CHAPTER 16

BEHAVIORAL CYCLES AND THE NEURAL TIMING OF INFANTICIDE AND PARENTAL BEHAVIOR IN MALE HOUSE MICE

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Infanticide is a unique form of intraspecific aggression. Most forms of intraspecific aggression rarely lead to the death of interacting animals, whereas infanticide — by definition — is the killing of conspecific young. Much of the early research on this controversial subject assumed that such behavior was maladaptive and symptomatic of grossly abnormal social conditions in nature or in the laboratory. In his classic studies of the Norway Rat, Calhoun (1962) observed that sociopathologic conditions, such as populations stressed by excessive crowding, can indeed lead to a severe social breakdown and thus a high incidence of infanticide.

In recent years, however, a prominent new view of infanticide has emerged: it is a violent but adaptive reproductive strategy found in a variety of mammals and other vertebrates (*e.g.* Hrdy, 1979; Hausfater and Hrdy, 1984). Field and laboratory studies have dramatically documented the reproductive advantages that accrue when an infant-killing male usurps the territory of another male (Hrdy, 1979; vom Saal and Howard, 1982; Packer and Pusey, 1984, this book). By killing the offspring of a defeated competitor, an infanticidal male benefits in two ways. First, he eliminates potential reproductive and resource 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.

The male house mouse, (*Mus domesticus* and *M. musculus*), has become the focus of much research concerning the socioecology, evolution and physiology of infanticidal and parental behavior in male rodents. Male house mice routinely attack and kill alien young whenever they encounter them, but an effective infanticidal strategy must allow a male to recognize when his own offspring might be present. Thus, a fundamental issue in the study of infanticide concerns the factors that prevent male mice from harming their own progeny. It is now widely accepted that multiple behavioral mechanisms — and combinations thereof — are responsible for inhibiting pup-killing behavior in male mice (*e.g.* Soroker and Terkel, 1988; Palanza and Parmigiani, 1991; Elwood, this book). The major theme of this chapter, however, involves our investigation of one of these inhibitory mechanisms — specifically, the dramatic changes in behavior toward offspring that are triggered by the act of ejaculation.

THE EJACULATORY PHENOMENON

In male house mice, the act of ejaculation during mating provides a fail-safe neural signal for timing the onset of paternity (vom Saal, 1985). The specific stimulus of ejaculation inhibits infanticide. However, a remarkable aspect of this phenomenon is that a male's pup-killing behavior often does not cease for many days after mating, but nearly always ceases by the time his own sired offspring would be born three weeks later. When infanticide ceases, males react non-aggressively toward pups and often express parental behavior similar to that of a newly lactating female. Furthermore, infanticidal behavior spontaneously re-emerges at a time that coincides with the weaning and dispersal of offspring (vom Saal, 1985). These timed behavioral changes — which result specifically from ejaculation — are clear-cut and remarkably consistent among various house mouse stocks (McCarthy and vom Saal, 1986; Kennedy and Elwood, 1988; Palanza and Parmigiani, 1991; Soroker and Terkel, 1988; Perrigo *et al.*, 1989a, 1990). This phenomenon has also been verified in the Norway rat, *Rattus norvegicus*, (Mennella and Moltz, 1988) so it may occur in other rodents as well. In terms of reproductive advantages, male mice and rats are thus likely to eliminate pups sired by a competitor, whereas copulation ensures that they do not harm their own pups during the period of their mate's lactation.

Our primary interest here is the unique neural timing mechanism that gauges the passage of time between ejaculation and the birth of pups in male house mice. The remainder of this chapter presents an overview of what we have learned from CF-1 stock males (*Mus domesticus*) about the

nature of the ejaculatory trigger and the timing mechanism that regulates time-delayed changes in their behavior toward pups. First, we have examined the role that pituitary and gonadal hormones play in the modulation of these behavioral changes. Second, we have employed experimental strategies where behavioral changes toward pups were carefully monitored when males were maintained and mated at various light/dark regimens, including free-running rhythm conditions of either constant light or constant dark. Third, we have tested the effects of remating and the effects of aging on the timing of pup-killing and parenting strategies. And finally, we have examined individual variation in the neural timing of these behaviors and correlated these results with hormonal events that occur during late fetal development. All in all, our research has suggested a variety of new phenomena to be studied, including the possibility of a circadian-based timing system that facilitates the long-term synchronization of behavioral changes.

