of 2 experimental groups: (i) 12-long-AGD females and 12-short-AGD females were separated by the wire barrier; (ii) a female of long ($N = 17$) or short ($N = 16$) AGD was separated by the wire barrier from a 90-day-old sexually naive male that had been individually housed for the previous 24 hr. At the end

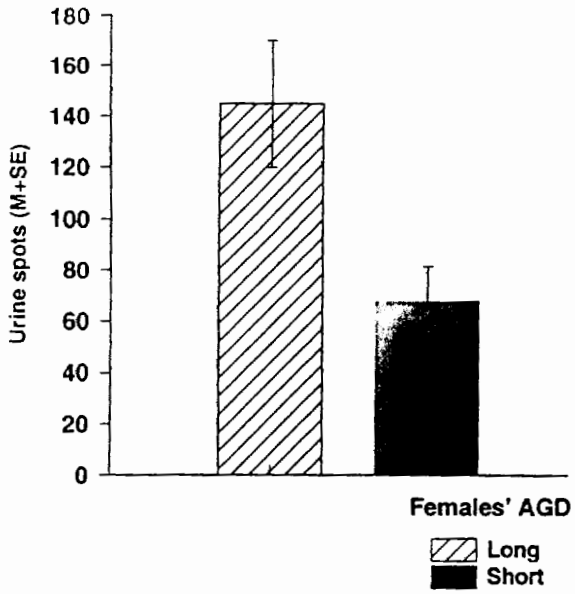

FIG. 3. Urine marking by males exposed to virgin females characterized by long or short AGD.

of the 1 h urine-marking test, the filter paper was removed and discrete urine marks (which fluroesce under uv light) deposited on it by females or males were counted (2).

The mean number of urine marks deposited by females of long- and short-AGD was compared in the presence of a male or a female stimulus animal of the opposite AGD. Results showed that female urine marking was not correlated with AGD whether females were housed across a wire partition from another female or a male. However, both short- and long-AGD females produced significantly more urine marks when in the presence of a male (about 50 marks/h) than when contacting a female (about 15 marks/h; male vs. female stimulus for both long and short AGD females, $P < 0.01$).

Figure 3 shows that urine marking by males differed in the presence of long- vs. short-AGD females, with males exposed to long-AGD females depositing a higher number of urine marks than males exposed to short-AGD females (t test, $t(28) = 2.8$, $P < 0.01$).

EXPERIMENT 4

MATERNAL AGGRESSION

During lactation female house mice become intensely aggressive toward unfamiliar conspecifics that intrude into the nest area. This behavior, referred to as maternal aggression, may have evolved to protect the offspring from being killed by conspecifics. In fact, infanticide by both intruder males and females has been well documented in house mice (e.g., 9,12,15). Maternal aggression appears to be a heterogeneous phenomenon and can be categorized as either offensive or defensive, based on context and/or characteristics of opponents (19). However, information concerning maternal aggression is based almost exclusively on studies with domestic mice.

The present study was designed to examine maternal aggression of wild mice in relation to AGD at birth. We also examined a number of other variables predicted to be correlated with maternal aggression, such as infanticidal tendency prior to becoming pregnant, length of time following delivery, and sex of the intruder. Finally, we examined whether postpartum estrus, which occurs either during the first or second night following delivery, would influence a female' s behavior toward a male intruder, as well as the intruder's behavior towards the female and her pups.

Intruder females were a subset of those categorized as medium-AGD at birth (Fig. 2) which had exhibited infanticide during the preliminary test for behavior toward pups. The intruder females were individually housed 3 days before being used in a test and were unfamiliar and genetically unrelated to the lactating females (this was also true for intruder males).

Intruder males were sexually naive males of the same stock. After weaning the males had been housed in unisexual groups of 4–6 individuals. At 90 days of age males were individually housed for 24 h and tested for their behavior towards a newborn pup following the same procedure described for females. Only infanticidal males were used as intruders (83% of the tested males killed the pup). This test for infanticide took place 3 days before the test during which the male was used as an intruder into the cage of a lactating female; all males remained individually housed until being used as intruders.

