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Individual Variation in the Neural Timing of Infanticide and Parental Behavior in Male House Mice

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PERRIGO, G., L. BELVIN AND F. S. VOM SAAL. *Individual variation in the neural timing of infanticide and parental behavior in male house mice*. PHYSIOL BEHAV **50**(2) 287–296, 1991.—In male house mice (*Mus domesticus* and *M. musculus*), the act of coital ejaculation provides a fail-safe neural signal for timing the birth of their offspring. A unique aspect of this phenomenon is the extraordinary latency that can occur between the stimulus (ejaculation) and its adaptive neural response (male mice cease killing pups and behave parentally toward them). Thus the inhibition of infanticide is routinely time-delayed for many days after mating. In the absence of mating, cohabitation with a female will not inhibit infanticide in CF-1 stock males (*M. domesticus*), whereas the birth of pups in the male's home cage will inhibit infanticide. But with regard to the ejaculatory phenomenon, which also includes the spontaneous reemergence of infanticide 50–60 days after mating, this entire behavioral cycle toward pups can occur in the total absence of regular time cues from a light/dark cycle following ejaculation. However, exposure to photoperiodic (L:D 12:12) or constant light (LL) accelerated the transition time from infanticide to parenting after ejaculation, while in constant dark (DD), the transition time to parenting was significantly prolonged. The time interval between ejaculation and the inhibition of infanticide, which varied among individuals first mated at 6 months of age, was repeatable when the same males were remated at 9 months of age; however, when males were again mated at 18 months of age, the time interval between ejaculation and parenting was dramatically prolonged. In general, coital ejaculation triggers a neural timing system that cannot be explained by any presently known physiological mechanism. Our results do suggest, however, that the neural timing variation observed among individuals is influenced by sex steroid exposure during late fetal development.

Infanticide Parental Ejaculation Fetal position Mouse *Mus* Light/dark cycle Aging Circadian LL DD

INFANTICIDE, defined as the killing of conspecific young, is a violent but adaptive behavioral strategy found in a variety of male mammals (8). Field and laboratory studies have dramatically documented the reproductive advantages that accrue when an infant-killing male usurps the territory of another male (9, 19, 37). By killing the offspring of a defeated competitor, an infanticidal male benefits in two ways: First, he eliminates potential competition with his own offspring, and second, once a female's own young are killed, she rapidly ovulates again and mates with the usurper male.

An effective infanticidal strategy must prevent a male from harming his own offspring. In the male house mouse, ejaculation provides a fail-safe neural time signal for assessing when his own sired offspring are present in the environment (34). The specific stimulus of ejaculation during mating can inhibit infanticide in male mice. However, the male's pup-killing behavior often does not cease for many days after mating, but nearly always ceases by the time his own offspring would be born three weeks later. When infanticide ceases, most males react parentally toward pups and behave similar to a newly lactating female. Furthermore, infanticidal behavior spontaneously reemerges after offspring are weaned (34). These timed behavioral changes, which result specifically from ejaculation during mating, occur consistently among various house mouse stocks (12, 17, 20, 25, 30). This phenomenon has also been verified in the Norway rat, *Rattus norvegicus*, (18) and it may occur in other rodents as well.

Figure 1 is a schematic representation of this phenomenon as observed in CF-1 stock house mice (*Mus domesticus*) from our laboratories. The male's behavioral cycle toward pups has four distinct phases:

1) *Premating*. In virgin males, half of all individuals spontaneously kill pups while the other half do not harm them. These latter males either "Parent" pups (40%) or they "Ignore" them (10%). By definition, a parental male retrieves a pup to his nest where he incubates it and keeps it warm (5,34). Males who "Ignore" pups neither harm them nor parent them.

2) *Ejaculation and pregnancy of mate*. Ejaculation during

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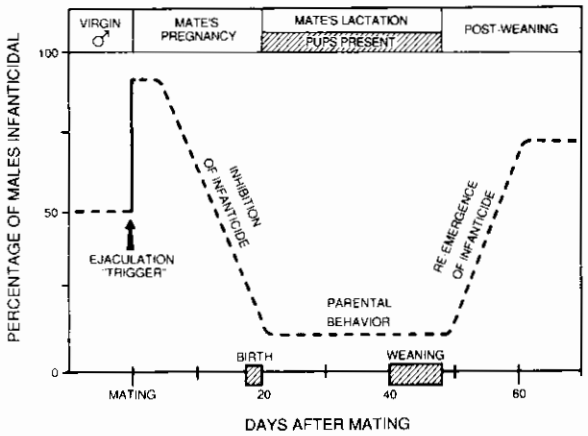

FIG. 1. A unique behavioral cycle. Schematic representation of temporal changes in behavior toward pups resulting from coital ejaculation in CF-1 stock male house mice.

mating intensifies pup-killing behavior. Virtually all males will now attack and kill pups immediately after mating; they will continue to kill pups during part or most of their mate's pregnancy.

3) *Lactation of mate.* By the time pups are born, 19–20 days after mating, infanticide has ceased and most males behave parentally toward pups. They remain parental throughout their mate's lactation.

4) *Postweaning of offspring.* Between 50 and 60 days after mating, many males spontaneously begin killing pups again. The reemergence of infanticidal behavior thus coincides with the weaning of pups.

Remarkably, this entire behavioral cycle occurs even when a CF-1 male is kept totally isolated from his mate and deprived of any female cues whatsoever following ejaculation (26,34). Furthermore, the inhibition of infanticide occurs after mating even when a male's pituitary and testes are removed (23). We are unaware of any other similar neural phenomenon in mammals where such dramatic shifts in adaptive behavior are timed to occur so many days, indeed weeks, after a specific stimulus such as coital ejaculation.

While coital ejaculation appears to be the primary mechanism inhibiting infanticide in CF-1 stock males, there is considerable variation in the occurrence of infanticide and parental behavior and the mechanisms that inhibit infanticide among the widely different genetic stocks of house mice used to study these behaviors (12, 14, 26, 30, 32). Female cues, for example, can also effectively inhibit infanticide in males of other stocks. However, in previous experiments with CF-1 stock males, we did not specifically test for the presence of these redundant inhibitory mechanisms. Thus, in the first experiment reported here, we briefly examined whether pup killing and parenting strategies could be manipulated by exposing virgin CF-1 males to chemical and tactile stimuli from females.