The Time-Course of Behavioral Changes Triggered by Ejaculation

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

(1) *Pre-mating.* In virgin males, half of all individuals spontaneously kill pups while the other half do not harm them. These latter males either "Parent" pups (about 40%) or they "Ignore" them (about 10%). By definition, a parental male retrieves a pup to his nest where he incubates it and keeps it warm (vom Saal, 1985; Elwood, 1986). Males who "Ignore" pups neither harm nor parent them.

(2) *Ejaculation and Pregnancy of Mate.* Ejaculation intensifies pup-killing behavior. Virtually all males will 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 ceases and most males behave parentally toward pups. They remain parental throughout their mate's lactation.

(4) *Post-weaning of Offspring.* Between 50 and 60 days after mating, many males spontaneously begin killing pups again. The re-emergence of infanticidal behavior thus coincides with the weaning of pups.

Remarkably, this entire behavioral cycle toward pups occurs even when a CF-1 male is kept totally isolated from his mate and deprived of any

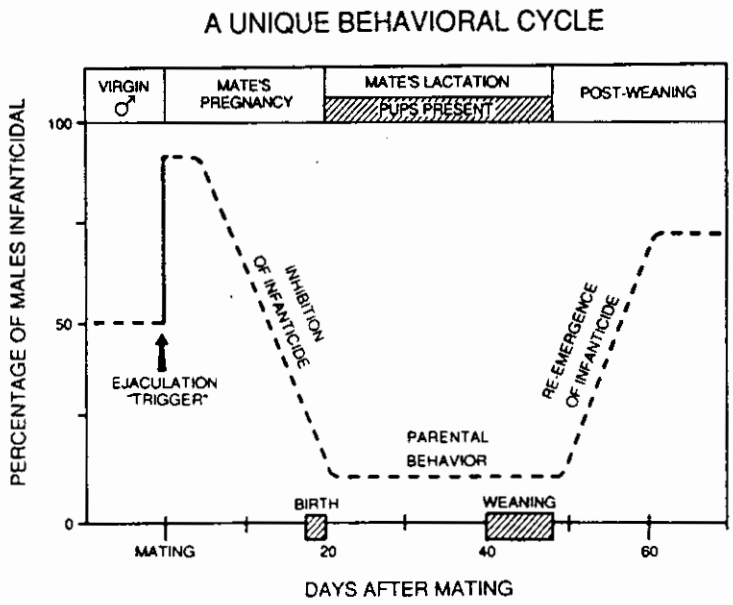

Figure 1 Schematic representation of temporal changes in behavior toward pups resulting from ejaculation in CF-1 stock male house mice.

female cues whatsoever following ejaculation (vom Saal, 1985; Perrigo and vom Saal, 1989). Furthermore, the inhibition of infanticide occurs after mating even when a male's pituitary and testes are removed (Perrigo *et al.*, 1989a). 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.

The Measurement of Infanticide and Parental Behavior

When a male house mouse encounters a neonate he either attempts to kill it or he does not harm it. These are clear-cut, unambiguous responses. We can easily assess a male's behavior by placing a 1–3 day old pup in his home cage. If a male is infanticidal, he will typically approach the pup, rattle his tail, and suddenly lunge at and attempt to kill the pup with rapid

bites to the head and back. This is an acute and dramatic response, so we immediately intervene and try to rescue the pup from attack as quickly as possible.

The opposite response from infanticide is parental behavior. A Parental male will typically groom the pup about the head and genitals before retrieving it to his nest. Interestingly, when a parental CF-1 male is allowed to incubate a pup, he appears sedated and is largely inattentive to disturbance. When the small subset of CF-1 males who "Ignore" pups are tested repeatedly, some may become infanticidal while others begin retrieving and incubating pups (vom Saal, 1985). "Ignorer" males thus appear to straddle a neutral behavioral state between infanticide and true parental behavior (Perrigo and vom Saal, 1989).

Recently, however, we have modified the above test procedure so that injuries to live pups are virtually eliminated (Perrigo *et al.*, 1989b). Test pups are now 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 screen-encased pup is quiescent, secure and completely buffered from attack. Thus, when an infanticidal CF-1 male encounters a screen-protected pup, he routinely attacks and repeatedly bites at the screen, without injuring the neonate (Perrigo*et al.*, 1989b). If, however, the male does not show any intent to harm the pup, the next step is to introduce an unprotected pup for 30 minutes and determine whether the male ignores the pup, or, if he is truly Parental. While this humane test procedure seems to be a reliable assessment of infanticidal tendencies in CF-1 stock males, a screen-protected pup has not proven to be an effective testing paradigm in either wild stock (unpublished observation) or other laboratory stock house mice (Elwood *et al.*, 1990).

