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Time-Contingent Change in Infanticide and Parental Behavior Induced by Ejaculation in Male Mice

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VOM SAAL, F. S. *Time-contingent change in infanticide and parental behavior induced by ejaculation in male mice.* PHYSIOL BEHAV 34(1) 7–15, 1985.—About 50% of sexually-naive male CF-1 mice (*Mus musculus*) commit infanticide (kill young). But, 80–90% of male mice commit infanticide between 1–4 days after mating. Between 12 and 50 days after mating infanticide is inhibited and most males (80–100%) behave parentally toward young (they build a nest, retrieve and groom the young, and hover over the young to keep them warm). Beginning at 60 days after mating, infanticide is again facilitated and parental behavior is inhibited (70–80% of males commit infanticide). The facilitation and inhibition of infanticide as a function of time after mating is mediated by the act of ejaculating rather than by mounting and intromitting during mating or by cohabiting with a female either before or after mating. The experience of committing infanticide either prior to mating or within the first 4 days after mating does not influence the facilitation of parental behavior that occurs 12 days after mating. But, repeated contact with young after males become parental serves to maintain parental behavior for an extended period of time. The age of the young (newborn–20 days old) utilized as stimulus animals does not influence these time-dependent changes in the behavior of male mice after ejaculation. This phenomenon is unique in that a response (infanticide) to a novel stimulus (young) is facilitated, then inhibited, and then facilitated again as a function of time between an event (ejaculation) and exposure to the novel stimulus. A male's own offspring would be born about 19 days after mating and be weaned by 50 days after mating, which is almost exactly the time during which parental behavior is facilitated and infanticide is inhibited in male mice. It is possible, therefore, that this phenomenon may represent a mechanism by which male mice are inhibited from killing their own offspring.

Infanticide Pup killing Parental behavior Maternal behavior Ejaculation Sexual selection

BECAUSE infanticide, the killing of conspecific young, has generally been viewed as an aberrant behavioral response to a breakdown in social structure, until quite recently few systematic studies of the variables which might influence infanticide in any species had been conducted (c.f., [10]). This view of infanticide was based in large part on the observation by Calhoun [6] of aberrant behavior, including infanticide, in rats confined in an environment in which population density was allowed to increase without intervention. Hrdy [11] has presented a contrasting hypothesis: that infanticide might be an adaptive behavior under certain circumstances. The view that infanticide has evolved because it is adaptive in some situations does not mean that all acts of infanticide must be considered to be adaptive, since there is little argument that a breakdown in social behaviors and an increase in both infanticide and aggression between adults occurs during times of social stress in rats and mice, as well as other rodents [6, 7, 18].

The experiments described in this paper were undertaken following the chance observation that male mice used as colony studs were significantly less likely to commit infanticide and were more likely to behave parentally toward young than were sexually-naive males. Subsequent to this initial finding, vom Saal and Howard [35] reported that, in addition to sexual experience, dominance status also influenced the behavior of male mice toward young, and other recent articles have reported similar findings [4,13]. The present experiments were designed to analyze in detail the phenomenon of time-contingent change in the behavior of male mice toward young following mating. I report here that this change in the behavior of male mice toward young is induced by the act of ejaculating during coitus. The inhibition of infanticide and facilitation of parental behavior represents a transient behavioral change beginning at 12 days and ending at 60 days after mating, which is almost exactly the time during which male mice might encounter preweanling young that they had sired.

GENERAL METHOD

Animal Housing

The animals used in these experiments were CF-1 house mice (*Mus musculus*), which have been maintained as an outbred stock in a closed colony since 1979. The original

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TABLE 1

THE PERCENT OF NAIVE MALE MICE THAT COMMITTED INFANTICIDE, BEHAVED PARENTALLY TOWARD OR IGNORED TWO NEWBORN MICE THAT WERE PLACED INTO A MALE'S CAGE FOR 30 MIN

Number of Days Between First and Second Test	First Test			Second Test		
	Infanticide	Parental	Ignore	Infanticide	Parental	Ignore
4	43	53	4	43	57	0
8	47	40	13	43	43	14
14	53	27	20	43	40	17
35	47	40	13	60	40	0
60	43	30	27	43	57	0
Mean for all Groups	46.6	38	15.4	46.4	47.4	6.2

The males were tested for their behavior toward young and then randomly assigned to groups (30 males/Group) and retested after different time periods. All males were housed individually throughout the experiment.

breeding stock was purchased from Charles River Farms (Wilmington, MA). All males were reared in mixed-sex litters (10 young per litter) and weaned when 23 days old, at which time they were housed 5/cage with male sibs. When the males were 35 days old, they were housed individually in 18×29×13 cm polypropylene cages to avoid fighting, since dominance status influences the behavior of male mice toward young [13,35]. Animal rooms were maintained at 25±1°C on a 14L:10D cycle, with lights on at 0600 hr.

