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Fetal, Hormonal and Experiential Factors Influencing the Mating-Induced Regulation of Infanticide in Male House Mice

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PERRIGO, G., W. C. BRYANT AND F. S. VOM SAAL. *Fetal, hormonal, and experiential factors influencing the mating-induced regulation of infanticide in male house mice.* PHYSIOL BEHAV **46**(2) 121–128, 1989.—When a male house mouse encounters a neonate he either attempts to kill it or he does not harm it. An unusual aspect of his response is that adaptive, time-dependent changes in behavior toward pups result from a unique stimulus-response system triggered specifically by ejaculation. In virgin male CF-1 mice, about 50% of all males are spontaneously infanticidal when they encounter a pup while the other 50% are typically "parental." The stimulus of ejaculation causes virtually all males to kill pups; however, by the time offspring are born three weeks after mating, infanticide is inhibited and almost all males now behave parentally toward pups. Our experiments examine fetal, experiential, and hormonal factors influencing the changes in infanticidal and parental behavior that occur in male mice as a result of mating. Males who developed in utero between two female fetuses, and were thus exposed to relatively low testosterone concentrations during fetal development, were significantly more likely to exhibit infanticide—both before and after mating—than were males who developed between two male fetuses. Concurrent exposure to testosterone appears required in order for naive males to exhibit infanticide and for spontaneously parental males to become infanticidal after ejaculation. In contrast, neither testosterone nor pituitary hormones appeared responsible for the timed inhibition of infanticide occurring by three weeks after mating, since castrated and hypophysectomized males showed a response pattern similar to intact males. The mating-induced inhibition phenomenon appears to be a neurally timed and mediated response that operates independently from pituitary hormone secretions or *changes* in gonadal hormones resulting from mating.

Infanticide Parental behavior Ejaculation Intrauterine position Aggression House mouse Hypophysectomy Stimulus-response

MOST forms of intraspecific aggression rarely lead to the death of interacting animals, whereas infanticide, by definition, is the killing of conspecific preweanling young. Much of the early work in this controversial area assumed that infanticide was maladaptive and symptomatic of abnormal or sociopathologic conditions in nature or the laboratory. Calhoun (4), in his classic studies of the Norway rat, noted that sociopathologic conditions, such as excessive crowding, can indeed lead to a severe social breakdown and thus a high incidence of infanticide. In recent years, however, a prominent new view of infanticide has emerged: such behavior can be adaptive and routinely occurs as a well-defined behavioral strategy in a variety of mammals and other vertebrates (10). Field studies of African Lions (18,23) and Indian langurs (12), and laboratory studies with both wild-trapped and domesticated stocks of house mice (15,37) have dramatically documented the reproductive advantages that accrue when an infanticidal male usurps the territory of a rival. By killing infants, a conquering male eliminates his competitor's offspring and re-establishes the breeding cycle of females for his own reproductive benefit (10,12).

The male house mouse, in particular, has become the focus of much research regarding the neural and endocrine substrates responsible for infanticide (26). The physiology of the male's infanticidal strategy is especially exciting, since the behavior of male house mice toward newborn pups is governed by a unique stimulus-response system triggered by ejaculation during coitus (33). In sexually-naive CF-1 mice about fifty percent of males exhibit infanticide. The act of ejaculation promotes infanticide in most males; however, by the time a male's own sired offspring

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would be born around three weeks after mating, infanticide is inhibited in virtually all males. When infanticide ceases, most males express parental behavior which is similar to lactating females. While the proportion of sexually-naive male mice that exhibit infanticide differs widely between different stocks of mice (19, 26, 34), the mating-induced phenomenon is well-defined and remarkably consistent among both wild (15,24) and laboratory stocks of house mice (9, 33, 37). This phenomenon also occurs in the Norway rat (17) and possibly other rodents as well. Thus, virtually all male mice and rats are likely to eliminate pups following mating but are inhibited from harming their own progeny during their mate's lactation. We know of no other time-dependent phenomena in mammals where a dramatic shift in adaptive behavior has evolved to occur many days after a specific stimulus such as ejaculation.