Finally, it should also be emphasized that a male's reaction toward a newborn pup is a generalized, non-specific 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 (vom Saal and Howard, 1982; Svare *et al.*, 1984; vom Saal, 1985; McCarthy and vom Saal, 1986). Male mice also have spontaneous ejaculations nearly every night (Huber and Bronson, 1980), but this event does not in any way influence their behavior toward pups.

Female Cues *Per Se* Do Not Inhibit Infanticide in Virgin CF-1 Males

As noted in the introduction, there are multiple inhibitory mechanisms that prevent a male mouse from harming his offspring, and cohabitation

with a female will effectively inhibit male infanticidal behavior in some stocks of house mice. With regard to the ejaculatory phenomenon, however, the CF-1 mouse is an ideal choice of study, mainly because virgin CF-1 males appear totally insensitive to female cues *per se* as an inhibitor of infanticide. To illustrate this point, two groups of adult, virgin CF-1 males were identified as infanticidal on a pretest and then exposed to an array of chemical and tactile cues from either pregnant or nonpregnant females.

In the first phase of this experiment, a wire mesh bottom cage containing either three females, 10–11 days pregnant, or three nonpregnant females was suspended over each male's cage; thus, female urine and wastes showered freely on the males below. Pregnant females were changed every seven days so that none would deliver pups while suspended overhead (nonpregnant females were also changed every seven days). Each male was tested with a pup beginning at the time of first exposure to females suspended overhead (Day 0), and then retested every three days thereafter until day 21 (pups would have been born by now if the males had been allowed to mate).

In the second phase of this experiment, males in both groups were now allowed to cohabit with either a pregnant female or a sexually unreceptive, ovariectomized female. Likewise, pregnant and ovariectomized females were also replaced every seven days. During this phase of the experiment, testing with a pup occurred every seven days. Finally, after 21 days of cohabitation, all females in both groups were replaced with a pregnant female scheduled to give birth 10–12 days later. One more test with a pup occurred on Day 28 and each female was allowed to give birth in the male's own cage. When litters were born, cages were checked daily each morning over the next 10 days for any evidence of pup-killing. Throughout both phases of this experiment, a control group of virgin infanticidal males was also tested on the same schedule. The control males were not exposed to any female cues whatsoever.

As shown in Figure 2, which visually summarizes all of the results of this experiment, only the delivery of pups in the male's own cage inhibited infanticidal behavior in the virgin males ($P < .0001$ as compared with the control males). Only one litter was killed in the virgin male Groups 1 and 2, and previous experiments have established that if a male mouse kills one pup he will also kill the entire litter (vom Saal and Howard, 1982; Palanza and Parmigiani, 1991). 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. Evidence from Parmigiani's

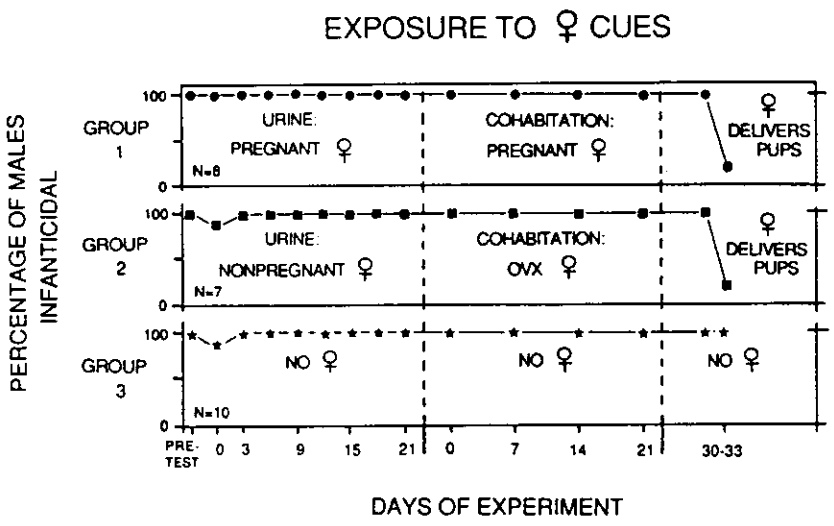

Figure 2 The response toward newborn pups when virgin infanticidal males were exposed to various combinations of chemosensory and tactile cues from female mice (OVX = ovariectomized). A zero to 100 percentage scale is shown for each of the three groups.

laboratory suggests that 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 (see Palanza and Parmigiani, 1991; Parmigiani *et al.*, this book). Regardless of these results, the act of ejaculation is still the primary stimulus that inhibits infanticide and regulates the timing of parental behavior in CF-1 males. Figure 2 also confirms that even when tested repeatedly with a pup, this procedure does not in any way inhibit infanticide among virgin males.