Testing Procedure

All males were mated in their home cages. Unless specifically indicated, cages were changed at weekly intervals. The infanticide testing procedure consisted of placing two 1-day-old mice into the cage of a male and then examining the pups 30 min later. In none of the experiments described herein were males tested with young that they had sired. One of three possible behavioral responses was recorded at this time: (1) infanticide—one or both pups were dead; (2) parental behavior—one or both pups were in a nest with the male hovering over them, and the pups were warm, or (3) ignore—neither pup was in the nest or wounded, and both pups were cold (newborn mice cannot thermoregulate). In many cases, of course, these pups were not totally ignored, but this term is used to indicate that no evidence of disturbance of the pups was evident at the end of the 30-min test period. Within limits, the length of time that the young are left with the male does not appear to influence the proportion of adult male mice that are observed to exhibit infanticide (15 min–24 hr; F. vom Saal, unpublished observation; [26]). Either infanticide or parental behavior occur almost immediately after young are introduced into a male's cage. Also, neither the age (newborn, 7-days old, 10-days old) nor the sex of the young influences the tendency of males to exhibit infanticide or parental behavior [26,35].

In these experiments 2 pups were placed into each male's cage. In subsequent experiments, we have utilized 1 pup as the stimulus with no difference in the frequencies of behaviors reported below (M. McCarthy and F. vom Saal, unpublished observation). In addition, in other studies with both CF-1 and wild male mice ([35]; M. McCarthy and F. vom Saal, unpublished observation), we have observed that males that kill 1 pup eventually kill all pups when males come in contact with an entire litter of young. On repeated tests of the same males (see Table 1), males that killed one of the 2 pups on one test usually killed both pups on the other test. Thus, in the studies reported here males that killed either 1 or 2 pups were classified as infanticidal and males that behaved parentally toward either 1 or 2 pups were classified as parental.

Other testing procedures have also been utilized in some previous studies of infanticide. For example, in some experiments males were allowed to enter chambers containing females and their nursing young. The frequency of infanticide in naive or sexually-experienced CF-1 male mice does not differ based on whether pups are placed into a male's cage or a male is placed into a cage containing a female and her nursing litter [35]. In contrast, in wild male mice, the testing procedure has a marked effect on whether males commit infanticide (M. McCarthy and F. vom Saal, unpublished observation).

Moving males from one cage to another is stressful, and this could potentially interact with other variables in influencing the behavior of male mice toward young. The procedure of placing the young into a male's cage was thus utilized in all of the experiments described in this paper so that the greatest number of variables were controlled. This procedure not only involves the least amount of distrubance to the male, but results in a minimal loss of life of young relative to procedures involving placing a male with a female and her entire litter.

Statistical Analyses

An important issue in studies of infanticide is the unit of analysis that should be used. In all of the studies which I will describe, the behavior of each male served as the unit of analysis. In some previous studies (c.f., [13, 14, 19]) the total number of young killed in all litters within an experimental condition has been presented, and each death within a single litter has been treated as an independent act of infanticide, even though all young within a litter were killed by a single male. This method of analysis violates the assumption of the Chi Square statistic that all events are independent, and conclusions concerning rodent infanticide based on these studies are open to question.

TABLE 2

THE PERCENT OF DIFFERENT GROUPS OF MALE MICE (30 MALES/GROUP) THAT COMMITTED INFANTICIDE, BEHAVED PARENTALLY TOWARD OR IGNORED TWO NEWBORN MICE THAT WERE PLACED INTO A MALE'S CAGE FOR 30 MIN

		Days after Mating							
	Premating	4	12	20	35	42	50	60	90
Infanticide	50	83	10	10	17	30	23	77	73
Parental	23	17	83	80	63	63	57	17	23
Ignore	27	0	7	10	20	7	20	6	4
χ^2 vs. Premating group:		10.8	21.3	19.6	10.8	10.6	7.4	5.6	6.8
Significance level:		$p<0.005$	<0.001	<0.001	<0.005	<0.005	<0.05	<0.05	<0.05

The males were tested at different times after mating with a female.

METHOD AND RESULTS

Age of Males at Initial Testing

Experiment 1. The tendency for male mice to commit infanticide changes during adolescence, but the ontogeny of infanticide varies in different strains of mice. In C57BL/6J mice, no change in the frequency of infanticide is observed after males reach 55 days of age (70% commit infanticide), while in DBA/2J male mice, a dramatic increase in the frequency of infanticide occurs between 65 days of age (20% commit infanticide) and 135 days of age (65% commit infanticide; 28). No change in the proportion of the F_{1-4} male offspring of wild mice that commit infanticide (80–90%) occurs after the males are 60 days old (M. McCarthy and F. vom Saal, unpublished observation).