The temporal changes we observe in the frequency of infanticide and parental behavior in males from our CF-1 laboratory stock can be partitioned into several distinct phases: 1) *Premating*—where about 50% of all virgin CF-1 males exhibit spontaneous infanticide when they encounter pups, while about 50% exhibit either spontaneous parental behavior or they do not harm pups; 2) *Ejaculation*—where infanticide is enhanced as soon as mating occurs such that 85–90% of CF-1 males will now attack and kill pups during part of most of their mate's pregnancy; and 3) *Mid- to Late Pregnancy*—where infanticide is inhibited such that 80–90% of all CF-1 males shift to parental behavior prior to the birth of pups. This unique response pattern occurs even when CF-1 males are isolated and deprived of female cohabitation immediately after mating (33). Vom Saal (33) also identified a fourth phase: between 50 and 60 days after mating most parental CF-1 males suddenly become infanticidal again, and this shift coincides with the time when pups are normally weaned and depart from their natal environs. The studies presented here, however, are concerned only with the male's response to pups when sexually-naive and the mating-induced changes in infanticide occurring between ejaculation and the birth of his pups.

Our experiments examine how a male's behavior toward pups is hormonally-programmed during fetal development. Specifically, the experiments address some of the experiential and hormonal factors influencing infanticide and parental behavior in sexually-naive and newly-mated adults. Some of our results have unusual physiological interpretations and suggest the evolution of unique hormone-behavior interactions correlated with the social and reproductive structure of typical wild populations of house mice.

GENERAL METHOD

Animal Stocks, Housing and Surgery

All animals used were domestic stock CF-1 house mice, *Mus domesticus* (2,16). Males were isolated by 50 days of age and kept in $28 \times 18 \times 12$ cm cages with wood shaving bedding, and supplied with Purina Mouse Chow and water ad lib. Room temperature was maintained at $22 \pm 2°C$. Animals were not used in experiments until about 100 days of age. Except where specifically noted, all surgery was performed with Metofane anesthesia. Specific methodology will be described with each experiment.

Assessment of Infanticide and Parental Behavior

When a male house mouse encounters a neonate he either attempts to kill it or he does not harm it. These are clear-cut responses. We assess a male's behavior by quietly placing a 1–5-day-old pup at one end of the male's home cage farthest from his nest. If a male is infanticidal, he will typically approach the pup, rattle his tail, and suddenly lunge at and kill the pup with rapid bites to the head and back. This is an acute and dramatic response, so we cannot always intervene on behalf of the pup. Pups are quickly rescued and, if necessary, humanely euthanized. If a male does not attempt to kill the pup, the pup is left in the male's cage for 30 minutes. Most males who are not infanticidal exhibit parental behavior. Parental males groom the pup about the head and genitals and retrieve it to their nest where they incubate the pup and keep it warm. There is also a small subset of males (10–15%) who ignore pups, neither harming them nor exhibiting typical parental behavior (33). Pup-ignoring males appear to straddle a neutral behavioral state between infanticide and true parental behavior (19).

In addition, a male's reaction toward a pup seems to be a nonspecific response with no evidence for kin recognition. Previous studies have shown that neither the sex, age (1–10 days old) nor paternity of the pup has any discernible influence on a male's propensity to exhibit either infanticide or parental behavior (15, 26, 33, 37). Male mice also have spontaneous ejaculations nearly every night (13), but this does not influence their behavior toward pups.

Pretesting of Sexually-Naive Males

Seventy adult virgin males (80 days of age) were pretested with a neonate as described above. Testing this large number of animals was necessary to individually classify males as either infanticidal or parental for use in later experiments. The results were as follows: 32 males (46%) exhibited spontaneous infanticide, 27 males (39%) exhibited spontaneous parental behavior and 11 males (15%) ignored the pup. This frequency distribution replicates previous findings in virgin CF-1 males (33). These males were retested in the ensuing experiments after an interval of about two weeks. CF-1 male mice are remarkably consistent in their behavior toward young when retested after an interval of anywhere from one week to two months (33).

We have also tried to find a more humane alternative to the use of live pups. In a pilot experiment, 13 CF-1 males who behaved parentally and 16 CF-1 males who were infanticidal when tested at 80 days of age were retested 1-week later with a warm, freshly-euthanized pup (CO_2 chamber). There was no correlation between the two tests: 8 of the 13 parental males (62%) either severely bit or cannibalized the dead pup, while 4 of the 16 infanticidal males (25%) ignored the dead pup. Since we have been unable, at present, to find a reliable testing procedure which does not involve a live neonate, we have instead made every attempt to minimize the number of animals used in each of the following experiments.

EXPERIMENTAL SERIES

Experiment 1: Fetal Programming of Infanticide and Parental Behavior in Relation to Intrauterine Position

House mice produce multiple offspring in litters. The intrauterine position phenomenon refers to the fact that fetuses are positioned randomly in the uterine horns and exposed to different sex steroid concentrations in relation to whether they develop next to same sex or opposite sex fetuses (28, 35, 36). Blood testosterone levels during days 17–18 of gestation are significantly higher in fetuses that develop between two males (2M individuals) than fetuses that develop between two females (0M individuals). Fetuses that develop between a male and female (1M individuals) show intermediate concentrations of blood testosterone. The scheme used for classifying the position of male and female fetuses in the uterine horns is shown in Fig. 1.