The Effects of Gonadal and Pituitary Hormones

As will be detailed later, differential exposure to sex steroids during fetal development clearly influences the way adult male mice behave toward young. Some steroid-sensitive behaviors are "organized" during perinatal development and do not require the presence of specific gonadal hormones in order for the behavior to occur in adulthood (Beatty, 1979; vom

Saal, 1983). But other behaviors may be "sensitized" during perinatal development and therefore require the presence of gonadal hormones in adulthood in order for the behavior to occur ("activation").

When virgin CF-1 males are castrated and allowed to interact with a pup for the first time, males behave parentally (Perrigo *et al.*, 1989a). If, however, these same castrated males are implanted with a 1 cm Silastic capsule containing 5 mg of crystalline testosterone (dissolved in .02 cc of sesame oil) and retested for their behavior toward pups several days later, half of the previously parental males will exhibit infanticide. As depicted earlier in Figure 1, this finding mimics the typical 50/50 proportion of spontaneously infanticidal versus non-infanticidal males observed whenever a large random sample of gonadally intact virgin CF-1 males are tested. Removal of the testosterone capsule abolishes infanticide. In summary, concurrent exposure to testosterone is required for a virgin male to exhibit infanticide.

The act of ejaculation causes a dramatic surge in LH (luteinizing hormone) and testosterone in male mice (Coquelin and Bronson, 1980), so we tested whether the hormonal changes triggered by mating might be responsible for mediating changes in a male's behavior toward pups. Twenty-two spontaneously infanticidal males were hypophysectomized (their pituitary gland was removed) and castrated, and, in order to maintain both their ability to mate and exhibit infanticide (vom Saal, 1983), they were also implanted with a 5 mg testosterone capsule (see above). Half of the males were allowed to mate while the other half were not. When tested with a pup at 20 days after mating, only 1 out of 12 mated males exhibited infanticide while 6 out of 10 non-mated males still exhibited infanticide ($P < .05$; Perrigo *et al.*, 1989a). Males who were hypophysectomized and mated thus showed a post-mating inhibition pattern identical to that observed in previous experiments using intact males (vom Saal, 1985). This finding revealed that the mating-induced inhibition of infanticide is a neurally-timed and mediated response, operating independently from pituitary hormone secretions or changes in gonadal secretions resulting from of mating (Perrigo *et al.*, 1989a).

How Do Mated Males Keep Track of Time?

Since the stimulus of ejaculation results in an unusually prolonged sequence of behavioral changes, a mated male must somehow be able track his mate's pregnancy and thus stop killing pups and behave parentally at the appropriate time. This prompted us to ask how mated males could measure the passage of time after mating. Because CF-1 males do not need female cues or typical hormonal cues in order to exhibit this response, this

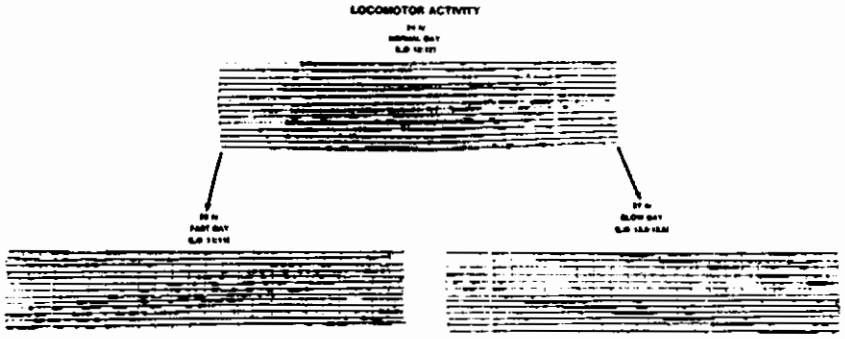

Figure 3 Daily locomotor patterns in a representative *fast day* male (left) and *slow day* male (right); 24 hr strips from an event recorder are pasted consecutively over several days. The dark bars in each strip represent when the animal was active on a running wheel. All individuals began their entrainment at a normal L:D 12:12 cycle; the top picture represents a typical activity pattern of a standard 24 hr day. The stair-step patterns generated in the lower pictures demonstrate that the *fast day* animal and the *slow day* animal were entrained to their respective photocycles.

clearly suggested that the efficacy of their strategy depended on a unique neural timekeeper.