Male CF-1 mice were tested for infanticide when 60 days old, and 30-days later the same males were retested when 90 days old. Fewer male mice committed infanticide when tested at 60 days of age (N=204; infanticide=29%, parental=55%, ignore=16%) than when tested again at 90 days of age (N=204; infanticide=49%, parental=46%, ignore=5%). When the data for the 60-day old males were compared to those obtained from another group (N=150) of 90-day-old males (see Experiment 2; Table 1, mean for all groups), the 60 day old males were significantly less likely to commit infanticide and were more likely to behave parentally than were the 90-day old males, $\chi^2(2)=12.7$, $p<0.01$. Since there is a change in the tendency of CF-1 male mice to commit infanticide or behave parentally between 60 and 90 days of age, but no further change in behavior toward young occurs between 90 and 150 days of age ([32] see Experiment 2, Table 1), in all of the following experiments, males were initially tested when about 90 days old.

The Effect of Mating Experience on Infanticide and Parental Behavior

Experiment 2. To determine whether males would exhibit the same behavior toward young on repeated tests, and also to determine whether retesting males at different intervals influenced behavior toward young, 150 sexually-naive males were tested for infanticide and then retested at 4, 8, 12, 35 or 60 days after the first test (30/Group). With few exceptions, males that committed infanticide on the first test committed infanticide on the second test (Table 1). This experiment thus serves as a control for age at testing and length of time of isolation for the males tested at different intervals after mating in Experiment 3.

Experiment 3. The data presented in Table 2 reveal the remarkable effect that mating experience has on the tendency of male CF-1 mice to either commit infanticide or behave parentally toward young. In this experiment 240 male CF-1 mice were paired with females for 3 hours, after which time the females were removed from the males' cages and examined for vaginal plugs (Day 0=the day of mating). The males were then randomly assigned to groups to be tested for infanticide at different intervals after mating (30/Group). The behavior of these groups of males toward newborn young was compared with the behavior of a group of 30 sexually-naive males (premating group). Four days after mating there was a significant increase in the proportion of males committing infanticide (83%) compared to sexually-naive males (50%). However, 12 days after mating just the opposite was the case: there was a significant decrease in the proportion of males that committed infanticide and an increase in the proportion of males that behaved parentally relative to the premating group. This inhibition of infanticide was also observed in males tested at 20, 35, 42, and 50 days after mating. Between 50 and 60 days after mating, however, the facilitation of infanticide and inhibition of parental behavior was again observed. Males tested at both 60 and 90 days after mating were significantly likely to commit infanticide than were sexually-naive males.

Experiment 4. This experiment was conducted to determine whether results similar to those described in Experiment 3 would be obtained when the same males were tested for infanticide prior to mating and then retested with young at different intervals after mating. Ninety males were tested for infanticide, allowed to mate with a female, and then retested for their behavior toward young 1, 8 or 20 days after mating (30 males/Group). The results presented in Table 3 reveal that infanticide was enhanced relative to premating levels 1 day after mating, and all of the males that did not commit infanticide 1 day after mating (17% parented or ignored the young) had also not committed infanticide on the pretest. Males tested 8 days after mating behaved as if they

had not mated at all: only 1 out of 30 males did not exhibit the same behavior on both the pre- and post-mating test. Finally, most of the males tested 20 days after mating behaved parentally. Again, all males that committed infanticide 20 days after mating had also committed infanticide on the pretest. These findings reveal that there are a few male mice that do not change in their behavior toward young 1 or 20 days after mating, but, instead, exhibit the same behavior toward young both before and after mating. Chi-square analysis performed on the post-mating data again confirmed that there was a significant effect of length of time since mating on the behavior of males toward young.

Experiment 5. In a situation in which a male mated with one female and then between 2 and 3 weeks later mated with a second female, it would clearly not be adaptive for the second mating to facilitate infanticidal behavior, since the male might well come into contact with young produced by his first mating. To examine this situation, 14 naive males were tested for their behavior toward young (43% committed infanticide, 50% behaved parentally, and 7% ignored the young). These males were then mated with first one female (Day 0) and then with a second female 15 days later. Four days after the second mating (19 days after the first mating) infanticide proved to be inhibited and parental behavior facilitated (14% of the males committed infanticide and 86% behaved parentally). The behavior of these males toward young differed significantly from that of male mice 4 days after mating for the first time (83% committed infanticide and 17% were parental; Table 2; Day 4 Group; $\chi^2(1)=19.2$, $p<0.001$). It appears, therefore, that the facilitation of infanticide within 4 days of mating only occurs in sexually-naive males. In addition, the second mating 4 days prior to exposure to young does not interfere with the inhibition of infanticide at the time that the young produced by the first mating would be born.

Experiment 6. In Experiments 3 and 4 all males were tested with newborn young at different intervals after mating. The objective of both this and the next experiment was to determine whether the behavior of males would change as a function of time since mating if the young were near the actual age that the male's own offspring would be at the time of testing. Initially, it was hypothesized that males might commit infanticide when exposed to newborn young 30 days after mating but behave parentally toward young that were near the age of the males' own offspring. However, the results of Experiment 3 revealed that this was not the case.

