BRAIN P.F. and D. BENTON (Eds): THE BIOLOGY OF AGGRESSION – ISBN 90-286-2851-7

THE INTRAUTERINE POSITION PHENOMENON IN MICE

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ABSTRACT. Adult male mice (Mus musculus) are extremely aggressive, but female mice are typically described as being non-aggressive. Many of our models of human behavior are derived from animal models. The degree to which aggression is sexually dimorphic is thus of theoretical significance as well as of importance to both biopsychologists and ecologists who study the behavior of mice. This paper describes experiments concerning the intrauterine position phenomenon in mice, i.e., the capacity of male and female fetuses to have their development modified via exposure to steroids secreted by contiguous litter-mates. These experiments have revealed that there is considerable variability in aggressiveness among adult female mice. Differences in aggressiveness between females appear to be due to differences in circulating androgen levels during prenatal life based on the proximity of a female fetus to male fetuses in utero. Comparisons of male mice that developed in utero between either two male fetuses or two female fetuses have revealed differences in prenatal estrogen titers and in adult sensitivity to both progesterone and testosterone. Differences in the exhibition of infanticide and parental behavior have also been recorded.

1. INTRODUCTION

Most studies concerning the influence of gonadal hormones on aggression have involved pharmacological and/or surgical intervention after parturition in mice. Two reasons for this are that it is difficult, or in some cases impossible, to experiment with fetuses, and 25 years ago it was reported that injecting a female mouse with testosterone propionate (TP) shortly after birth rendered the female non-cyclic in adulthood (the androgen sterility

syndrome; 1). It was thus assumed that the critical period for sexual differentiation of neural areas mediating behaviors such as aggression also occurred shortly after birth in mice as well as in rats (2,3). It is now apparent, however, that there is no absolute critical period during which the organization of aggression must occur in mice as was originally proposed. But, there does appear to be a period of maximum sensitivity to the organizational effects of androgen on aggressive behavior during the perinatal period. For example, in a study concerning the effects of prenatal and/or neonatal exposure to androgen on adult intermale aggression, I found that exposure to androgen during the last third of gestation resulted in an increase in sensitivity to the aggression-inducing action of testosterone (T) in adulthood. But, exposure to androgen shortly after birth had a greater sensitizing effect than did prenatal androgen exposure. Thus, intermale aggression in mice is more readily modified by androgen exposure just after birth than before birth (4).

2. THE INTRAUTERINE POSITION PHENOMENON IN FEMALES

In the ecological and experimental literature concerning aggression in mice, it is occasionally mentioned that aggression between females has been observed (5,6,7). But, in discussions of aggression in mice, females are still referred to as non-aggressive (8). I was always intrigued by the fact that there were adult albino female mice in my colony (both Rockland-Swiss and CF-1 mice) that would attack other females when they were housed together. This observation, together with the observation that female mice that developed in utero between male fetuses (2M females) had longer anogenital spaces at birth than did females that did not develop next to a male fetus (0M females), led to the hypothesis that intrauterine position might influence interfemale aggression in mice. The tissue between the anus and the genital tubercle elongates in response to androgen and provides a sensitive bioassay for prenatal androgen exposure (9). Subsequently, vom Saal and Bronson (10) reported that 2M female mouse fetuses have significantly higher amniotic fluid and blood titers of T than do 0M female fetuses. This finding provided direct evidence that 2M females are exposed to higher levels of T than are 0M females due to their contiguity to male fetuses. Presumably, the excess T in the amniotic fluid and blood of 2M females results from T secreted by male fetuses' testes passing across the placental membranes via the amniotic fluid.

To test whether intrauterine position influenced adult aggression between female mice, the offspring of time-mated females were delivered by cesarean section, and 2M and 0M females were saved. Pairs of adult 0M and 2M females that were in diestrus were observed for 30 min. Significantly more of the 2M females were aggressive toward and established dominance over the 0M females (9).

Thus, elevated T levels during prenatal life due to intrauterine contiguity to male fetuses significantly increases spontaneous aggressiveness toward other females in mice. 2M female mice have also been found to urine mark their environment at a higher rate than do 0M females (9). Urine marking by male mice living in deme (family) units serves to delineate territorial boundaries (11). These data, taken together, suggest that 2M females may be more territorial than 0M females. Further support for this hypothesis is provided by the finding that lactating 2M female mice are significantly more aggressive than lactating 0M females toward a female intruder into the nest area containing the young (9). The traditional bias is that prenatally androgenized females should be at a reproductive disadvantage. But, 0M and 2M female mice do not differ in their capacity to ovulate, mate, produce and raise healthy young when housed individually (9). It is proposed, however, that 2M females, by virtue of their aggressiveness, may have a reproductive advantage over other less aggressive females, particularly when population density is high and competition for resources is intense (12). In experiments involving freely growing populations of mice in seminatural environments, it has been observed that a small percentage of the females are reproductively active. The reproductively-active females appear to take part in territorial defense along with the dominant male in the deme. The subordinate females either are unable to ovulate and mate, are unable to sustain a pregnancy, or if they do deliver offspring, are unable to successfully protect and care for the young (5,6). This is surprising given the general assumption that in rapidly cycling species such as Mus musculus, all females are either pregnant or lactating (13).

While more 2M females may reproduce successfully when population density is high, it is possible that 0M female mice may be at a reproductive advantage over other females when population density is low. This hypothesis is supported by the findings that 0M females enter puberty sooner and have shorter estrous cycles than 2M females. 0M females also emit a more potent attractive cue than 2M females and are more sexually arousing to males than 2M females (9,10,14,15). In addition to 0M and 2M females, 1M female mice that were positioned in utero between a male and a female fetus also have been examined in some experiments. 1M females are intermediate between 0M and 2M females in terms of the length of the anogenital space, timing of puberty and aggressiveness (unpublished).