Our primary interest, however, is the neural timing mechanism that gages the passage of time between coital ejaculation and the birth of pups. In a previous experiment (25), male CF-1 mice were entrained to a 22 h (L:D 11:11) versus 27 h (L:D 13.5:13.5) daylength. Entrainment was verified by monitoring day-by-day locomotor activity in a running wheel. At 20 days after mating, males in the 22 h day had ceased killing pups, while the majority of males in the 27 h day were still infanticidal. Males in the 22 h day, however, had experienced four more light/dark cycles at 20 days after mating than did their counterparts in a 27 h day. Because the sudden transition from infanticide to parental behavior was matched with the number of light/dark cycles rather than the amount of real time experienced after mating, this suggested that a photoperiodic timing mechanism was coupled with the ejaculatory event (25). In a similar vein, male Norway rats maintained at L:D 12:12 also show a sharp change from pup killing to parenting between 18 and 20 days after mating (18).

Thus, in the next series of experiments reported here, we employed different lighting regimens to further examine whether male mice have evolved a photoperiodically linked mechanism that measures the passage of time after mating. Behavioral changes toward pups were thus monitored closely when males were housed and mated in free-running conditions of constant dark (DD) or constant light (LL). Individual variation in the timing of pup-killing and parenting behaviors, the effects of remating, and the effects of aging were also examined.

GENERAL METHOD

Animal Stocks and Housing

All animals used were CF-1 stock house mice maintained at L:D 12:12 from birth. Males were grouped 4–5 per cage at weaning (23 days of age). When 50 days old, all males were separated and individually housed in $28 \times 18 \times 12$ cm cages with corn cob bedding, and supplied with Purina mouse chow and water ad lib. Room temperatures were maintained at $22 \pm 2°C$. Males were not used in experiments until they were at least 150 days of age. Specific methodology is described with each experiment.

Assessing Whether a Male Is Infanticidal or Parental

When a CF-1 male encounters a neonate, he either attempts to kill it, or he does not harm it. These are clear-cut, unambiguous responses. We tested a male's reaction to a pup by quietly placing a 1–3-day-old newborn at one end of his home cage, farthest from his nest. To protect the pup from injury, the pup was placed within a tube made of 1.5 mm^2 wire mesh screen. A tube 4–5 cm long and 1.5 cm in diameter is large enough to slide a neonate comfortably inside. The pup is quiescent, secure and completely buffered from attack (24).

When an infanticidal CF-1 male encountered a screen-protected pup, he typically attacked and repeatedly bit at the screen, but without injuring the neonate (24). If the male did not show any intent to harm the pup, the next step was to introduce an unprotected pup for 30 minutes to determine whether the male was truly parental. If he did not ignore the pup, a parental male would groom the pup, retrieve it, and incubate it in his nest. While this humane test procedure is a reliable assessment of infanticidal tendencies in CF-1 stock males (24), a screen-protected pup has not proved to be an effective testing paradigm in wild stock house mice (unpublished observation).

It should also be emphasized that a male's reaction toward a newborn pup is a generalized, nonspecific response. Neither the sex, age (1–10 days old) nor relatedness of the pup appear to have any discernable influence on a male's tendency to exhibit infanticide or parental behavior (17, 32, 34, 37). Male mice also have spontaneous ejaculations nearly every night (10), but this event does not in any way influence their behavior toward pups. Thus intravaginal ejaculation is the specific neural trigger that initiates the behavioral changes described in Fig. 1 (34).

EXPERIMENTAL SERIES

Experiment 1: Will Female Chemosensory Cues, Cohabitation, or the Birth of Pups Inhibit Pup Killing in Unmated Male CF-1 Mice?

As noted in the introduction, cohabitation with a female will effectively inhibit male infanticidal behavior in some stocks of house mice. Thus we examined whether pup-killing behavior could also be inhibited in infanticidal CF-1 males by exposing them to a range of chemical and tactile cues from pregnant and nonpregnant females.

Forty-five virgin males (5 months old) maintained at L:D 12:12 were pretested with a 1–3-day-old pup as described above. Twenty-five of these males exhibited infanticide (56%) and were partitioned into three experimental groups: Group 1 (N = 8) consisted of males exposed to cues from pregnant females; Group 2 (N = 7) consisted of males exposed to cues from nonpregnant females; and Group 3 (N = 10) consisted of control males who were not exposed to female cues at all. The two-part experiment was done as follows.

Part A: Chemical cues. A cage with a wire mesh bottom was suspended 12 cm above the top of each virgin male's cage. Group 1 males had three females, 10–11 days pregnant, suspended overhead; thus, urine and wastes showered freely on the males below. Pregnant females were replaced every 7 days so that none would deliver pups while suspended overhead. Likewise, Group 2 males had three nonpregnant females suspended above, also replaced every seven days. Group 1 and 2 males were tested with a pup three hours after the first group of females was placed in the boxes overhead (Day 0, two h after lights on) and then retested at the same time every three days thereafter until Day 21 (offspring would have been born by this time had the males been allowed to mate). Group 3 controls had an empty wire bottom cage suspended overhead; they were also tested with a pup on the same schedule.

Part B: Female cohabitation and delivery of pups. If a Group 1 or 2 male continued to exhibit infanticide at Day 21, he was then allowed direct contact with a female. Thus a pregnant female (sexually unreceptive) was placed in the cage of each infanticidal male in Group 1 (immediately after the Day 21 test of Part A). Pregnant females were again replaced every 7 days so that none would give birth while cohabiting with the male; this also prevented any males from mating with their live-in females during postpartum estrus. An ovariectomized female, also sexually unreceptive, was placed in the cage of each male in Group 2 (these females were also replaced every 7 days). The testing procedure was the same as Part A, except that each male was only tested every 7 days thereafter until Day 21, including the Group 3 controls. If a Group 1 or 2 male continued to exhibit infanticide at Day 21 of female cohabitation, a pregnant female was then allowed to deliver pups in his cage. Thus, when pregnant and ovariectomized females were replaced on Day 21, each infanticidal male in Groups 1 and 2 received a pregnant female scheduled to give birth 10–12 days later. One more test with a pup occurred on Day 28 of cohabitation. When litters were born on Day 30–33 of cohabitation, cages were checked daily each morning over the next 10 days for any evidence of pup killing. Control males were also tested for infanticide on Day 28 and 33.