A group of 30 males was tested with newborn young 20 days after mating (when their own offspring would just be born), and these same males were then also retested with 10-day-old young 30 days after mating. At ten days of age, mouse pups are not as mobile as are mice that are older than 13 days of age. Mice become very active beginning on Day 13 after birth coincident with the opening of their eyes (which are closed prior to this time).

When tested with 10-day-old mice, only one of the males did not exhibit the same behavior previously exhibited toward newborn young on Day 20 after mating (Day 20 Group: infanticide=10%, parental=73%, ignore=17%; Day 30: infanticide=7%, parental=73%, ignore=20%). The behavior of these males toward 10-day-old young on Day 30 after mating was compared to the behavior of sexually-naive males toward 10-day-old young (N=20; infanticide=55%, parental=25%, ignore=20%), and these two groups of males were found to differ significantly, $\chi^2(2)=16.0$, $p<0.001$.

Experiment 7. In this experiment the behavior of males toward male and female young which were close to the age of weaning (which is thought to occur between 3–4 weeks of age in wild mice) was examined. The age of onset of puberty in mice is influenced by population density, but in the laboratory, in male and female mice the onset of puberty typically occurs between 30 and 50 days of age [2, 30, 31]. Males were tested 40 days after mating for their behavior toward 20-day-old young, one of the young being a male and one a female (the male's own offspring would be 20 days old at this time). Previously, it was reported that male mice do not behave differently toward newborn male and newborn female mice [26,35]. In this experiment each male was observed during the 30-min test period.

Six of the 30 males that were tested (20%) attacked the young severely enough to inflict wounds, although none of the males in this experiment actually killed the young within the 30-min test period, and no difference in behavior toward the male or the female young was observed. The remaining 24 males (80%) did not exhibit biting attacks toward the 20-day-old young, although most of these males appeared aroused, and rough grooming was observed. Rough grooming has generally been considered to be a component of intermale aggressive behavior in mice [31], but males were equally likely to exhibit this behavior toward male or female 20-day-old young. Retrieving and hovering over the young by the males was not observed, but the young were very active during the test period and did not remain in the nest.

Twenty sexually-naive males were also tested for their behavior toward 20-day-old young. In this experiment a male and a female 20-day-old mouse were placed into each male's cage. The behavior of each male was observed during the

TABLE 3

THE PERCENT OF MALES THAT COMMITTED INFANTICIDE, WERE PARENTAL TOWARD OR IGNORED TWO NEWBORN MICE THAT WERE PLACED INTO A MALE'S CAGE FOR 30 MIN

	Pretest	Retest 1 Day After Mating
Infanticide	47	83
Parental	33	13
Ignore	20	4
	Pretest	**Retest 8 Days After Mating**
Infanticide	47	50
Parental	43	43
Ignore	10	7
	Pretest	**Retest 20 Days After Mating**
Infanticide	43	23
Parental	43	77
Ignore	14	0

All males were administered a pretest and then allowed to mate with a female one week later. The males were then randomly assigned to three groups (30 males/Group) and retested at different intervals after mating.

*1 vs. 8 vs. 20 days after mating: $\chi^2(4)=25.9$, $p<0.001$.

TABLE 4

THE PERCENT OF A SINGLE GROUP OF 30 MICE THAT COMMITTED INFANTICIDE, BEHAVED PARENTALLY TOWARD, OR IGNORED TWO NEWBORN MICE THAT WERE PLACED INTO A MALE'S CAGE FOR 30 MIN

	Days After Mating								
	4	12	20	35	50	60	70	80	90
Infanticide	83	10	0	13	13	17	20	20	24
Parental	17	87	93	77	80	73	77	77	73
Ignore	0	3	7	10	7	10	3	3	3

These males were first tested 4 days after mating with a female (see Table 1; Day 4 group) and then were retested at 8–15 days intervals.

test period, and no difference in behavior toward male or female young was observed. Fifteen of 20 sexually-naive males (75%) exhibited biting attacks toward the 20-day-old young (regardless of the sex of the young) which was indistinguishable from aggression that is observed toward submissive adult males (the young exhibited the typical submissive posture when attacked, and the attacks were directed toward the flanks). Five males (including one of the males that exhibited biting attacks) were observed to mount the young: 2 juvenile males and 3 juvenile females were mounted. Finally, one male neither mounted nor attacked but only exhibited rough grooming toward the young.

In summary, 80% of males that had mated 40 days prior to coming in contact with 20-day-old young were inhibited from exhibiting biting attacks toward the young during the 30-min test period, but 75% of sexually-naive males attacked 20-day-old young (mated vs. naive males; attack vs. no attack; $\chi^2(1)=14.9, p<0.001$). In neither case did the sex of the young influence the behavior of the adult males. Thus, mating-induced inhibiton of infanticide occurs in response to newborn young between 12 and 50 days after mating (see Table 2), as well as in response to young that are the age that the male's own young would be at 30 and 40 days after mating.