Previous studies have shown that the frequency of infanticide

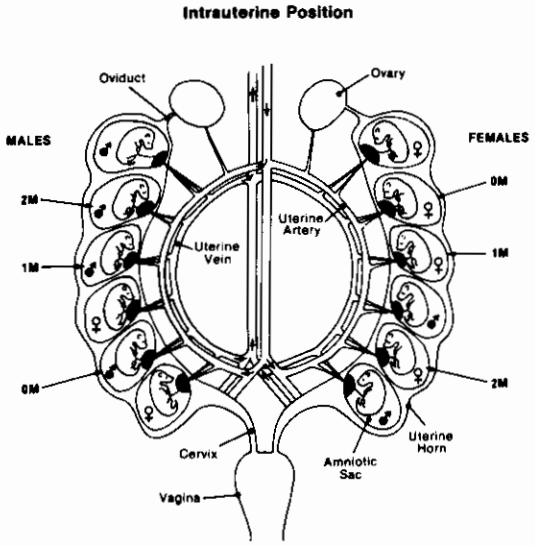

FIG. 1. Diagram of the uterine horns and vascular supply of a pregnant mouse at term. The labels 0M, 1M and 2M refer to the number of male fetuses an individual is contiguous to: 0M = between 2 females; 1M = between a male and female; 2M = between two males.

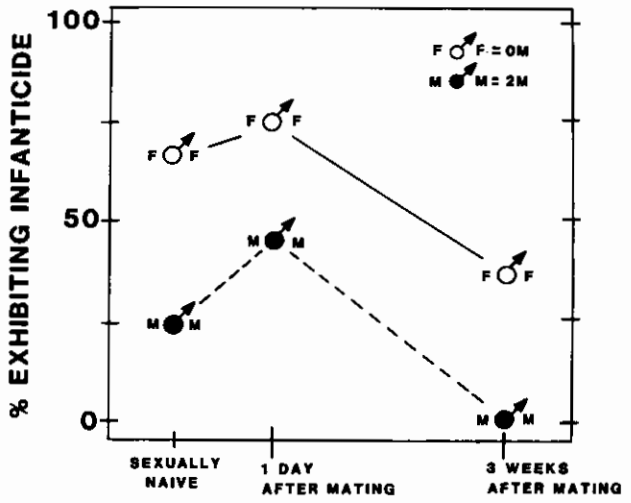

FIG. 2. Percentage of 2M versus 0M males exhibiting infanticide before mating, one day after mating and three weeks after mating.

and parental behavior observed among adult virgin males is correlated with an individual's intrauterine position (31). Thus, in the following experiment, we tested whether individual variation among males in infanticide and parental behavior as a result of mating might also be influenced by prior intrauterine position. Our objective here was to compare mating-induced changes in behavior toward pups exhibited by males obtained from 2M versus 0M intrauterine positions (since 1M individuals have always been found to show intermediate characteristics between the 2M and 0M extremes, ethical considerations did not warrant the sacrifice of additional pups by testing 1M males).

To obtain males from known intrauterine positions, pregnant CF-1 females were sacrificed by decapitation on day 19 of pregnancy several hours before the time of normal parturition (28). Pups were removed sequentially from the uterine horns by Cesarian section and classified in accordance with the scheme in Fig. 1. Male 2M and 0M pups were identified and raised in randomly mixed litters by foster mothers giving birth the same day. All pups were weaned at 23 days of age. When adult (80 days of age), 19-0M and 15-2M virgin males were each tested with a newborn pup at three time points: several days before mating, and at both 1 and 21 days after mating. Mating was accomplished by placing several estrous females in the male's home cage for four hours and was verified by the presence of a copulatory plug.

Figure 2 reveals significant differences between 2M versus 0M males in regard to the frequency of infanticide and parental behavior. When sexually naive, 0M males were three times more likely to exhibit infanticide than their 2M counterparts: 63% versus 20%, respectively ($\chi^2 = 4.7$, $p < 0.05$). This replicates previously reported differences in infanticide between 0M and 2M virgin CF-1 males (31). By one day after mating, infanticide was elevated in both the 0M and 2M groups; several parental males became infanticidal as a result of the stimulus of ejaculation. By three weeks after mating—when a male's own offspring would be born (Day 19 of pregnancy in CF-1 mice)—100% of the 2M males exhibited parental behavior, whereas 37% of the 0M males remained infanticidal ($\chi^2 = 4.9$, $p < 0.05$). Thus, developing in utero between 2 female fetuses (0M individuals) appears to render a high proportion of male mice insensitive to the inhibitory effects of mating on infanticide.