The experimental apparatus consisted of two polyethylene cages (each $45 \times 25 \times 15$ cm) connected by a 20-cm tunnel, which could be closed by a removable partition. One of the cages was provided with a polyethylene box ($10 \times 7.5 \times 7$ cm) containing nest material in which females could deliver their litters. The nest box was covered by a removable transparent lid and rested directly on the Aspen bedding. The entrance to the nest box was provided via a 5-cm diameter tunnel which was 7-cm long. The nest box was provided to simulate burrows of mice living in natural conditions and to provide the female a defensible nest area. The second cage served to provide the intruder an area outside of the female's home cage in which to escape if attacked by the female. The cages were covered by a stainless steel lid on which food (Purina) and a water bottle were placed.

Females of short- and long-AGD were paired with adult males of the same wild stock. A few days before delivery each female was individually housed in the experimental apparatus. For tests with both male and female intruders, a few minutes before introduction of an intruder, the lactating resident was confined in the cage containing the nest box by interposing partitions at the ends of the tunnel connecting the two cages. The intruder (marked on the back by a white marker) was then placed into the cage not containing the nest box. After 2 min the partitions were removed, and the animals were allowed to interact.

Male Intruder Test

Twenty-two short-AGD and 20 long-AGD lactating females were used in this experiment. One half of the long- and short-AGD females had shown infanticidal behavior on the pretest, while the remaining females had been noninfanticidal. Each female underwent three consecutive intruder tests on days 1, 2, and 4 postpartum. This was done to examine the female's behavior towards male intruders during postpartum estrus and, subsequently, when maternal aggression is more intense (26). To maximize the likelyhood of females being in postpartum estrus, intruder tests were conducted between 1700–2300 h on day 1 and 2 postpartum. On day 4 postpartum tests took place between 1000–1600 h.

Detailed observation and recording of behaviors lasted 10 min, but the animals were continuously monitored to check for the occurrence of infanticide during the following 50 min. In

maternal aggression tests, the amount of stress imposed on mothers and pups was minimized by intervening and removing the intruder as soon as any pup was attacked (4).

Female Intruder Test

On day 4 postpartum between 1700 and 2300 h, a randomly selected subset of lactating females (10 short-AGD and 8 long-AGD) were tested for maternal aggression against a previously infanticidal, medium-AGD female intruder. For each female, there was at least a 5-h period between the earlier test with the male intruder and the test with the female intruder.

Behavioral Analysis

The following behaviors of lactating females were recorded using an Esterline Angus multi-channel event recorder: (i) Proportions of intruders attacked; (ii) Latency to attack (i.e., the time from the initial contact to first biting attack); (iii) Accumulated attacking time (i.e., the total duration of biting attack); (iv) Tail rattling [i.e., the total duration of rapid lateral quivering or thrashing of the tail (threat component of agonistic behavior)]; (v) Fear/defence (i.e., total duration of fear-related behaviors, such as contact-related immobility, upright defensive posture, vocalizations, and startle responses); (vi) Social investigation (i.e., total duration of sniffing and grooming the intruder); (vii) Maintance behavior (i.e., the total duration of self grooming, feeding, and drinking); (viii) Nest-oriented behavior (i.e., total duration on nest, crouching over pups, suckling rearranging nest material); (ix) The total duration of nonsocial activities (i.e., exploration of the cage).

In order to assess whether females would differ in their success at protecting their litters as a function of their AGD at birth, the occurrence of, and latency to, infanticide by male intruders, as well as counterattack by the male to attacks by the lactating female resident, were recorded. Because a test session was immediately terminated if the intruder attacked a pup, the total duration of test sessions could vary. Thus, duration data for all behaviors were calculated as a ratio where the time (in s) spent in a given behavior was divided by the totat duration of time of recording behaviors, which could be a maximum of 600 s. This ratio was then converted to percent ($\times 100$) so that the data for each behavior represented the percent of the test session engaged in the behavior. The total for percent time engaging in the behaviors that were recorded was less than 100%, reflecting the fact that there were periods during which the test animal was immoble.

Statystical Analysis

Chi square analysis was conducted to compare the proportion of animals that exhibited a behavior. Latency measures were analyzed by nonparametric tests (i.e., Kruskall Wallis and Mann–Whitney), since latency data were not normally distributed. Duration data for behaviors were analyzed by ANOVA using the two-factor, repeated measures analysis available on the Statistical Analysis System (SAS), general linear model.