Edwards [9] reported that about 60% of gonadectomized male Swiss-Webster mice attacked 25–30 day-old young. In contrast, when adult mice are gonadectomized, there is a marked decrease in aggressiveness toward other adult males [31]. Edwards' [9] findings, as well as the present results, indicate that aggression toward 20–30-day-old-young is not influenced by the same variables as adult intermale aggression, since aggression toward prepubertal mice is influenced by neither the sex of the stimulus animal nor the circulating concentrations of gonadal steroids in adult males.

Experiment 8. In this experiment evidence was obtained that the repeated opportunity to behave parentally toward young results in the maintenance of parental behavior once it is initiated in male mice. The 30 males that had been tested for infanticide 4 days after mating (see Table 2; Day 4 group) were repeatedly exposed to newborn young 12, 20, 35, 50, 60, 70, 80 and 90 days after mating. Each test lasted for 30 min. The results presented in Table 4 reveal that between the fourth and twelth day following mating there was a complete reversal of behavior: infanticide was facilitated and parental behavior was inhibited on Day 4, while on Day 12 infanticide was inhibited and parental behavior was facilitated. The repeated exposure to young served to maintain parental behavior 90 days after mating (73% of the males behaved parentally; see Table 4; Day 90), while most of the males tested for the first time 90 days after mating committed infanticide (73% committed infanticide; see Table 2; Day 90 group). This difference is statistically significant, $\chi^2(2)=22.5, p<0.001$.

The results of Experiments 2–8 demonstrate that most sexually-naive male mice that are tested for their behavior toward young at 90 days of age and then retested at a later time exhibit the same behavior toward young on both tests, regardless of the interval between tests. But, males that mate with a female change in their behavior toward young as a function of the duration of the time between mating and exposure to young. Mating-induced facilitation of parental behavior begins at 12 days and continues through 50 days after mating. This facilitation of parental behavior is observed whether the stimulus animals are newborn pups or pups that are near the age that the actual offspring of the male would be at the time of testing. Finally, the experience of committing infanticide either prior to mating or within the first 4 days after mating does not influence the facilitation of parental behavior that occurs 12 days after mating. But, males given the opportunity to parent young for brief periods at 10 day intervals beginning 12 days after mating do not exhibit the decline in parenting and facilitation of infanticide which would otherwise occur by 60 days after mating.

Mechanism of Facilitation and Inhibition of Infanticide and Parental Behavior

Three experiments that were designed to investigate the particular aspect of mating which produces the changes in male behavior described above have been completed.

Experiment 9. Thirty male mice were placed with 2 diestrous, non-receptive females for 24 hr. The absence of mating was verified by examination of the females after 12 hr and 24 hr for the presence of vaginal plugs. Three weeks later the males were tested with 2 newborn young: 53% of the males committed infanticide, 27% behaved parentally, and 20% ignored the young. Cohabiting with a female but not mating thus has no significant effect on either infanticide or parental behavior in male mice (compared to premating group, Table 2).

Experiment 10. Thirty males were pretested for their behavior toward young (56% committed infanticide and 44% behaved parentally). Two weeks later the males were allowed to mount (at least 20 times with intromissions) a sexually-receptive female, but the females were removed before the males ejaculated. The males were retested 1 day

and 20 days later for their behavior toward young. Little change in behavior relative to the pretest occurred in these males at either 1 day (infanticide=56%; parental=40%; ignore=4%) or 20 days (infanticide=40%; parental=50%; ignore=10%) after mating.

Experiment 11. Thrity males were pretested for their behavior toward young (43% were infanticidal, 47% were parental, and 10% ignored the young). Two weeks later the males were allowed to ejaculate when paired with a sexually-receptive female. As soon as the males finished ejaculating (within 2 sec after the end of post-ejaculatory pause, during which male and female mice remain motionless; [21]), the males were re-housed individually in clean cages. This procedure prevented any post-ejaculatory contact with the female partner or her soiled bedding. One day after ejaculating 83% of these males committed infanticide, 14% behaved parentally, and 3% ignored newborn young, while 20 days after ejaculating 3% committed infanticide, 87% behaved parentally, and 10% ignored newborn young.

Taken together, the results of Experiments 9–11 demonstrate that contact with females without mating, or mounting and intromitting without ejaculating, does not influence the behavior of males toward young. But, males that ejaculate during coitus and have no post-ejaculatory contact with the female partner or her odor exhibit a change in their behavior toward young both 1 and 20 days after mating. Furthermore, the results of Experiment 11 confirm the findings in Experiments 4 and 8 that the experience of killing young either before mating or within 4 days after mating does not influence the inhibition of infanticide and facilitation of parental behavior 20 days after mating in male mice.