We speculated that mated males could keep track of time either: 1) by measuring the absolute amount of time passing after ejaculation, or 2) by assessing the number of light/dark cycles experienced after ejaculation. Since photoperiodic variation in nature always provides infallible temporal cues for entraining daily (circadian) and seasonal cycles of feeding, breeding, metabolism and movement, we suspected the latter hypothesis. To test both possibilities, we used an experimental paradigm that allowed us to distinguish between absolute time (a standard 24 hr day) versus the number of light/dark cycles experienced: CF-1 males were thus housed at artificially fast (L:D 11:11 = 22 hr.) versus artificially slow (L:D 13.5:13.5 = 27 hr) daylengths (Perrigo *et al.*, 1990).

"Fast" versus "slow" time

One hundred adult CF-1 males were placed in light-tight, coffin-sized boxes illuminated inside with a 15-Watt fluorescent lamp (L:D 12:12 was

their initial light/dark cycle). Fifty males in each group were slowly adapted over a 25 day period to the 22-hour *fast day* cycle or the 27 hr *slow day* cycle by either increasing or decreasing the length of their light and dark exposure by several minutes each day. To verify behavioral entrainment, the locomotor patterns of several randomly chosen males in each group were monitored in cages with a running wheel interfaced to an event recorder (Figure 3). Males were allowed to mate and then screened for infanticide one day after ejaculation. Parental males were discarded from the experiment while the remaining infanticidal males (about 85% in both the *fast* and *slow day* groups) were retested with a pup between 16 and 25 absolute (24 hr) days after mating. The rationale behind our test procedure is illustrated by the timeline diagram in Figure 4. Specifically, half of the *fast day* males were retested at 16.3 absolute days (=18 light/dark cycles) and half were retested at 20 absolute days (= 22 light/dark cycles) after mating, while half of the *slow day* males were retested at 20 absolute days (=18 light/dark cycles) and half were retested at 24.8 absolute days (= 22 light/dark cycles) after mating. Our objective here was to directly compare both groups at 20 absolute days after mating and also control for the number of equivalent light/dark cycles experienced by both groups (18 versus 22 cycles).

Figure 5 shows the post-mating inhibition of infanticide graphed in two complementary perspectives: First, in relation to the number of absolute (24 hr) days experienced after mating, versus second, in relation to the number of light/dark cycles experienced after mating. When viewed side-by-side, the graphs suggest the presence of a unique neural timekeeper. At 20 absolute (24 hr) days after mating there was a significant difference in the frequency of infanticide between the*fast* and *slow day* groups (13% versus 61%, respectively; $P < .005$), suggesting that mated males did not rely on the amount of absolute time after mating as a cue to inhibit infanticide. Likewise, no differences were noted in the frequency of infanticide with both groups matched for experiencing the same number of light/dark cycles. This suggested that photoperiodic cues synchronized the shift in behavior, since a sudden transition from violent to benevolent behavior toward pups occurred as a function of the number of light/dark cycles experienced after ejaculation rather than the amount of absolute time (24 h days) experienced (Perrigo *et al.*, 1990).

The fact that dramatic shifts from infanticide to parental behavior in male mice parallel the behavioral and temporal dimensions of pregnancy in females is in itself interesting. Infanticide is also a fundamental component of the behavioral repertoire of female house mice — virtually all pregnant wild-stock females kill pups up to the time of parturition, at which

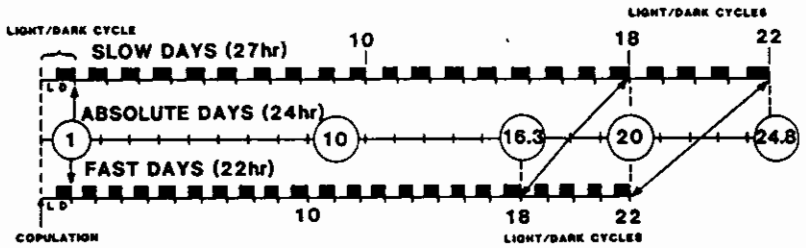

Figure 4 The relationship between absolute time (24 hr days) and the increasing desynchronization of light/dark cycles experienced by both groups during the course of this experiment. The alternating dark bars on the fast and slow time scales represent the dark phase of the repeating light/dark cycle. By 20 absolute days after mating, *fast day* males had experienced 4 more light/dark cycles than *slow day* males.