Results

As shown in Fig. 2, which visually summarizes the results of both parts of Experiment 1, the delivery of pups in the male's own cage was the only treatment that inhibited infanticidal behavior in the unmated males, $\chi^2(2) = 18.1$, $p < 0.0001$. Only one litter was killed in the virgin male Groups 1 and 2, and previous experiments have demonstrated that if a male CF-1 mouse kills one pup he will also kill the entire litter (37). All surviving litters appeared healthy (8–14 pups per litter) and there was no evidence of bitten or missing pups over the next 10 days, nor was there any evidence of wounds, indicating that a female had attacked the male to defend her litter.

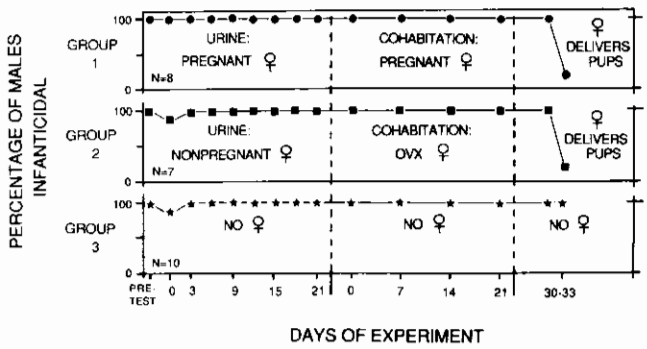

FIG. 2. Exposure to ♀ cues. Summary of Experiment 1. The response toward newborn pups when virgin males were exposed to various combinations of chemosensory and tactile cues from female mice (OVX = ovariectomized). A zero to 100 percentage scale is shown for each of the three groups. The pretest established that only infanticidal males were used. In Part A of this experiment (exposure to chemical cues) males were tested every three days for 21 days. In Part B of this experiment (cohabitation and the delivery of pups) males were instead tested every 7 days until the female delivered on Day 30–33. Day 0 in Part B was a test that occurred 2 hours after a cohabitant female was first placed in the male's cage.

Two fundamental points need to be emphasized here: First, Fig. 2 clearly establishes that, in the absence of mating, female exposure does not inhibit infanticide in CF-1 stock males; and second, for the purpose of the remaining experiments, neither does repeated testing with a pup, even at three day intervals, result in any inhibition of infanticide.

Experiment 2: Postmating Changes in Male Infanticidal and Parental Behavior in the Presence or Absence of Photoperiodic Cues

As described in the introduction, previous findings suggested that coital ejaculation triggers a photoperiodically influenced timing mechanism that allows a male mouse to synchronize parental behavior with the presence of his pups (25). The following experiment examined the timing of these behaviors when male mice were housed, mated and tested at typical husbandry conditions of L:D 12:12 versus free-running rhythm conditions of constant dark (DD) or constant light (LL). Our purpose here was to establish whether mated males would exhibit this phenomenon in the absence of entraining light/dark cues and, if so, whether males kept in different free-running conditions and thus exhibiting different circadian periods ["Aschoff's Rule"; see (1,28)] would undergo the transition from pup killing to parenting at different times after mating.

Pretesting and partitioning of virgin males. As described in Fig. 1, half of all virgin CF-1 males spontaneously kill pups whenever they encounter them, while the other half will usually retrieve and parent them. Previous experiments have already established that whether or not a virgin CF-1 male is infanticidal

or parental appears to be programmed by in utero variation in sex steroid exposure during late fetal development (23, 26, 33). These behavioral differences apparently result from testosterone emanating from fetal siblings, and therefore depends on whether a male fetus develops next to same or opposite sex fetuses (35,36). Since a major purpose of these experiments was to examine individual variation in timing, it was absolutely essential to control for this physiological variation by identifying whether a virgin male was infanticidal or parental before he was delegated to an experimental treatment. The necessity of doing this will be obvious from our results.

Ninety-one virgin male mice (5 months of age and maintained at L:D 12:12) were, for the first time, pretested for their behavior toward a 1–3-day-old pup. The 91 males were classified as follows: 46 were infanticidal (51%), 33 were parental (36%), and 12 males ignored the pup (13%). This replicates previous findings (23,34). One day later, the pretested males were evenly distributed among three rooms maintained at one of three lighting regimens: constant light (LL), L:D 12:12, or constant darkness (DD). Thirty males were delegated to each lighting condition and partitioned as follows: 15 Infanticidal males, 11 Parental males and 4 Ignorer males. A partially covered 34-watt fluorescent light was the illumination source in the L:D 12:12 and LL rooms (range of 20–30 lux as measured by incidental light with a Gossen Luna-Pro meter at the bottom and top, respectively, of the cage rack where males were housed).

Assessing the time-course of behavioral changes toward pups after ejaculation. After three weeks of accommodation to their lighting condition, each male had two female CF-1 mice (50 days of age) placed in his cage (beginning at the time of lights on at 1200 h in the L:D 12:12 treatment; females were placed in the DD and LL cages at the same time). Two females were used to increase the likelihood that all males could be mated in as few days as possible. All females were removed after three hours. CF-1 males inject an unmistakable copulatory plug upon ejaculation, so the presence of a vaginal plug confirmed that a male had mated. After five days, 28 out of 30 males in each group had mated, resulting in a distribution of 14–15 Infanticidal, 10–11 Parental and 3–4 Ignorer males within each experimental group. Upon confirmation of ejaculation (Day 0), each male was immediately tested with a pup and then retested between 1400 and 1500 h every three days thereafter until Day 30 after mating. Two more retests were done at Day 60 and Day 90 after mating. All observations in DD were done with a 15 watt "safe" red light that remained on continuously throughout the experiment in all three rooms.

Results

Figures 3 and 4 show complementary perspectives of how each individual male behaved toward pups during the entire 90 day test period following mating. These data are hierarchically sorted to reveal the timing variation among individuals during the first 30 days of the experiment. The Fig. 3 matrix represents an overview of timing variation resulting from each light treatment in toto, whereas the Fig. 4 matrix focuses on timing variation among males subdivided in relation to their pretest behavior toward pups. The differences in the timing of transitions in behavior shown here are striking. Statistical differences were assessed with Chi-square analyses comparing the frequency of infanticide (KILL) versus parenting (P) and ignoring (Ign) among the groups at each test day. For Chi-square analysis purposes, parenting and ignoring behaviors were combined into one Noninfanticidal category.