DISCUSSION

Pregnancy in house mice lasts between 18 and 20 days, depending on the strain. Young are typically weaned from 3 to 4 weeks after birth and, depending on population density, may remain within the natal territory or disperse [2, 15, 16]. A male mouse would thus typically be in contact with its own nursing young between 3 and 7 weeks after mating. The finding that between 2 and 7 weeks after mating there is a significant inhibition of infanticide in male mice is thus striking in that males tend not to be infanticidal at those times that they would be likely to come in contact with nursing young that they had sired. Within the first 4 or after 60 days following mating, there is actually a facilitation of infanticide in previously mated mice compared to sexually-naive males. Thus, infanticide is facilitated at times when a male would encounter the nursing young of another male, but infanticide is inhibited during times that a male might encounter its own nursing young.

In addition to influencing infanticide in male mice, mating also influences parental behavior. Between 2 and 7 weeks after mating, most male mice: build a nest similar to that built by a female just prior to parturition, retrieve the young, stimulate micturition in the young by licking the genitals periodically, and hover over the young to maintain their body temperature. These behaviors are sometimes referred to by the sex-linked term, maternal behavior. But, the present experiments demonstrate that these behaviors occur naturally (and predictably) in male as well as female mice. Therefore, at least in mice, it is inappropriate to label these behaviors as maternal, which suggests that they are female-typical but not male-typical behaviors. The term parental behavior is preferred to describe the complex of behaviors listed above since it identifies that the behaviors are exhibited by animals of both sex.

The results of some previous studies have suggested that infanticide and the complex of behaviors collectively referred to as parental behavior do not just represent opposite ends of a behavioral continuum [31, 32, 33, 34]. For infanticide to be exhibited, androgen must be present in the circulation, but the response to androgen is modulated by hormones during early life. In contrast, parental behavior is not mediated by circulating androgen, although the tendency for male mice to behave parentally is modulated by hormones during early life. The regulation of infanticide and parental behavior thus appears to be different, although in most cases the two behaviors represent alternative responses to young (only very rarely has a male been observed to first wound a pup and then also exhibit parental behavior toward the pup).

The behavior of males that are in the "infanticidal mode" or in the "parental mode" provides a striking contrast. Males that are infanticidal become highly aroused and exhibit tail vibrating (rattling) and eye-squinting in response to the presence of young. The males then lunge at the young and inflict multiple wounds. CF-1 males virtually never eat young that they kill (exhibit cannibalism), and infanticide is not synonymous with cannibalism, although in some mouse strains cannibalism is observed [27]. Prior to exhibiting an attack toward another adult male mouse, an aggressive male will also exhibit the same types of behaviors (tail rattling, eye squinting) observed in male mice just prior to committing infanticide. In contrast, male mice that exhibit parental behavior methodically begin building a deep nest once the young are placed into the cage. Also, once males have retrieved the young into the nest and are hovering over the young, they appear somewhat sedated and have to be pushed out of the nest so that the young can be examined. This is in marked contrast to the behavior of isolated male mice that are not handled; such males typically become very aroused when distrubed and will bite someone who attempts to touch them.

The results of Experiment 8 demonstrate that once male mice become parental as a result of having mated, the repeated opportunity to behave parentally (even for periods of only 30 min at intervals of 10–15 days) can serve to interfere with the facilitation of infanticide that would otherwise occur 60 days after mating in male mice, and an inhibition of infanticidal behavior and facilitation of parental behavior is maintained for an extended period of time. In a previous experiment, 33 males were tested 20 days after mating: 15% were infanticidal while 76% were parental. The same males were then tested 70 days later (90 days after mating): 88% were infanticidal while 12% were parental [35]. Thus, when males were repeatedly exposed to young at intervals of 10 days (Experiment 8; Table 4), parental behavior was maintained 90 days after mating, but when the interval between exposure to young was 70 days, the initial experience of parenting young 20 days after mating did not serve to maintain parental behavior, and most males committed infanticide 90 days after mating.

When the litters of female rats [5,22] or mice [25] that have just delivered are replaced daily with newborn young, behavior charcteristic of females that have just delivered a litter is maintained for extended periods of time (several months). Thus, the young serve as stimuli for eliciting parental behavior in adult females, and the characteristics of the young influence the behavior of the mother regardless of the duration of time following parturition. This phenomenon is

thus different from that reported here, since the characteristics of the young (newborn, 10 days old or 20 days old) do not appear to influence the inhibition of infanticide following mating in male mice.

Males which mount and intromit, but do not ejaculate, when paired with a female do not change in their behavior toward young, while males that ejaculate but have no post-ejaculatory contact with a female do change in their behavior toward young. It is thus proposed that some aspect of ejaculating during coitus (such as vaginal stimulation of the engorged penile cup which forms during ejaculation; [21]) may serve as the "neural trigger" for the facilitation, inhibition, and then facilitation of infanticide following mating in male mice. It is interesting that penile engorgement at ejaculation has a significant effect on sperm motility within the uterus and serves as a "neural trigger" for the continued release of prolactin and the maintenance of pregnancy in rats and mice [1, 20, 21]. Male CF-1 mice have spontaneous ejaculations almost every night [12]. These ejaculations (which may be physiologically different from ejaculations during coitus) obviously do not lead to the changes in behavior toward young that occur after ejaculating during coitus in CF-1 male mice.