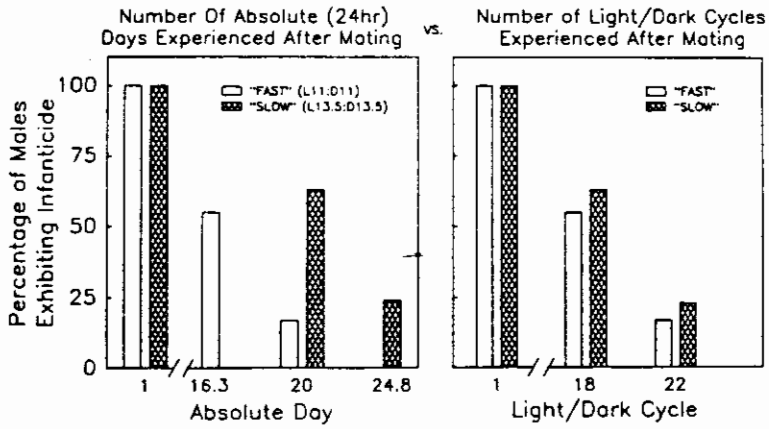

Figure 5 The percentage of male mice maintained at *fast* (=22 hr) and *slow* (=27 hr) days exhibiting infanticide when graphed in relation to absolute (24 hr) days versus the number of light/dark cycles experienced after ejaculation.

time they become parental (McCarthy and vom Saal, 1985; Soroker and Terkel, 1988). Thus, both sexes exhibit infanticide and seem to share a common suite of parental behaviors expressed at the time pups are born. But female house mice rely on the cues from developing fetuses and always seem to gauge the length of pregnancy in absolute time (Lanman and Seidman, 1977). Even when entrained to extreme light/dark cycles mimicking a 20 versus 28 hour day, female house mice still give birth the same number of absolute days after insemination (Davis and Menaker, 1981).

Male mice have apparently evolved a novel timekeeping solution for synchronizing their behavior toward pups with the duration of their mate's pregnancy. Some photoperiodically mediated phenomena in rodents, such as the preovulatory surge in LH, and hence, the organization of estrous cycles, regularly occur at 4–5 day multiples of daily light/dark cycles (Alleva *et al.*, 1968; Fitzgerald and Zucker, 1976). Unlike our present finding, however, these events are mediated by cyclic changes in the secretion of pituitary and gonadal hormones. As described in the previous section, the mating-induced inhibition of infanticide can occur in male mice even in the absence of pituitary hormones or changes in gonadal secretions.

How Does the Neural Timing System Operate in the Presence or Absence of Photoperiodic Cues?

The above results suggested that ejaculation triggers a photoperiodically mediated timing mechanism that can synchronize a male's parental behavior with the presence of his pups (Perrigo *et al.*, 1990). 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 — just before the birth of their offspring at 22 days (Mennella and Moltz, 1988). The most obvious physiological explanation of this result would be a circadian-based timing mechanism, which, in the absence of entraining photoperiodic cues, would exhibit an endogenous rhythm with a period of about 24 hours.

Thus, 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 hypothesis was that males kept in different free-running conditions might undergo the transition from pup-killing to parenting at different times after mating.

Pretesting and partitioning of virgin males

As noted earlier, 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 also shown 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 (vom Saal, 1983; Perrigo and vom Saal, 1989; Perrigo *et al.*, 1989a). These behavioral differences apparently result from testosterone emanating from fetal siblings and thus depends on whether a male fetus develops next to same or opposite sex fetuses (vom Saal and Bronson, 1980; vom Saal, 1989). Thus, we controlled for this physiological variation by identifying whether a virgin male was infanticidal or parental before he was delegated to an experimental treatment. The necessity for doing this will be obvious from our results.

Ninety-one virgin male mice (5 months of age and maintained since birth at L:D 12:12) were, for the first time, pretested for their behavior toward a 1-3 day old pup. The males were classified as follows: 46 were infanticidal (51%), 33 were parental (36%), and 12 males ignored the pup (13%). This replicates previous findings (vom Saal, 1985; Perrigo *et al.*, 1989a). One day later, the pretested males were evenly distributed among three animal rooms maintained at one of three light/dark treatments: constant light (LL), L:D 12:12, or constant darkness (DD). Thirty males were delegated to each light 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 both the L:D 12:12 and LL rooms (range of 20-30 Lux as measured at the bottom and top, respectively, of the cage rack where males were housed).