Following the first test on day 1 postpartum, some females ceased to care for the pups, and in some cases the entire litter disappeared. The loss of litters occurred between the end of testing on one day and the time that animals were examined on the next day; in no case was this observed to occur by an experimenter. Maternal behavior of wild-type females is very sensitive to any disturbance, expecially during the first 24 h following parturition (12). As a consequence, the number of experimental subjects on the different test days postpartum varied (i.e., short-AGD females): $N = 22, 17, 16$; long-AGD females: $N = 20, 19, 17$, on days 1, 2, 4 postpartum, respectively.

RESULTS

Only the data obtained on day 4, when both male and female intruders were tested, are presented in Table 1. With regard to differences based on postpartum day of testing (data not shown),

TABLE 1
BEHAVIORS OF LONG- AND SHORT-AGD LACTATING FEMALES IN RESPONSE TO MALE OR FEMALE INTRUDERS

	Long AGD		Short AGD		ANOVA
Sex of intruder	Male	Female	Male	Female	
Proportion of attack	8/8 (100%)	8/8 (100%)	7/10 (70%)	9/10 (90%)	-
Latency to attack	2.5 (1–10)	70 (2–420)	12 (1–600)	75 (1–600)	Mann Whitney test F vs. M, $p < 0.02$
Attack	4.8 ± 1.5	8.6 ± 1.6	4.9 ± 2	10.5 ± 2.4	AGD: ns Intr: $p < 0.02$ $F = 5.6$ AGDxIntr: ns
Tail Rattling	7 ± 3.1	4.8 ± 2.2	0.9 ± 0.6	2 ± 1	AGD: $p < 0.02$ $F = 6.9$ Intr: ns AGDxIntr: ns
Fear/defense	1.6 ± 0.7	0.1 ± 0.07	3.7 ± 0.9	0	AGD: $p < 0.1$ $F = 2.5$ Intr: $p < 0.001$ $F = 18$ AGDxI: $p < 0.07$ $F = 3.$
Social investigation	0.1 ± 0.13	1.4 ± 1.3	0.2 ± 0.06	4.1 ± 1.2	AGD: ns Intr: $p < 0.006$ $F = 8.6$ AGDxI: $p < 0.1$
Maintenance	15.7 ± 5.7	13.7 ± 4.7	15.3 ± 4.2	15.5 ± 4.8	ns
Non Social	50 ± 11.8	56 ± 11.3	60 ± 6.6	61 ± 6	ns
Nest	2 ± 1	1.5 ± 0.8	7.4 ± 4.4	0.77 ± 0.4	ns

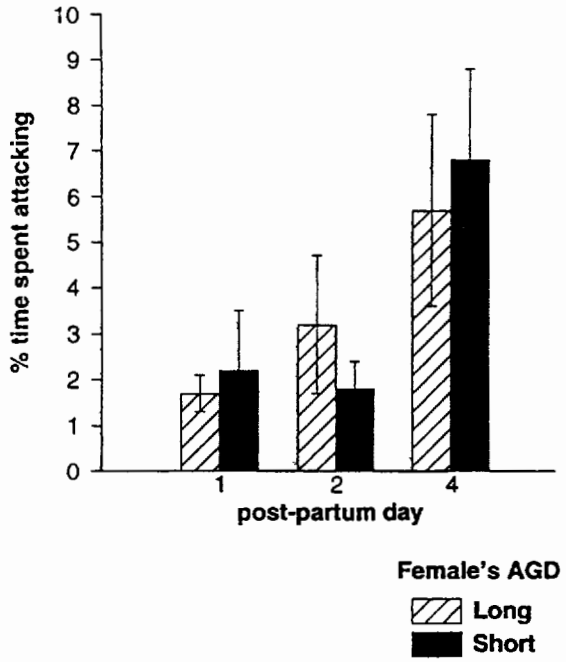

FIG. 4. Accumulated attacking time (M ± SE) towards male intruders by lactating females tested on different postpartum days. ANOVA: AGD: ns; day: $F = 5.4$ $p < 0.05$; AGDxday: ns.