Transitions in behavior after mating. As shown in the Fig. 3 matrix, there were significant overall group differences ($p=0.05$ or less) in the frequency of infanticide on Days 6, 9, 12, 27, 60, and 90 after mating (Chi-Square values and probabilities are reported in Fig. 3). In general, the group differences resulted from a higher frequency of infanticide in the DD males. Also obvious in Fig. 3 is the irregular pattern of transition from pup killing to parenting observed in some males. This transition occurred smoothly between consecutive test days in many individuals, but in some males, transient and spontaneous episodes of parenting or pup killing occurred sporadically throughout the course of testing. Furthermore, within the first 30 days after mating, the majority of "Ignore" events occurred at an interface between infanticidal to parental transitions. The frequency results from the L:D 12:12 group in Fig. 3 also match with previous experiments where CF-1 males were maintained at L:D 12:12 and tested for infanticide only one or two times between Days 1 and 21 after mating (34), instead of every three days as in this experiment. This suggests that repeated testing with a pup does not influence the timing of infanticide inhibition after mating.

Behavior of pretested Parental and Ignorer males. As shown in the Fig. 4 matrix, which specifically partitions males according to their pretest behavior toward pups, coital ejaculation triggered pup killing in virtually all of those males classified as Parental or Ignorer in the pretest. (Mere exposure to females, without ejaculation, does not induce pup killing in virgin Parental males; see data in Table 1.) The transition back to parental behavior occurred rapidly in the LL and L:D 12:12 males, but was significantly prolonged in the DD males. These groups differed ($p=0.05$ or less) on Days 3, 6, 9, 12, and 15 (Chi-Square values and probabilities are shown in Fig. 4). Significant group differences disappeared at Day 18, coinciding with the birth of pups, but reappeared again at Day 27. By 60 days after mating (pups would have been weaned by this time) many of the parental males, especially those in DD, became pup killers again.

Behavior of pretested Infanticidal males. Figure 4 also reveals that among those males classified as Infanticidal in the pretest, there were no significant behavioral differences except for Day 60 and Day 90 after mating (100% of the DD males were infanticidal again by Day 90). Nevertheless, the majority of pretested Infanticidal males in each group still expressed parental behavior within 21 days after mating. Some pup-killing males, however, never expressed parental behavior at all.

Effects of light. One further point is visually obvious in Fig. 4, namely that males who were categorized as either Parental or Ignorer on their pretest underwent transitions to parental behavior more rapidly than their Infanticidal counterparts. Thus all males were ranked according to the day after mating when they expressed their first bout of parental behavior (Parental and Ignorer males were combined as one Noninfanticidal category). Indeed, Mann-Whitney U-tests revealed significant transition time differences between the two pretest categories of males (Infanticidal versus Noninfanticidal) in both the LL ($U=23.5$, $p<0.005$) and L:D 12:12 treatments ($U=35.5$, $p<0.005$). In sharp contrast, no significant transition time differences were noted between the two pretest categories of DD males ($U=80.5$, $p>0.40$). This suggests that pretested Noninfanticidal males responded to the stimulus of light differently than pretested Infanticidal males: the presence of light in the LL and L:D 12:12 treatments accelerated the postmating transition to parenting among Noninfanticidal males, whereas in the absence of light (DD), there were no significant behavioral differences among pretested Infanticidal and Noninfanticidal males.

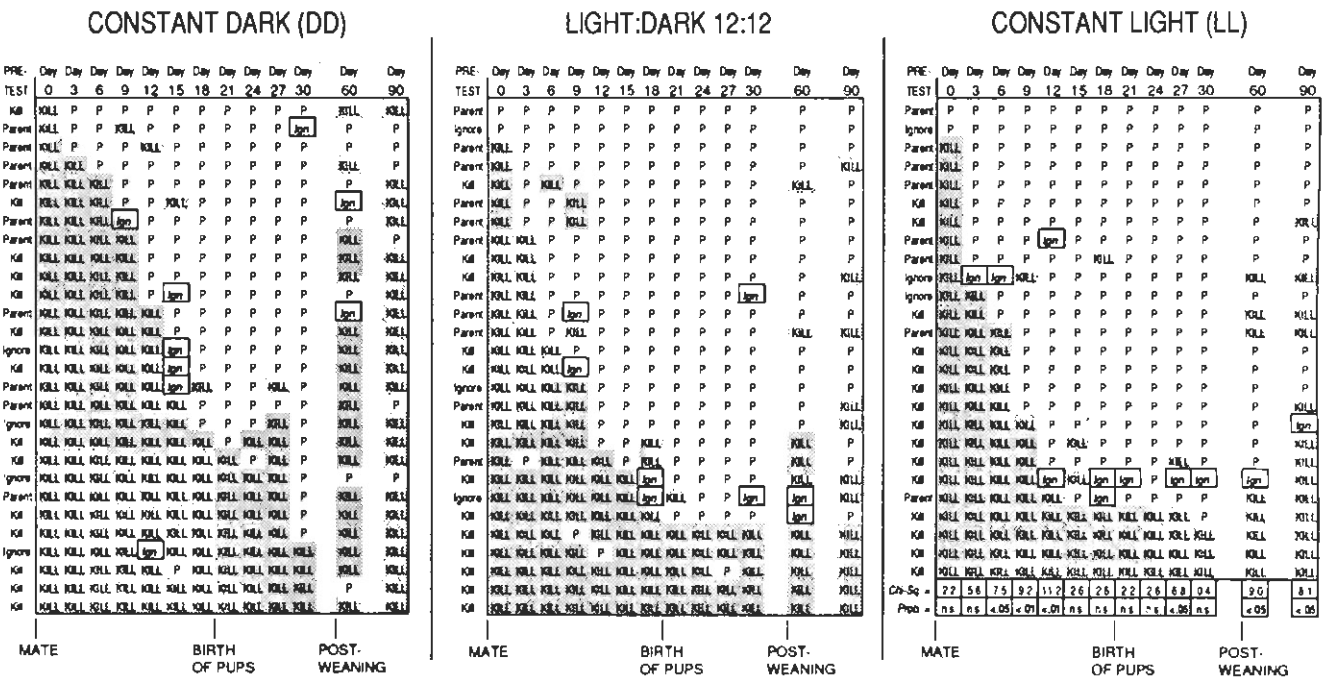

FIG. 3. The hierarchically sorted response patterns of individual male house mice who were mated and tested for their behavior toward a newborn pup (KILL = infanticidal; P = parental; Ign = ignored pup) at conditions of constant dark (DD), L:D 12:12 and constant light (LL). Males were tested immediately after ejaculation (Day 0) aud every three days thereafter until Day 30; two more tests occurred at Day 60 and Day 100 after mating. The behavior of each male prior to mating is shown in the Pretest column. Chi-Square values ($df = 2$) and probabilities (n.s. = not significant) comparing the frequency of Infanticidal versus Noninfanticidal behavior among all three groups at each test day are reported at the bottom of the columns under the LL group. Two of the pretested Parental males in the LL treatment died during the second week after mating, so they are absent from these results.