After three weeks of accommodation to their light/dark 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 hrs in the L:D 12:12 treatment; matings in the DD and LL rooms were also done at the same absolute time). All females were removed after three hours. The presence of a vaginal plug confirmed whether a male had ejaculated. This procedure was repeated for five days until most of the males in each group had mated, resulting in a distribution of 14-15 Infanticidal, 8-10 Parental and 3-4 Ignorer males within each of the three experimental groups. Upon confirmation of ejaculation (Day 0), each male was immediately tested with a pup and then retested between 1400 and 1500 hrs 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 red light, which, for control purposes remained on constantly in all three treatment rooms.

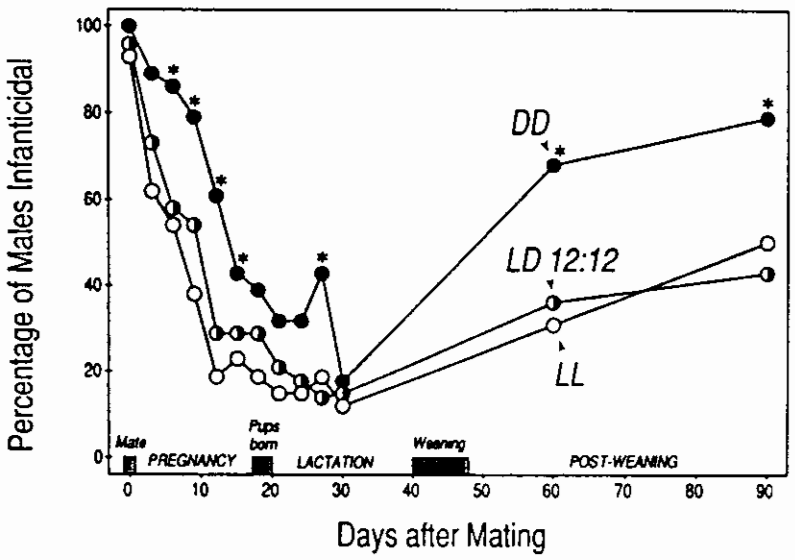

Figure 6 The hierarchically sorted response patterns of individual male house mice who were mated and tested for their behavior toward a newborn pup (KILL=infanticidal; P=parental; Ign=ignored pup) at conditions of constant dark (DD), L:D 12:12 and constant light (LL). Males were tested immediately after ejaculation (Day 0) and 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.

The time-course of behavioral changes

Figures 6 and 7 show complementary perspectives of how each treatment group and pretest behavior of males behaved toward pups during the entire 90 day test period following mating. The raw data in the Figure 6 matrix are hierarchically sorted for the first 30 days of this experiment to reveal the complete range of behavioral and timing variation for each individual within each light treatment, whereas the Figure 7 graph focuses on the overall pattern of timing variation among males subdivided in relation to their pretest behavior toward pups. The behavioral differences