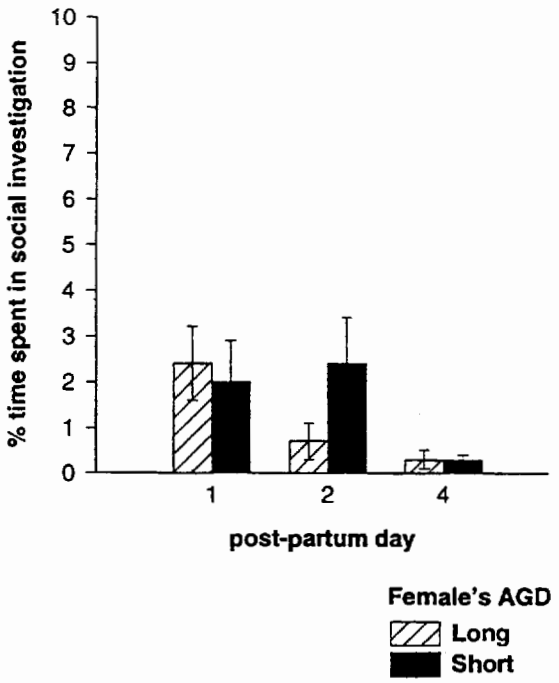

FIG. 5. Social investigation (M ± SE) of male intruders by lactating females of short or long AGD in different postpartum days. ANOVA: AGD: ns; day: $F = 3.4$ $p < 0.03$. AGDxday: ns.

the proportion of long- and short-AGD females attacking, and the time spent in different behaviors during the test with a male intruder, did not differ significantly as a function of day postpartum for (i) tail rattling; (ii) fear/defense; (iii) nest oriented; (iv), maintenance; and (v) nonsocial behaviors. In contrast, between days 1 and 2 vs. day 4 postpartum, the total duration of attack behavior significantly increased (by over 300%; $P < 0.01$; Fig. 4) while social investigation significantly decreased (by 85%; $P < 0.01$; Fig. 5) for both long- and short-AGD females. Latency to attack also decreased significantly for both long- and short-AGD females from day 1 and 2 to day 4 (by 90%; $P < 0.05$). Long-AGD females displayed significantly more tail-rattling toward male intruders than short-AGD females on postpartum days 2 and 4 ($p < 0.03$; see Fig. 6 for data on postpartum day 4).

Contrary to what is typically observed in domestic stocks of mice (such as the Rockland Swiss; 26), where very little aggression is observed until day 3 postpartum, wild-type females showed a relatively high rate of attack even during the first 48 h following delivery, when postpartum estrus typically occurs. Attacks by females toward male intruders were commonly accompanied by vocalizations (typically associated with fear) and defensive postures. Bites delivered on the intruder's body were rarely vicious, since females often were observed initiating attacks but without actually biting the male. The resident females often remained at the entrance tunnel leading to the nest box, lunging towards the male, that typically tried to enter the nest box.

There was no relationship between a female's AGD and success at protecting her offspring from male infanticide. In spite of maternal attacks, the majority of males succeeded in entering the nest box and attacked a pup. If the test had not been immediately terminated at this time, prior studies using both Swiss (21) and wild-type (12) mice have shown that the entire litter would have been killed in every instance once an attack on one pup is initiated. Since the test was terminated at this time, there was no basis for assessing whether females with long- vs. short-AGD's might have differed in the likelihood of protecting and rearing at least some of their offspring once a pup was attacked.

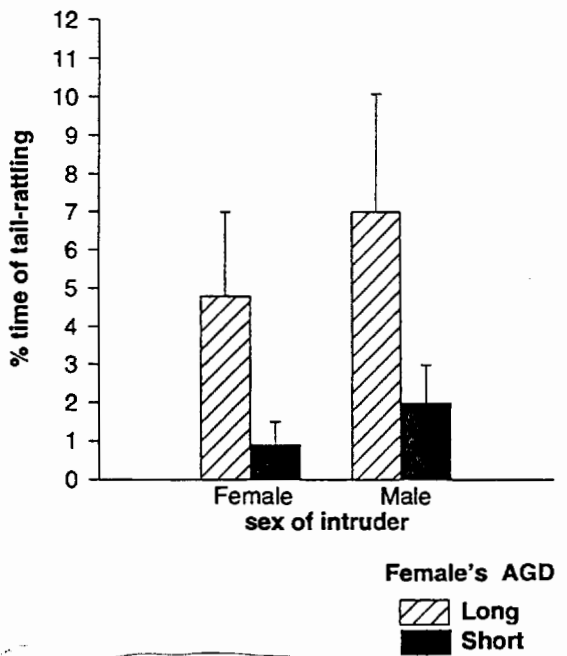

FIG. 6. Accumulated attacking time (M ± SE) toward male intruders by lactating females with different potential for infanticide as virgins.