Experiment 2: The Effects of Castration and Testosterone Replacement on Infanticide and Parental Behavior in Sexually-Naive Male Mice

The previous experiment revealed that differential exposure to sex steroids during fetal development dramatically influenced the behavior of male mice toward young both before and after mating. Some steroid-sensitive behaviors are "organized" during perinatal development and do not require the presence of specific gonadal hormones in order for the behavior to occur in adulthood (1,30). In contrast, other behaviors may be "sensitized" during perinatal development and therefore require the presence of gonadal hormones in adulthood for the behavior to occur ("activation"). The objective of this experiment was to verify that testosterone would be required for an adult male that had not previously encountered a pup to exhibit infanticide.

Ten adult, naive males were castrated. When tested with a pup for the first time ten days later, all 10 males behaved parentally. Each male was then implanted with a 1-cm long silastic capsule (0.062 in i.d., 0.125 in o.d.: Dow Corning # 602-285) containing 5 mg of testosterone dissolved in 0.02 cc of sesame oil. When tested ten days after implantation, 5 males (50%) killed pups and 5 males still behaved parentally. The testosterone capsules were removed and four days later all ten males again behaved parentally toward pups (Fig. 3). When viewed in conjunction with the results from the pretest of all virgin males (see the General Method section), these findings demonstrate that about 50% of gonadally-intact CF-1 males and 50% of castrated CF-1 males given replacement testosterone will exhibit infanticide when they encounter a pup. Both castration and the removal of testosterone support in castrated males abolished infanticidal behavior.

Experiment 3: Prior Experience With Pups and the Effect of Castration

The results of the previous experiment show that once males had been castrated and implanted with testosterone, there were no effects of infanticidal experience on their subsequent behavior

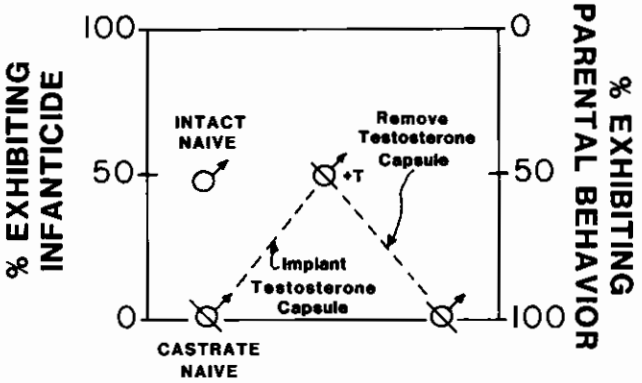

FIG. 3. The effects of castration and testosterone replacement on infanticide and parental behavior. All males were castrated before their first test with a pup. The percentage of intact naive males exhibiting infanticide on their first test with a pup (from pretest data in the General Method section) is shown here for comparison purposes.

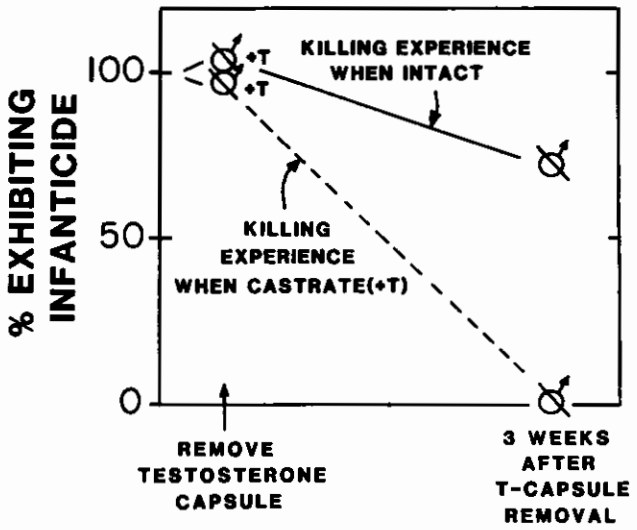

FIG. 4. Experiential effects of infanticide with and without the gonads. The group with killing experience after castration (see Experiment 2) is redrawn here from Fig. 3 for comparison purposes.

toward young. In the following experiment, however, we asked whether intact males with experience killing pups before being castrated would exhibit the same responses as their counterparts in Experiment 2.