Does testing in light versus dark influence behavior toward pups? One potential pitfall in the apparent light-mediated differences noted above is that males might be more prone to kill pups depending on whether they are tested in the dark or in the light. Specifically, DD males were always tested in darkness, while the L:D 12:12 and LL males were always tested with the lights on. In a follow-up experiment, 58 virgin males were thus tested for their behavior toward a newborn pup at opposite times of day: 29 males were tested at three hours after lights on while 29 males were tested in darkness three hours after lights off. Males in this experiment were 5 months of age, maintained since birth at L:D 12:12. The results in Table 2 show there were no differences in pup killing or parenting at opposite times of day (66% of the males were infanticidal when tested with the lights on, while 55% were infanticidal when tested with the lights out; $\chi^2 = 1.3$, $p > 0.50$). Thus testing in either light or dark (a 12 h phase difference) did not seem to influence how a male behaves toward pups.

Experiment 3: Period of Free-Running Locomotor Activity in DD Versus LL: Test of Circadian-Linked Timing Hypothesis

After Experiment 2 was completed (90 days after mating), eight LL males and ten DD males were randomly chosen and placed in cages with a running wheel interfaced to an Esterline-Angus event recorder. Free-running activity patterns were recorded for 40 days, at which time we estimated the period (τ = tau) of each male's activity cycle by eyefitting a line through his activity onsets during the last 15 days of activity. The purpose of this experiment was to examine whether a correlation existed between the period (τ) of a circadian activity/rest cycle and the timing of postmating behavioral changes in either DD or LL.

Results

Our results were consistent with "Aschoff's Rule" (1,28); thus, the free-running activity cycle of DD males was considerably shorter, about 1 h less, than the cycle of LL males. The period (τ) of the activity/rest cycle in DD males was 24.13 ± 0.05 h (mean $\pm$ SEM) while in the LL males, $\tau = 25.15 \pm 0.17$ h, $F(1,16) = 38.4$, $p < 0.0001$. Within the eight LL males, however, there was a significant positive correlation between the period (τ) of an individual's activity cycle and the number of days elapsed between ejaculation and their first expression of parental behavior ($r^2 = .75$, $p < 0.05$). In contrast, no such correlation existed in DD animals ($r^2 = .16$, $p < 0.25$). These results neither support nor refute the involvement of a circadian-based timing mechanism, they only suggest a correlation in constant light.

Experiment 4: Repeatability of Mating-Induced Changes in Behavior and Retest of Individual Males at 12 Hour Intervals

The following experiment asked two questions: first, whether the effect of first mating and the timing of inhibition observed in Experiment 2 is repeatable within individuals, and second, how consistent is the transition from infanticidal to parental when retests occur at much shorter time intervals (every 12 h). Six males who showed rapid transition times from infanticidal to parental behavior were thus selected from each of the three lighting conditions and mated for a second time at 100 days af-

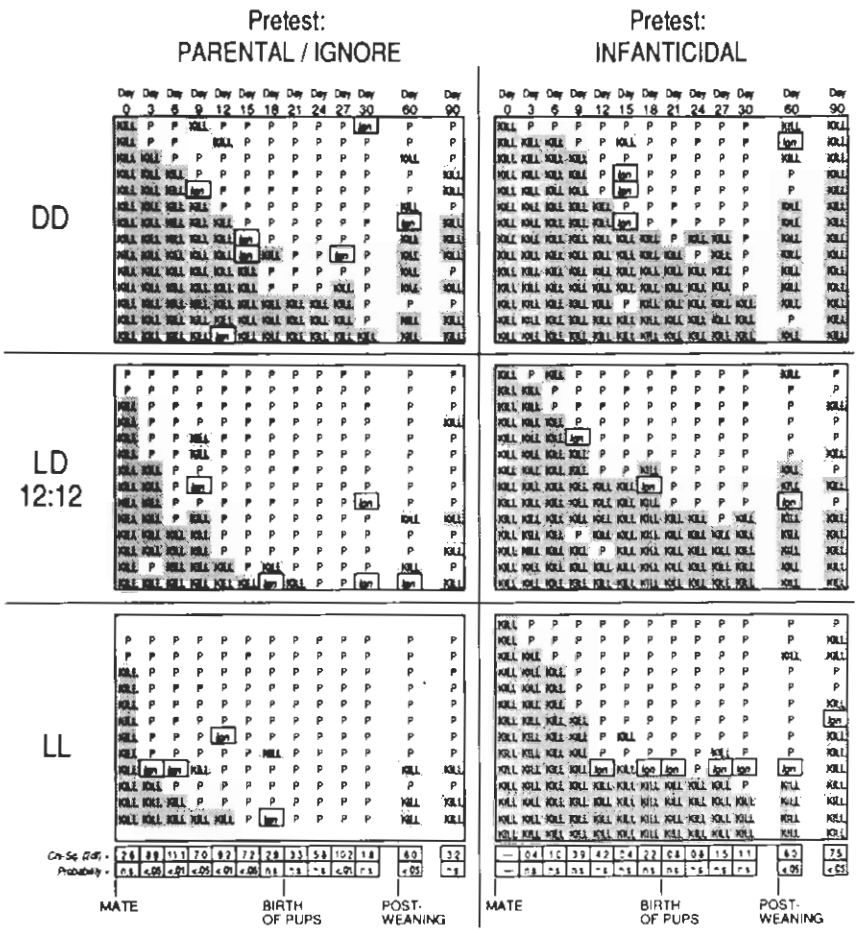

FIG. 4. These are the same data as shown in Fig. 3, but sorted and graphed from a different perspective. Thus males are now subdivided in relation to their pretest behavior toward pups. Chi-Square values ($df = 2$) and probabilities (n.s. = not significant) comparing the frequency of infanticidal versus noninfanticidal behavior at each test day are reported at the bottom of the columns within each Pretest category.

ter their first mating (same mating procedure as noted in Experiment 2). The first test with a pup occurred immediately after ejaculation was confirmed. Unlike Experiment 2, however, in which retests occurred at three day intervals, each retest in this experiment occurred instead at 12 h intervals until the end of Day 12. Thus subsequent tests in the L:D 12:12 room occurred at the time of lights on (1200 h) and at the time of lights off (2400 h). Tests in the DD and LL rooms were done at the same time. This resulted in all three groups being tested at either exactly (L:D 12:12) or, because of the presumed free-run in DD and LL males, at approximately 12 h differences in their circadian phase.