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For day 1 postpartum, about 25% of males attacked a pup within the first 10 min of observation, and about 75% of males attacked a pup within the 1 h test period; similar results were observed on test days 2 and 4. These data suggest that maternal attack was rarely successful in preventing male infanticide, although the complex housing condition employed (with two connected cages and the protected location of nests) could provide a relatively defendable nest area and an environment into which the male could escape when attacked, in comparison to the usual laboratory tests conducted in one small cage.

The data in Table 1 show differences in the behavior of lactating long- and short-AGD females according to sex of intruder. Fear/defense behaviors were significantly influenced by the sex of intruder for both long- and short-AGD females, with both categories of lactating residents showing more fear/defense behaviors when confronting male than female intruders ($P < 0.001$). A significant effect of the sex of intruder was found with regard to social investigation, with lactating females showing more social investigation toward female than male intruders ($P < 0.01$).

For both long- and short-AGD females, the latency to attack was shorter toward male than female intruders ($P < 0.02$). Females of long- and short-AGD did not differ in terms of the amount of time spent attacking the intruders, but with intruder females, there was a significantly greater total duration of attacks by both long- and short-AGD lactating females than was observed with intruder males ($P < 0.05$). Female intruders were often severely injured, whereas males rarely showed visible wounds.

Due to the very high rate of attack toward the female intruders, we decided not to extend the observation beyond the initial 10-min period during which behaviors were recorded (in order to determine whether infanticide would occur). However, given the intensity of attack toward female intruders, infanticide by the intruder females would have been unlikely, because the intruder would probably have been killed.

Figure 6 shows that there was significantly more tail rattling by long- than short-AGD females in response to both male and female intruders on Day 4 postpartum ($P < 0.05$).

All females had been tested for infanticidal behavior as virgins, and as mentioned previously, this behavior did not relate to AGD. However, we examined whether there was a relationship between behavior toward a pup while a virgin and postpartum aggression toward male intruders. Lactating females that had been infanticidal as virgins showed a greater total duration of attack toward male intruders than noninfanticidal females (Fig. 7). No difference in attack towards female intruders (on day 4 postpartum) as a function of being infanticidal or noninfanticidal as virgins was found.

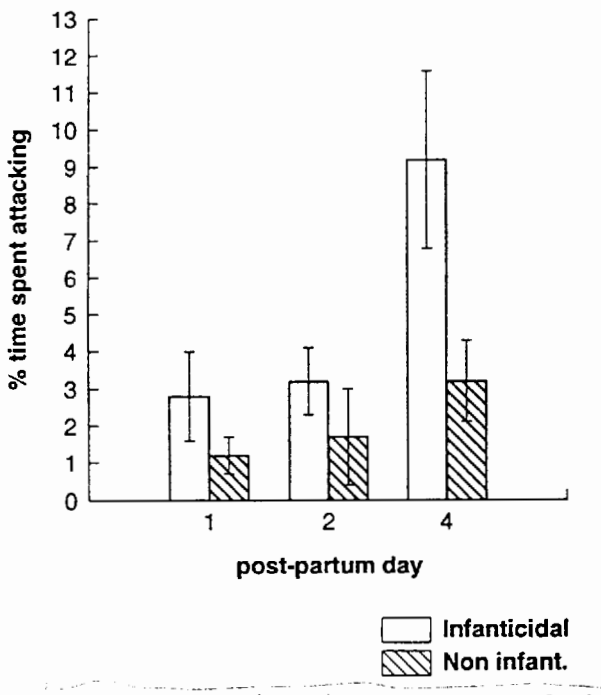

FIG. 7. Amount of tail rattling (M ± SE) displayed by lactating females characterized by long or short AGD towards male or female intruders.

GENERAL DISCUSSION

The results of Experiment 1 showed that intrauterine position is correlated with anogenital distance (AGD) at birth in wild female mice and that anogenital distance at birth can thus be used as a bioassay for prenatal exposure to testosterone. Recently, Vandenbergh and Huggett (personal communication) have extended this observation and found that anogenital distance at weaning (corrected for body weight and referred to as the anogenital distance index) can also be used as a predictor of fetal testosterone exposure; female mice catagorized at puberty as having a different anogenital distance index differed in the age at first estrus and in the sex ratio of the first two litters produced.