We castrated 11 of the spontaneously infanticidal virgin males pretested earlier and implanted each male with a silastic capsule containing 5 mg of testosterone (see Experiment 2). The 11 experienced killers were retested with a pup ten days later. All 11 males were still infanticidal. The testosterone capsules were removed and, when tested three weeks later, 8 males still killed pups (73%) while only 3 males (27%) now behaved parentally. Even though the males tested in this experiment exhibited infanticide twice prior to being tested for their behavior toward young in the absence of testosterone, while in Experiment 2 males only had one opportunity to exhibit infanticide prior to removal of testosterone, these results suggest an unusual behavioral interaction between experience with pups and the presence of intact gonads (Fig. 4). Specifically, if males have had experience killing a pup before castration, most males are likely to continue killing pups even in the absence of testosterone.

Experiment 4: The Effect of Castration on the Mating-Induced Inhibition of Infanticide

Experiments 2 and 3 established the role of testosterone and pup-killing experience in supporting infanticidal behavior in sexually-naive males. The following study examined the effect of gonadectomy, and hence, the absence of testosterone, on the inhibition of infanticide in CF-1 males following mating.

We castrated 16 of the pretested infanticidal males and implanted each with the standard 5-mg testosterone capsule in order to maintain mating behavior. Ten days later, all 16 males still exhibited infanticide. Each male was then allowed to mate with an estrus female; after mating was confirmed, the testosterone capsule was removed. When tested four days later, 12 (80%) of the 16 males still killed pups. However, most of the remaining infanticidal males did indeed change their behavior towards pups by 21 days after mating: only 5 males (30%) now killed pups while the remaining 11 males (70%) behaved parentally ($\chi^2 = 5.49$, $p < 0.07$).

When compared to the 11 pretested infanticidal males described in Experiment 3 who were treated similarly, but had not been allowed to mate, fewer mated males exhibited infanticide 21 days after mating (Fig. 5). Although the difference was not statistically significant ($\chi^2 = 3.0$, $p < 0.09$), the marginal probability level here did not warrant the sacrifice of additional animals. These results are, however, consistent with the hypothesis that ejaculation can override the effects of prior infanticidal experience and thus inhibit pup-killing in castrated males.

Experiment 5: The Effect of Testosterone on the Facilitation of Infanticide After Mating

The objective of this experiment was to determine whether the facilitation of infanticide previously reported to occur in parental male mice within the first four days after mating (33) would also be observed in castrated males in the absence of testosterone.

Twenty intact virgin males that behaved parentally on the pretest were castrated and implanted with the standard 5-mg testosterone capsule. When tested 10 days later, 19 out of the 20 males behaved parentally. The 19 parental males were each allowed to mate with an estrous female. After mating was confirmed, the testosterone capsule was removed immediately from 10 of the males while the capsules were left in the other 9 males. Each male was then retested with a pup at 4 and 21 days after mating. None of the 10 males who had their testosterone capsules removed exhibited infanticide at either 4 or 21 days after mating. In contrast, three out of 9 (33%) of the males with the testosterone capsule killed pups at 4 days after mating; nevertheless, all 9 of these males still behaved parentally when tested at 21 days after mating.

The low percentage of males induced to exhibit infanticide at 4 days after mating does not allow any firm conclusions here; nevertheless, a postmating facilitation of infanticide occurred only in those parental males who still had testosterone capsules at the time of testing. This suggests that the presence of testosterone is required for parental male mice to exhibit infanticide as a result of ejaculation.

Experiment 6: The Effect of Hypophysectomy on the Mating-Induced Inhibition of Infanticide

Ejaculation is known to induce a dramatic surge in both

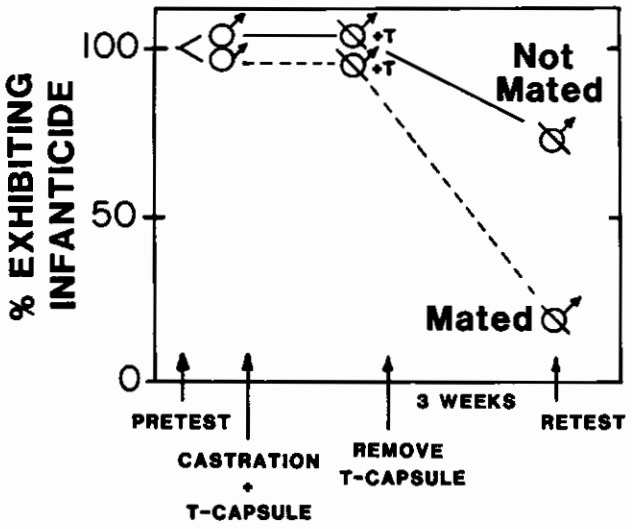

FIG. 5. The effect of gonadectomy on the mating-induced inhibition of infanticide. The not-mated group (see Experiment 3) is redrawn here from Fig. 4 for comparison purposes.