TABLE 1

RESPONSES TOWARD PUPS WHEN FIVE-MONTH-OLD NONINFANTICIDAL MALES (N = 9) WERE EXPOSED FOR THREE HOURS TO TWO FEMALES, BUT DID NOT MATE

	Exposure to ♀♀	
Response Toward Pup:	Before	After
Parental	8	8
Ignore	1	1
Infanticidal	0	0

Males were tested immediately after the removal of females. There was no change in a male's behavior.

TABLE 2

RESPONSES TOWARD PUPS WHEN VIRGIN MALES (5 MONTHS OLD) WERE MAINTAINED AT L:D 12:12 AND TESTED AT OPPOSITE TIMES OF DAY

	Time of Day	
Response Toward Pup:	Light	Dark
Parental	9 (31%)	10 (35%)
Ignore	1 (3%)	3 (10%)
Infanticidal	19 (66%)	16 (55%)

N = 29 for each group; $\chi^2(2) = 1.3$, $p > 0.50$.

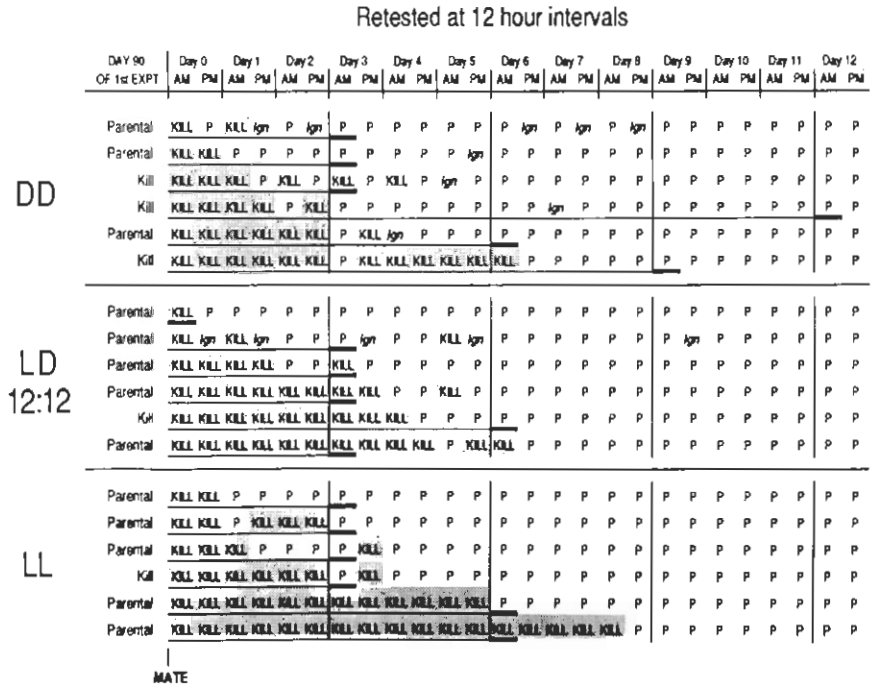

FIG. 5. The response patterns of individual male house mice (six from each of the three light treatments) who, after the completion of Experiment 2, were mated for a second time and then tested for their behavior toward a newborn pup (KILL = infanticidal; P = parental; Ign = ignored pup). Males were tested immediately after mating (Day 0) and retested every 12 hours thereafter until the end of Day 12 (AM = morning; PM = evening; see text). For comparison purposes, the heavy dark bar indicates at which of the 3-day test intervals parental behavior was first recorded in Experiment 2. Their last recorded behavior toward pups is also shown (Day 90 of Experiment 2, ten days before they were remated in this experiment).

Results

As shown in Fig. 5, a clean transition from pup killing to parenting sometimes occurred in as little as 12 hours, but only in five of the 18 males tested here. In contrast, the majority of males (13 out of 18) showed day-to-day fluctuations in their behavior toward pups before "locking in" to consistent parenting. The heavy dark bar shown in Fig. 5 indicates the test day in Experiment 2 (their first mating) when parental behavior was first noted. Since these males were tested at different intervals (every three days versus every 12 h in this experiment), we cannot make a legitimate statistical comparison here. However, with the exception of one of the DD males, the visual results agree remarkably well, timewise, with the pattern of changes observed when the same individuals were tested three months earlier. These data suggest that after a behavioral cycle is "reset," ejaculation during mating can trigger a new cycle of infanticidal inhibition, one that appears programmed to last about the same length of time within an individual.

Experiment 5: The Effects of Aging

Males kept in the L:D 12:12 condition remained individually housed until 18 months of age, at which time they were again allowed to mate. This provided a direct comparison between the same individuals when tested at one full year after their first mating experience (6 months versus 18 months of age). All surviving males (18 out of the original 28) were mated (same mating procedure as in Experiment 2) and then tested every three days for 30 days.

Results

As shown in Fig. 6, the transition to parental behavior after mating was more prolonged when the males were older. In fact, five of the older males (28%) never ceased killing pups. With regard to individual variation, there was no longer any correlation (as suggested by the previous experiment) between the time elapsed between mating and parenting when a male was 6 months versus 18 months of age ($r^2 = .07$, $p > 0.35$; $N = 13$, since five old males never exhibited parental behavior and could not be compared). In general then, the neural timing mechanism appears to attenuate with age.