3. THE INTRAUTERINE POSITION PHENOMENON IN MALES

Initially, it seemed unlikely that intrauterine position could have an effect on male mice. One reason for this assumption was the finding that exposure to a very high dose of TP injected into pregnant female mice (1.5 mg/day) during the last third of preg-

nancy failed to influence aggressiveness in male mice that were gonadectomized at birth and administered T in adulthood. The female offspring from TP-injected mothers were completely virilized, however (4). The conclusion was that supplemental androgen administration did not produce a "super male". Presumably, male mouse fetuses secrete sufficient androgen to be maximally virilized.

Recently, the levels of T, estradiol (E_2) and progesterone (P) were measured in the amniotic fluid of male mouse fetuses that were located either between two male fetuses (2M males) or between two female fetuses (0M males). Amniotic fluid titers of these steroids were also measured in female mouse fetuses. All fluids were collected on Day 17 of gestation (parturition occurs on Day 19). Males and females did not differ in their amniotic fluid levels of T or P, but females had significantly higher E_2 titers than did males. In addition, 0M males had significantly higher amniotic fluid E_2 titers than did 2M males, while no significant differences in T or P titers were found (unpublished).

This surprising finding stimulated a series of experiments comparing adult 0M and 2M male mice. In one set of studies, 0M and 2M males were gonadectomized immediately following cesarean delivery on Day 19 of gestation. In adulthood, these males were compared for the tendency to exhibit female sex behavior (lordosis) when injected with estradiol benzoate (EB) or EB and P. A 25µg /.05cc oil dose of EB was administered 52 h before the 0M and 2M males were paired for 30 min with an intact 1M male that had developed in utero between a male and a female fetus. One half of the 0M and 2M males also received a 200µg/.02cc injection of P 4 h before testing. Each animal was tested once per week for four weeks. By the third and fourth weeks of testing, significantly more of the 0M males receiving EB and P injections were mounted by the 1M males and were sexually receptive than were any of the 2M males or the 0M males receiving only EB. Thus, P facilitated sexual receptivity in 0M but not 2M males. Eight weeks following completion of this study, the same males received subcutaneous implants of 10mm silastic capsules containing 5 mg T/ .02cc oil and were tested for aggression toward an olfactory bulbectomized 1M male opponent. Olfactory bulbectomy renders males non-aggressive, but such males still elicit aggression. Testing occurred twice per week for five weeks. The 2M males required fewer days of exposure to T to exhibit a biting attack than did the 0M males. This study was replicated with another group of naive 0M and 2M males that had been gonadectomized at birth with the same results. Also, at autopsy after 35 days of T exposure, the 2M males had significantly heavier seminal vesicles than did the 0M males (unpublished). Taken together, these findings reveal that 2M male mice are more sensitive to T than are 0M

males both in terms of the induction of aggression and seminal vesicle growth, but that 2M males are less sensitive than 0M males to the effects of EB and P on the neural areas regulating lordosis behavior.

Intact adult 0M and 2M male mice were also compared for their aggressiveness. Pairs of 0M and 2M males were matched for age and weight and were observed for 10 min, after which they were left together for 24 h. There was no significant difference between 0M and 2M males in terms of the likelihood of establishing dominance in this experiment. Other 0M and 2M male mice were paired with an olfactory bulbectomized 1M male opponent and observed for 10 min, after which they were left together for 24 h. The 0M and 2M males did not differ in the intensity of aggression exhibited during the 10-min observation or in the number of wounds inflicted on the 1M male during the 24-h period (unpublished).

Intact naive, adult 0M, 1M and 2M male mice were also tested for the tendency to exhibit infanticide or parental behavior (retrieving and hovering over pups) toward newborn young. Infanticide has been proposed to reflect a breakdown in social structure, and thus has been considered a pathological or non-adaptive behavior (16). More recently, Hrdy (17) has proposed that infanticide may, under certain circumstances, increase the reproductive success of an individual. For example, in rapidly cycling species such as Mus musculus, if the dominant male in a deme dies, the male that replaces it would be unable to mate with a female that was nursing young until after weaning occurred due to lactational suppression of ovulation. But, if the new dominant male were to kill the young, then the female would rapidly ovulate. Under these circumstances pup-killing behavior should have positive selective value.

Two newborn mice were placed into the home cage of singly housed 0M, 1M and 2M male mice for 30 min. Significantly more of the 0M males killed the pups (63%) than did the 1M males (40%) or the 2M males (23%). Significantly more of the 2M males exhibited parental behavior (67%) than did the 1M males (37%) or the 0M males (27%; unpublished). This finding is actually consistent with the other findings from comparisons of 0M and 2M males. It is known that perinatal exposure to elevated levels of either EB or TP can increase the sensitivity of the neural substrate mediating aggression to the aggression-inducing action of T in adulthood (4,18). The 0M males have higher amniotic fluid titers of E_2 during fetal life but are less sensitive to the aggression-inducing action of T in adulthood. It has also been reported that perinatal exposure to EB or TP results in a decrease in sensitivity to the pup-killing inducing action of T in adulthood in mice (19,20). Thus, perinatal EB or TP administration to female mice has opposite effects on aggression and pup-killing when the

females are administered T in adulthood. The finding that 2M males are the least likely to exhibit pup-killing behavior in adulthood is thus consistent with the finding that they are more sensitive to the aggression-inducing action of T, but suggests that 2M males might be exposed to higher levels of T during fetal life, which is not supported by the available data. Alternatively, it may be that the higher levels of E_2 that were found in the amniotic fluid of OM male fetuses is having an opposite effect on adult aggressive and pup-killing behaviors than the effect observed when neonates are injected with EB.

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