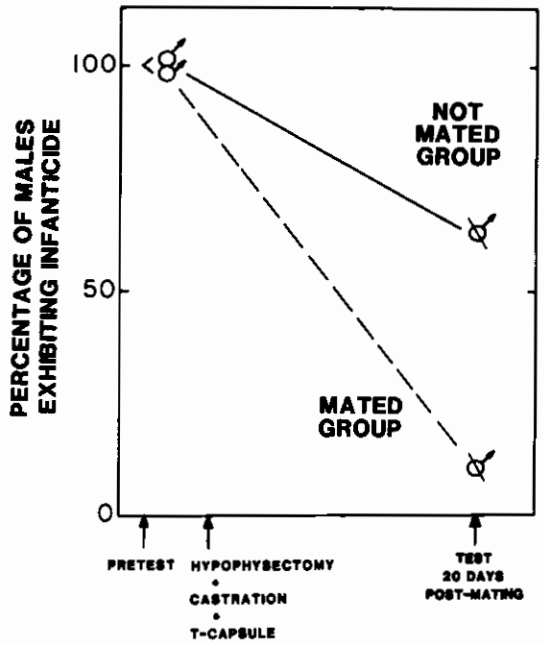

FIG. 6. The effect of hypophysectomy on the mating-induced inhibition of infanticide. All males were also castrated and implanted with a testosterone capsule (to maintain mating behavior and infanticide).

prolactin and LH (luteinizing hormone), which then leads to a surge in testosterone in male mice (3,7). The results from castrated males in Experiments 4 and 5 showed that the inhibition of infanticide after mating occurred in the absence of *changes* in testosterone secretion resulting from mating. We thus hypothesized that mating-induced changes in pituitary hormone secretion were also not responsible for triggering the postmating inhibition of infanticide. This issue was considered important since prolactin has widespread synergistic effects with steroids on behavior, particularly the maintenance of lactation and parental behavior in females (21,25), and infanticidal males that cease killing pups sometime during the interim between mating and the birth of their own offspring almost always exhibit parental behavior.

We examined the role of the pituitary by hypophysectomizing 22 pretested virgin males who were spontaneously infanticidal. Each male was anesthetized with Nembutal and hypophysectomized by aspirating the pituitary through a 16-gauge syringe. The syringe barrel was guided through the auditory canal to the base of the brain by using a Stoettling hypophysectomy stereotaxic apparatus (Model # H-200). While still anesthetized, each hypophysectomized male was also castrated and implanted with a 5-mg testosterone capsule to maintain mating behavior. Three weeks after surgery, 12 randomly chosen males were allowed to mate with an estrous female, while the remaining 10 males were not allowed to mate. The testosterone capsules remained in the males throughout the remainder of the experiment.

As shown in Fig. 6, a significant inhibition of infanticide occurred by three weeks after ejaculation in the mated group of hypophysectomized males: only 1 of the 12 mated males exhibited infanticide, but 6 out of 10 of the nonmated males continued to kill pups at this time ($\chi^2 = 4.54$, $p < 0.05$). The absence of the pituitary, however, did appear to diminish the expression of parental behavior in the mated group: only 5 of the 11 noninfanticidal males exhibited parental behavior while the remaining 6 males simply ignored the pup. Nevertheless, the results of this experiment confirm that the mating-induced inhibition of infanticide occurred independent of pituitary hormone secretions or *changes* in gonadal secretions resulting from mating. However, because castrated males in both this and the previous experiments had to be supplied with replacement testosterone in order to accomplish mating, we cannot be certain whether concurrent exposure to testosterone per se during ejaculation may be a permissive factor in initiating these behavioral changes.

GENERAL DISCUSSION

Our first conclusion here is that the behavior of male mice toward young—both before and after mating—is indeed influenced by whether an individual is positioned next to same or opposite sex fetuses in utero. As shown in Experiment 1, 2M males, those that underwent development between two male siblings, were far more likely to exhibit parental behavior than were their 0M male counterparts who developed between two female siblings. The intrauterine position phenomenon is a naturally-occurring developmental event that results in a gradient of sex steroid exposure among individuals during late fetal development. Differential exposure to testosterone among 2M (highest testosterone levels), 1M (intermediate testosterone levels) and 0M (lowest testosterone levels) positioned house mice has, in both sexes, been correlated with a wide range of behavioral, reproductive and morphological differences exhibited later in life (28, 31, 32, 35, 36). While much of the research on intrauterine position has been accomplished with laboratory stocks of house mice, position effects have been verified in wild-trapped house mice (unpublished observation), as well as rats, gerbils and pigs (5, 6, 20).