GENERAL DISCUSSION

Both sexes of house mice exhibit infanticide and express parental behavior at the time pups are born, but male mice lack the cues from developing fetuses that precisely regulate the timing of infanticidal and parental behavior in females (17, 25, 31, 34). While the use of different stocks of house mice among different laboratories was originally a source of considerable debate (and confusion) regarding the behavioral mechanisms that inhibit infanticide (5, 11, 14, 34), it is now extensively documented that multiple, redundant mechanisms have indeed

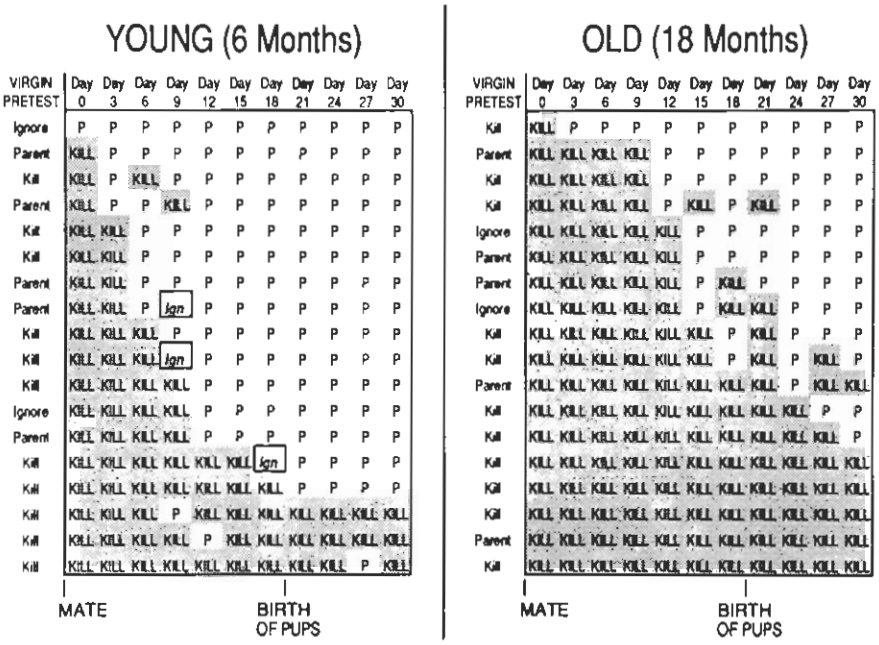

FIG. 6. Effects of aging. The hierarchically sorted response patterns of individual male house mice (maintained at L:D 12:12), who were mated and tested for their behavior toward a newborn pup (KILL = infanticidal; P = parental; Ign = ignored pup) when Young (six months of age; data from Experiment 1) and then retested again when Old (the survivors at 18 months of age). All males were tested immediately after ejaculation (Day 0) and every three days thereafter until Day 30.

evolved to inhibit pup-killing behavior in male mice. The degree to which these multiple inhibitory mechanisms are expressed apparently stems from genetic differences among various inbred and wild stocks (26).

The contrast in multiple, redundant mechanisms is especially evident in wild-trapped stocks of *Mus domesticus* and *M. musculus* [for a discussion of taxonomic differences see (15)]. Wild male *M. domesticus* trapped in Alberta, Canada behave similar to CF-1 males; their pattern of infanticide inhibition after mating in L:D 12:12, including the expression of parental behavior and reemergence of infanticide 60–90 days later, resembles that in Fig. 3; likewise, 20+ days of cohabitation with pregnant females does not inhibit infanticide either (unpublished observation). But in wild male *M. musculus* from Israel, ejaculation and female cohabitation are independent mechanisms; either mechanism, by itself, will effectively inhibit infanticide (30). In another muroid rodent, the Norway rat, ejaculation alone can inhibit infanticide, but so will chemosensory cues if virgin male rats are exposed only to the the soiled cage bedding of a pregnant female (18). This suggests an array of female counterstrategies to defend her litter from infanticidal attack [(18); see also (7,20)].

Our results in Experiment 1, however, demonstrate that CF-1 mice are a genetic stock in which virgin males appear insensitive to female cues per se as an inhibitor of infanticide. In contrast, the birth of pups in the presence of a male in his home cage appeared to inhibit infanticide (Fig. 2). Evidence from another laboratory suggests a direct tactile or chemotactile mechanism is involved here, because infanticide is inhibited only when the male has actual physical contact with the female and/or her pups during birth (21). In any case, the act of ejaculation during mating is still the primary stimulus that inhibits infanticide and regulates the timing of parental behavior in mated CF-1 males.

Evidence for Fetal Hormonal Programming of Individual Timing Variation and Responses to the Effects of Light

Since most nocturnal rodents see light only during short, crepuscular periods each day, one might argue that a housing condition of constant light, or even a typical photoperiodic laboratory condition of L:D 12:12, represents an unnatural light stimulus for a nocturnal species such as the house mouse. On the other hand, house mice in the laboratory are not as rigidly nocturnal as other rodents (22) and are frequently active during light hours. Furthermore, their reproduction is not under photoperiodic control (2,4).

Regardless of these potential arguments, Experiment 2 still revealed that the stimulus of light clearly influenced the timing of behavioral changes following coital ejaculation in male house mice. Photoperiodic (L:D 12:12) and constant light (LL) dramatically accelerated the inhibition of infanticide and emergence of parental behavior following mating, but mainly in those males who had displayed parental behavior when they were pretested as virgins (see Fig. 4). These results, together with prior findings, suggest a unique link between the way in which timing variation and differential responses to photic stimuli among adults are programmed by hormonal events during late fetal development.