The phenomenon which has been identified in these experiments is intriguing since the behavior exhibited by a male toward young is clearly contingent on the duration of time after a male has mated with a female, but for changes in the behavior of males toward young to occur, no prior contact with young is required. Also, the experience of committing infanticide either prior to mating (Table 3) or shortly after mating (before males enter the "parental mode" after mating; Table 4) does not appear to influence the inhibition of infanticide and facilitation of parental behavior which occurs 12 days after males ejaculate during coitus. If this phenomenon represented some type of conditioning process, then some effect of experience with young would be expected to have been observed in these experiments, although the experiments were not specifically designed to examine this issue. This phenomenon thus appears to be quite different from other phenomena in which changes in behavior as a function of time after an event have been reported to occur (see for example, the opponent-process model of motivation [24]). There is no precedent in the literature for the time-dependent changes that occur following mating in the behavior of male mice toward young. What is unprecedented is first the facilitation, then inhibition, and then facilitation of a behavioral response to a novel stimulus (young) solely as a function of time between an event (ejaculating) and exposure to the stimulus.

The terms facilitation and inhibition are used to describe the increase and decrease in the tendency for males to commit infanticide or behave parentally since it is hypothesized that these changes in behavior are the result of active processes, although the nature of these processes remains unknown. One possibility is that ejaculation during coitus has a long-term facilitating effect on infanticide, but for a limited period of time between 2 and 7 weeks after mating, parental behavior is facilitated and infanticide is inhibited. This would represent a delayed (and transient) response to ejaculating, while, except for this time infanticide is facilitated and parental behavior is inhibited by the act of ejaculating. Future experiments will determine whether males tested at intervals of greater than 3 months after mating will also be more likely to commit infanticide than sexually-naive males, or whether the facilitation of infanticide after mating will be found to begin decreasing beyond 3 months after mating.

There have been some suggestions that studies of behaviors that are proposed to be adaptive cannot be conducted with strains of mice reared for generations within the laboratory [3]. The question of whether the inhibition of infanticide after mating would also be observed in the offspring of wild *Mus musculus* (trapped in Columbia, Missouri) has thus been examined. One difference between laboratory strains of mice and wild mice is that virtually all sexually-naive wild male mice commit infanticide (87%; N=60) using the same test procedure described for the experiments in this paper. Sexually-naive wild mice have also been placed into a cage containing a lactating wild female and her 2-day old young, and 15 of 16 males (94%) committed infanticide. Other wild males were paired with females for 14 days and then housed individually. On the day after the female with which a male had been paired delivered young, the male was placed into the cage of an unfamiliar female that had also delivered young on the previous day, and 5% (N=20) of the males committed infanticide (M. McCarthy and F. vom Saal, unpublished observation). In these experiments with wild mice, the males cohabited with females for 2 weeks after mating, but cohabitation with an ovariectomized female for 14 days did not result in an inhibition of infanticide in wild male mice. The possibility that cohabitation with a female might have contributed to the inhibition of infanticide in wild male mice cannot definitely be ruled out. But, this would seem unlikely given that individual recognition of the female with which a male had mated did not mediate the change in behavior toward young after mating in wild males as wells as CF-1 males. Brooks and Schwarzkoff [4] have reported similar findings in experiments with C57B1/6J and DBA strains of mice. In contrast, individual recognition of females has been reported to influence infanticide in Swiss Webster mice [13], but this finding needs to be confirmed.

One might wonder why some form of individual recognition of nursing females should not occur in wild mice. In commensal populations of mice, a deme (breeding group) has been proposed to typically be composed of a single dominant male that sires virtually all offspring, a number of females, and a few subordinate males that do not reproduce [2, 8, 33, 35]. If there were many genetically-unrelated males within a deme that produced young at the same time, it would probably not be adaptive to have infanticide inhibited solely as a function of the time interval that has eleapsed since mating. Instead, some mechanism involving individual recognition of females with which mating had occurred might be anticipated to have evolved. The finding that the inhibition of infanticide and facilitation of parental behavior after mating does not involve individual recognition of an individual female with which either wild males or CF-1 males had mated thus appears consistent with the above premise that in mouse demes only one male sires young. This hypothesis also suggests that in species in which males are infanticidal but many reproductively-active males have access to fertile females, it is unlikely that an inhibition of infanticide based solely on the duration of time since mating would be found to occur.