Statistically, in any large random sample of CF-1 house mice (averaging 6 pups/uterine horn), 50% of all individuals are 1M while 20% are 0M and 20% are 2M (about 10% of individuals cannot be classified by this scheme) (28). A schematic illustrating the trimodal frequency distribution of 0M, 1M, and 2M phenotypes and their relative position along a generic response gradient is shown in Fig. 7. Whenever a correlation has been found relating a particular behavioral, somatic or reproductive characteristic to

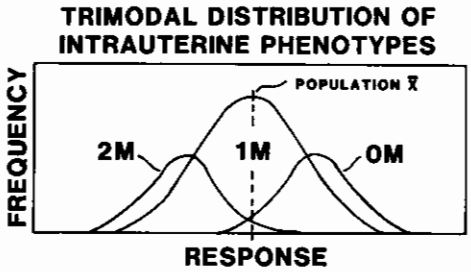

FIG. 7. Frequency distribution and generic response pattern of 2M, 1M and 0M individuals in a large population sample.

prior intrauterine position, 1M individuals have, on average, always been found intermediate in their characteristics between the 0M and 2M extremes. Thus, the population mean is centered on 1M individuals.

Because the adult responses of 2M versus 0M individuals are a consequence of being at opposite ends of a gradient of sex steroid exposure during fetal life, a primary conclusion about the temporal dynamics of infanticide and parental behavior is that adult male mice are programmed for different behavioral thresholds as a function of intrauterine position. Figure 8 represents a behavioral matrix illustrating our suggested scheme for relating increasing fetal testosterone exposure to one of four possible behavioral sequences exhibited by CF-1 males. The temporal changes in infanticidal and parental strategies are depicted here in three phases: 1) *Premating*, when males are virgin; 2) *Pregnancy*, defined as the time from ejaculation until a mate gives birth; and 3) *Lactation*, when a male's own sired pups are present and vulnerable to attack.

As illustrated by Fig. 8, 2M males receive maximal testosterone exposure during late fetal development. They are the most likely individuals to exhibit a "pure" parental strategy. In sharp contrast, 0M males receive minimal testosterone exposure during late fetal development, and they are the most likely individuals to exhibit a "pure" infanticidal strategy. We have observed that about 10–20% of CF-1 males are always parental and about 10–20% of males are always infanticidal, regardless of their mating history, while 60–80% of all males exhibit either a *PARENT* to *KILL* to *PARENT* sequence or a *KILL, KILL* to *PARENT* sequence during the respective premating, pregnancy and lactation phases (33,37). The frequencies of these four strategies match remarkably well, percentagewise, with the expected proportions of 2M, 1M and 0M phenotypes.

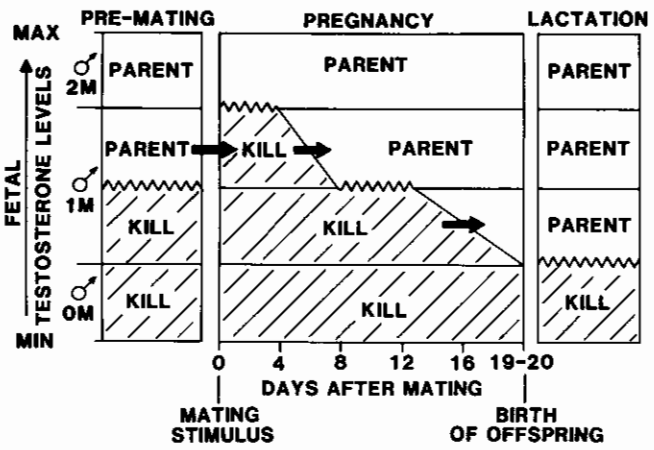

FIG. 8. Behavioral matrix relating increasing fetal testosterone exposure to the temporal changes in infanticide and parental behavior exhibited by adult CF-1 males.

Since there is substantial overlap in response characteristics between 2M and 1M adults and between 1M and 0M adults (see Fig. 7), one concept concerning Fig. 8 needs to be explicitly emphasized: our scheme does not imply that each of the three intrauterine phenotypes is locked-in to one of the specific behavioral patterns shown here; this diagram is only meant to visualize a probability distribution, like Fig. 7, showing which type of adult is most likely to exhibit spontaneous infanticide or parental behavior, and which type of adult is most likely to follow through on a specific behavioral sequence after mating.

A second point evident in Fig. 8 is that in previous studies where spontaneously parental CF-1 males became infanticidal as a result of ejaculation, most of them re-expressed their parental behavior within 8 days. Spontaneously infanticidal CF-1 males, on the other hand, typically do not cease killing pups and express parental behavior until 8 or more days after they mate (33). This suggests that fetal exposure to sex steroids also influences the timing of the inhibition of infanticide following ejaculation.