Previous experiments have established that CF-1 males who develop between two male fetuses, and are thus exposed to higher concentrations of testosterone, are significantly more likely to exhibit parental behavior both before and after mating than are their male counterparts who developed between two female siblings [(23,33); see also (29)]. This is known as the intrauterine position phenomenon (36), and describes the fact that, in mammals such as house mice that produce large litters, fetuses are positioned randomly in the uterine horns and are

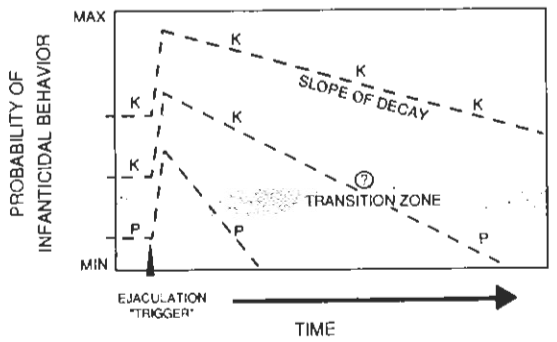

FIG. 7. Threshold and decay. A potential scheme for understanding how the postejaculatory inhibition of infanticide might operate in CF-1 stock males. Males who behave parentally (P) before they mate are thus stimulated by the act of ejaculation to exhibit infanticide. In males who are already infanticidal (K) before they mate, the stimulus of ejaculation simply intensifies their motivation to kill pups (see text). After ejaculation occurs, an inhibitory decay process is activated, which eventually eliminates infanticidal behavior by falling below the Transition Zone threshold, thus allowing parental behavior to occur. This model assumes that any one of three behaviors, Infanticide (K), Ignoring or Parenting (P), can occur when males are at or near their particular Transition Zone threshold.

therefore exposed to differential sex steroid concentrations depending on whether they develop next to same or opposite sex fetuses. As a result, an individual's intrauterine position has been correlated with a profound range of variation among reproductive, morphologic, and behavioral characteristics expressed when both sexes are adult, including adult-infant interactions (36).

The broad range of timing variation shown among all three treatment groups in Fig. 3 matches with the phenotypic variation in infanticide predicted by the intrauterine position model (23,26). In any large random sample of CF-1 males, there are always some individuals who simply do not respond to the stimulus of ejaculation. Some males always remain parental and, as noted above, these individuals most likely underwent fetal development between two male siblings. In direct contrast, some males always kill pups, regardless of how much time elapses after mating, and these individuals most likely underwent fetal development between two female siblings. Both subsets of CF-1 males represent about 10–15% of the population, and the data from Experiment 2 fit these expected proportions remarkably well. Among those few individuals in which mating per se does not seem to inhibit pup killing, the stimulus of ejaculation and female cohabitation are probably both required in order to inhibit infanticide (6,27).

A Proposed Neuroethological Model

Figure 7 is a scheme depicting the transitions in behavior toward pups resulting from mating in CF-1 males. The intense sympathetic stimulus of coital ejaculation triggers a sudden and dramatic behavioral change, which intensifies a male's motivation to kill pups. Thus infanticidal males remain infanticidal while virtually all parental males become infanticidal immediately after mating (e.g., see Figs. 4 and 5). Ejaculation also activates the time-delayed inhibition process. The neural substrate(s) governing infanticide thus appear to undergo an inhibitory decay, which, over the course of time, eventually diminishes a male's motivation to kill pups. Once an individual's threshold for the inhibition of infanticide is reached (Transition Zone), then parental responses can emerge. Previous experiments have shown that when CF-1 males are hypophysectomized, which eliminates the pituitary hormone prolactin [a facilitator of parental behavior; see (13)], they are significantly less likely to display parental behavior after mating and thus tend to ignore pups; hypophysectomy, however, does not prevent the postejaculatory inhibition of infanticide within 20 days after mating (23). Together, these findings suggest that the inhibition of infanticide and the occurrence of parental behavior are independent phenomena.

As implied by the previous section [see also (23,26)], the slope of a CF-1 male's behavioral decay is, to a large extent, programmed by hormonal events related to his fetal position, although light cues, genetic differences among individuals and social factors (e.g., synergistic female cues) undoubtedly influence this decay process too. With regard to aging, the time interval between coital ejaculation and parenting was relatively constant in individual young males (6 to 9 months of age), but in old males (18 months of age) this time interval was significantly longer; in fact, 28% of the old males never exhibited parental behavior. This suggests that the functioning of the timing system, shifts in response thresholds, and/or the need for redundant female cues also change during aging (27).

Also implicit in the Fig. 7 model is that when a male "ignores" a pup, he is at or near his behavioral threshold (Transition Zone) during the inhibition process [see also (23,26)]. Thus, if testing with a pup occurs during this transition, then any one of three behavioral states could be observed: Infanticide (K), ignoring, or parenting (P). The evidence for this comes from Experiment 4. By testing males at 12 h intervals, we observed fluctuations among all three behaviors during the transition phase. In fact, several males actually seemed to oscillate between infanticidal and noninfanticidal behaviors during the transition (Fig. 5).

Is There a Circadian Timing Mechanism?: Pros and Cons

As to how this behavioral transition is neurally timed remains perplexing. The results of a previous experiment clearly implied that coital ejaculation triggers a neural timing system that "counts" photocycles (25), which suggested a circadian-based timing function. However, the sample of 10 males in Experiment 3 who were given a running wheel in constant dark (DD) showed an average free-run of almost 24 h ($\tau = 24.1$ b). Yet, their transition to parental behavior was significantly delayed when compared to the L:D 12:12 group, which also exhibits a 24 h activity cycle under the lighting conditions provided in this experiment (unpublished observation). On the other hand, males kept in the free-running condition of constant, low intensity light (LL) did indeed measure the passage of time after mating differently than DD males. The inhibition of infanticide occurred more rapidly in LL males; in fact, the L:D 12:12 and LL groups both showed the same pattern in transition times. These findings are thus inconsistent with a circadian "day-counting" mechanism, since the period (τ) of free-running activity for the sample of 8 LL males in Experiment 3 was 25.2 h. This suggests that males in LL either should have taken longer to undergo the transition from infanticide to parental behavior after ejaculation or, at the very least, they should not have differed so dramatically from the DD males. Within the context of our present experiment, however, it is still not possible to eliminate whether a circadian timing system could have been "masked" by exposure to con-

stant light (3), nor do these results completely rule out the possibility of a covert rhythm that free-runs independently from activity/rest cycles.

Finally, we have made no attempt to explain how infanticidal behavior spontaneously reemerges two months after mating. In general though, the presence of light seemed to inhibit infanticide during this phase too. No simple physiological explanation can thus account for these time-delayed responses, and little more can be said here except that our present and past experiments suggest both parallels and paradoxes with widely studied behavioral and reproductive timing processes. Ablation of the suprachiasmatic nucleus, exposure to high intensity constant light (both of which disrupt circadian rhythms), or maintenance at skeleton photoperiods (e.g., two short entraining pulses of light daily) are several potential experiments that could distinguish whether light has a photoperiodic, direct, or some other peculiar regulatory role in the timing of this entire behavioral cycle.

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