The males tested in these experiments were isolated at 35 days of age and tested at various ages in adulthood. In a previous study [32], I reported that the frequency of infanticide (40–50%) in CF-1 male mice that were housed individually at 35 days of age and tested for their behavior toward young at 8 months of age (after 7 months of isolation) was not

significantly different from the frequency of infanticide exhibited by males isolated for less than 2 months. In addition, the same frequency of infanticide was observed in 3 to 4-month-old CF-1 males isolated 5 days prior to testing (M. McCarthy and F. vom Saal, unpublished observation). Thus, the length of isolation does not influence the likelihood that CF-1 males will commit infanticide. Being housed individually does influence the behavior of both C57BL/6J mice (L. Ghiraldi and B. Svare, personal communication) and wild male mice (M. McCarthy and F. vom Saal, unpublished observation). But, the effects of isolation on the behavior of male mice toward young in these experiments are dependent on the age of the males at the time of isolation and the testing procedure used.

The above findings suggest that the regulation of infanticide is complex and that strains of mice appear to differ in terms of which variables regulate infanticide and parental behavior. Another factor that influences the behavior of male mice toward young is dominance status, which has been found to influence infanticide in laboratory strains. Fighting and achieving dominance facilitates infanticide (82% of dominatnt CF-1 males commit infanticide) while subordination inhibits infanticide (23% of subordinate males commit infanticide [35]). Huck *et al.* [13] have reported similar findings. Thus, two different variables can result in an increase in the proportion of males that commit infanticide in laboratory mice, mating (within 4 days or after 60 days following mating about 80% of CF-1 albino mice commit infanticide) and achieving dominance. At these times, laboratory males resemble virgin wild male house mice in their behavior toward young (M. McCarthy and F. vom Saal, unpublished observation). What may have occurred during years of breeding in the laboratory is that there has been selection (by breeders) for males that are not infanticidal, and the frequency of infanticide in populations of sexually-naive laboratory male mice has gradually decreased. A similar situation may have also occurred in female mice, since fewer than 10% of laboratory female mice commit infanticide, while over 60% of non-lactating, wild female mice commit infanticide (M. McCarthy and F. vom Saal, unpublished observation). Since mating results in the inhibition of infanticide in both laboratory and wild male mice, this phenomenon does not appear to have been influenced by domestication.

Sexual Selection and the Concept of Infanticide as an Adaptive Behavior

Hrdy [11] has proposed four sets of circumstances under which infanticide could be adaptive. Her classification is based upon the nature of the benefit (e.g., access to mates, competition for food, etc.) to the infanticidal animal. The present experiments concern only one category of infanticide proposed by Hrdy: that relating to competition between males for access to estrous females, which is referred to as "sexually-selected" infanticide. Traits which enhance the ability of individuals to compete with members of the same sex for the chance to reproduce are thought to be subjected to sexual selection, which Darwin distinguished from natural selection (c.f., [29]).

Three predictions generated from the hypothesis that infanticide evolved through sexual selection [11,35] are that: (1) there must exist mechanisms to inhibit males from killing their own offspring; (2) the killing of an unfamiliar female's young must result in her ovulating and mating with the infanticidal male sooner than she could have had her young not been killed (ovulation is inhibited in lactating female mice); and (3) infanticide must be mediated, at least in part, genetically and thus be heritable. Previously, infanticidal male mice were observed to produce young at a faster rate than did non-infanticidal males, supporting the second prediction of the sexual-competition hypothesis [35]. The results of the experiments described here provide support for the first prediction of the sexual-competition hypothesis: that there is a mechanism which has evolved in mice to inhibit males from committing infanticide toward young that could possibly be their own offspring.

Female mice do not exhibit intense aggression toward intruders into the nest area until after postpartum estrus [25]. Perhaps this is an unavoidable consequence of the fact that a female mouse cannot simultaneously mate with and attack a potentially infanticidal male during postpartum estrus. Nevertheless, during the first 2 days after birth, newborn mice may be particularly vulnerable to infanticide by a new dominant male in a deme. One possible female counterstrategy to infanticide in this situation might involve communal nesting. Females with different aged young might share and jointly defend a common nest area. Since female mice in a deme are probably related, protection of another female's young should result in an increase in fitness. I have observed precisely this sort of communal nesting in a freely-growing population of CF-1 mice (F. vom Saal, unpublished observation) as have other investigators working with both laboratory and wild mice [17,23]. If, as has been proposed by Hrdy [11], infanticide has evolved due to the action of sexual selection in mice, then the adaptive significance of this behavior for both males and females cannot be ignored.

Svare *et al.* [27] have reported that the frequency of infanticide differs markedly in inbred strains of mice. These findings provide indirect support for the third assumption of the sexual-competition hypothesis: namely, that for infanticide to have evolved due to it being an adaptive behavior, at least some component of the variance must be mediated genetically. We are currently examining the heritability of infanticide and parental behavior in wild mice, and the results of this study should provide information relevant to the hypothesis that infanticide is an evolved behavior. Infanticide is regulated by fetal and adult hormone concentrations and by a host of experiental variables, such as dominance status, mating experience, housing density, and, most likely, other variables as well. Unfortunately, behaviors such as infanticide are likely to have a complex genetic basis and are therefore not easily examined using the tools of behavior genetics.

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