Another conclusion verified here is that a male mouse's propensity to exhibit infanticide is negatively correlated with intermale aggression. Experiments involving pharmacological manipulations during late fetal development have shown that exposure to elevated levels of testosterone greatly diminishes infanticide but greatly increases intermale aggression (19, 22, 27, 34). 2M males are thus more aggressive toward other males than are 0M males (36). Yet, as noted in Experiment 1, a high proportion of 2M males do not exhibit infanticide, whereas most 0M males do exhibit infanticide [see also (31)]. Since high testosterone exposure during fetal life has opposite effects on adult intermale aggression and infanticide, the inverse relationship between these two types of aggression suggests they are correlated behaviors (34). The results concerning infanticide may, offhand, seem counter to the classical sensitizing effects of testosterone during fetal life and its role in maintaining and potentiating aggression during adulthood, but an overview of the social structure of wild mouse populations offers a parsimonious explanation of how this unique behavioral dichotomy evolved.

In free-living situations, a dominant male usually defends a territory containing several breeding females. A 2M male may be more likely to remain in his natal territory by aggressively repelling or subordinating rival males (32), and there is evidence that virtually all offspring in a mouse deme are produced by the one dominant male (8). A predisposition for parental behavior would prevent a dominant 2M male from accidentally killing his own or related offspring. On the other hand, if a subordinate 0M male can supplant a dominant male and obtain mating after being dispersed from his natal deme, a strong infanticidal tendency would ensure the elimination of his competitor's pups. Mating alone does not seem to inhibit infanticide in many 0M male mice, but, although we have not yet tested this possibility, it is likely that these individuals will become parental before the birth of their progeny *provided* they are also allowed to cohabit with a female (9, 14, 24). When viewed in toto, the social organization of feral populations suggests that infanticidal behavior in male mice, and possibly other mammals, co-evolved in harmony with other hormone-mediated forms of aggression. Evolution has probably economized here: selection pressures have simply driven the physiological consequences of fetal testosterone exposure on intermale aggression and infanticidal behavior in totally opposite directions.

While exposure to elevated levels of testosterone in utero appears to greatly diminish spontaneous and mating-induced

infanticide, testosterone is still required for sexually-naive adults to exhibit this behavior. We found in Experiment 2 that castration eliminated infanticidal behavior among males inexperienced with pups; 100% of these males behaved parentally. But, when the same castrated males were given replacement testosteone, half of the previously parental males became infanticidal, resulting in the typical 50/50 distribution of spontaneously infanticidal versus noninfanticidal males we routinely observe in any large sample of CF-1 males from our colony [see also (33)]. This suggests that all male mice are capable of expressing parental behavior; however, fetal exposure to minimal steroid levels during gestation greatly increases the probability that a male will exhibit spontaneous infanticide.

In contrast to the second experiment, Experiment 3 demonstrated that if a gonadally-intact male is given the opportunity to kill a pup, castration does not eliminate this behavior in most males. When examined together, the results from Experiments 2 and 3 suggest two conclusions: first, that testosterone is required for a virgin male to exhibit infanticide upon his first encounter with a pup; and second, that the intact gonads somehow provide facilitating feedback for maintaining infanticidal behavior. The physiological interaction between the gonads and the initial experience of killing a pup is indeed unusual, since the presence of intact testes resulted in a markedly different behavior from that observed when only testosterone released from a silastic capsule was present.

Finally, the results of Experiment 5 suggest that testosterone is required for spontaneously parental males to become infanticidal as a result of mating. When testosterone was removed immediately after ejaculation, all parental males continued to behave parentally. In spontaneously infanticidal males, however, the results from both Experiments 4 and 6 suggest that neither testosterone nor the pituitary influenced the timed inhibition of infanticide that occurred by three weeks after mating. Thus, the mating-induced inhibition phenomenon seems to be a neurally-mediated response that operates independent of pituitary hormone secretions or *changes* in gonadal secretions associated with mating and ejaculation.

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NOTE ADDED IN PROOF

A major ethical issue of the General Method section concerned the potential injury to the live pups used in these experiments. We have since found a humane and accurate test procedure for assessing infanticide and parental behavior. By enclosing a live pup within a protective wire mesh screen, neonates are buffered from attack, and no injuries occur (see Perrigo, G.; Bryant, C.; Belvin, L.; vom Saal, F. S. The use of live pups in a humane, injury-free test for infanticidal behaviour in male mice. Anim. Behav.; in press).