The results of Experiment 3, in which females catagorized at birth as having long- or short-AGD were compared in adulthood, showed that males housed across a wire mesh partition from a long-AGD female deposited a higher number of urine marks than those exposed to a short-AGD female. Conversely, female urine marking was not related to AGD whether females were exposed to a male or a female stimulus animal of the opposite AGD. These results are consistent with the hypothesis that females with a long AGD are more androgenized than females with a short AGD with regard to the release of pheromonal cues (28,30).

The increased urine marking by males in the presence of long-AGD females may be due to long-AGD females releasing urine containing a different quality or quantity of pheromonal cues relative to short-AGD females (1). In this view, long-AGD females would stimulate male counter-marking as a response to pheromonal cues produced following the exposure to a higher level of testosterone during fetal life and thus associated with male-type stimuli. Males tend to urine mark at higher rates when in the presence of a strange male as opposed to a strange female (1). Male urine marking may provide females with the opportunity to assess male social status, since females can discriminate male social status on the basis of urinary cues (8) and prefer to mate with dominant males (6,22).

Regardless of their AGD at birth, females produced far more urine marks when in presence of a male than another female. This confirms previous results of Maruniak et al. (11) on laboratory mice and recent data of Palanza et al. (18) on wild mice. Increased urine marking in response to male cues may function to advertise territory occupancy to other females, thus suggesting that male cues can stimulate competition among females (18).

While in CF-1 mice, 2 M females were found to urine mark at higher rates than 0 M females (30), in this experiment AGD at birth did not predict rates of urine marking. It is thus possible that in this wild stock of mice, prenatal exposure to androgens does not affect this behavior in females, even though it appears to

affect the production of pheromonal cues that stimulate urine marking in males. The relative importance of intrauterine position phenomenon in influencing a variety of behavioral and physiological elements may, in fact, vary in relation to the gene pool of a particular mouse population (25,35).

Experiment 4 showed that after delivering a litter, long-AGD females displayed more tail rattling (a component of mouse agonistic behavior) towards intruders of both sexes in comparison to short-AGD females. However, other measures of aggression by lactating female towards intruders of either sex were not related to AGD at birth. Aggression by females, as well as aggression by males, has been shown to be related to exposure to testosterone during fetal life in laboratory stocks of mice (27). Specifically, previous studies have shown that 2 M female mice exhibited more intense aggressive behavior when nursing young than did 0 M females (10,30). Again, genetic difference can account for the discrepancy between previous findings on domestic mice and ours on a wild stock with regard to the intensity of aggression.

Contrary to what is commonly observed in most laboratory stocks of mice in which little or no aggression is observed until 2–3 days postpartum (26), in this experiment we observed that lactating females displayed relatively high rate of attack toward male intruders during the first 24–48 h after delivery, which encompasses the period of postpartum estrus. However, the behavior of the lactating female toward male intruders did vary between days 1 and 2 and day 4 postpartum in that the intensity of attack increased while social investigation decreased.

Anogenital distance at birth did not relate either to a females' infanticidal potential when virgin or to the intensity of aggression displayed by females when lactating. Nevertheless, a relation between these two behavior was found, as females which were infanticidal as virgins were more aggressive towards male intruders than noninfanticidal females. Aggression toward adults and infanticidal behavior when confronted with a newborn pup can both be viewed as forms of intraspecific competition. Levels of different forms of aggression, such as intermale aggression, infanticide by males or females, and maternal aggression, covary within and between different lines and strains of mice, thus suggesting that these different forms of aggressive behavior can be all related to the level of intraspecific competition within a population (20).

A major difference between the present study and experiments on laboratory stocks of mice, is that both male and female intruders represent a potential threat for the female's offspring. Aggression by lactating female mice toward other females, as well as males, can thus be a critical behavior to ensure survival of the litter. In accordance with previous studies on the laboratory Swiss line (21), wild lactating females respond differentially to intruders of differing sex, displaying more social investigation toward female but more fear-related behaviors toward male intruders. Contrary to what is observed in laboratory stocks (13,21), attacks on females were more intense and relentless than the attacks on male intruders. None of the female intruders exhibited infanticide during maternal aggression tests, whereas most of male intruders attacked the lactating female and her pups. Maternal aggression thus appears to be successful in defeating, and preventing infanticide by, other females but not by males. This finding, which confirms previous studies (e.g., 3,16,23), questions whether maternal attack on male intruders serves only as a counterstrategy to infanticide. It has been proposed that maternal aggression towards males could also serve to assess the quality, in terms of fighting ability, of males that will become the female's future mates (16,17,23).

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08496

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