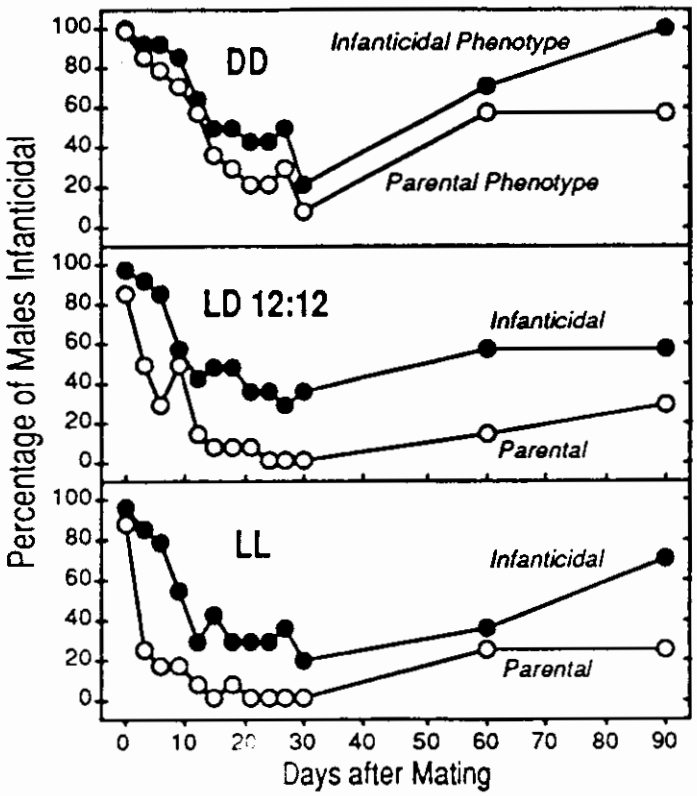

Figure 7 The temporal pattern of behavioral changes observed when males within each treatment group are graphed in relation to their behavioral phenotype as virgins (Infanticidal or Parental), which is correlated with testosterone exposure during late fetal development (see text). Males in each group were ranked according to the day after mating when they expressed their first bout of parental behavior. Mann-Whitney U-tests revealed significant transition time differences between the two phenotypes in LL ($p < 0.005$) and L:D 12:12 ($p < 0.005$), whereas in the absence of light (DD), there were no significant differences ($p > 0.40$). (Redrawn from Perrigo, *et al.*, 1991).

shown in these figures are striking. Statistical differences were assessed with Chi-square analyses comparing the frequency of infanticidal versus noninfanticidal behavior among the groups at each test day (for analysis purposes, parenting and ignoring behaviors were combined as noninfanticidal).

Transitions in behavior after mating

As shown in the Figure 6 matrix, there were significant overall group differences ($p = .05$ or less) in the frequency of infanticide on Days 6, 9, 12, 27, 60, and 90 after mating. In general though, the group differences resulted from a higher frequency of infanticide in the DD males. Also obvious in Figure 6 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 the interface between infanticidal to parental transitions.

The frequency results from the L:D 12:12 group in Figure 6 also match previous experiments where CF-1 males were tested for infanticide only one or two times between days 1 and 21 after mating (vom Saal, 1985), 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 males

As shown in the Figure 7 graph, which partitions males according to their pretest behavior toward pups, ejaculation immediately triggered pup-killing in virtually all of those males classified as Parental in the pretest. It should also be emphasized here that mere exposure to females, without ejaculation, does not induce pup-killing in virgin Parental males (Perrigo *et al.*, 1991). 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 ($p = .05$ or less) on Days 3, 6, 9, 12, and 15. 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 7 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.

The effects of light

One further point is visually obvious in Figure 7, namely that males who were categorized as Parental 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. Indeed, Mann-Whitney U-tests revealed significant transition time differences between the two pretest categories of males in both the LL ($p < .005$) and L:D 12:12 treatments ($p < .005$). In sharp contrast, no significant transition time differences were noted between the two pretest categories of DD males ($p > .40$). 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 post-mating transition to parenting among parental phenotypes, whereas in the absence of light (DD), there were no significant behavioral differences among pretested infanticidal and parental males.

The period of free-running locomotor activity in DD versus LL: Test of the circadian-linked timing hypothesis

After the above experiment 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 any correlation existed between the period (τ) of a circadian activity/rest cycle and the timing of post-mating behavioral changes in either DD or LL.

Our results were consistent with "Aschoff's Rule" (Aschoff, 1960; Pittendrigh, 1960); thus, the free-running activity cycle of DD males was considerably shorter, about 1 hr less, than the cycle of LL males. The period (t) of the activity/rest cycle in DD males was $24.13 \pm .05$ hrs (meaan $\pm$ sem) while in the LL males, $\tau = 25.15 \pm .17$ hrs ($p < .0001$). These results, and the results from the previous experiment, do not in themselves support the hypothesis of a circadian timing mechanism keeping track of daily cycles experienced after mating (Perrigo *et al.*, 1990). If this had been the case, then a post-mating shift to parental behavior should have occurred more

rapidly in DD males because of their shorter free-run period. Or, at the very least, there should have been no differences between the LL and DD groups. Figures 6 and 7, however, clearly suggest the opposite result. It should also be noted that the act of ejaculation has no effect on the phase (ϕ) nor period (τ) of the free-run when CF-1 males are maintained in DD (unpublished observation).

Within the eight LL males, however, there was a significant positive correlation between the period of an individual's activity cycle and the number of days elapsed between ejaculation and their first expression of parental behavior ($r^2 = .75$, $p < .05$). In contrast, no such correlation existed in DD animals ($r^2 = .16$, $p > .25$). These results are unique, but they neither support nor refute the involvement of a circadian-based timing mechanism, they only suggest a correlation in constant light. Nor, as will be discussed later, do they eliminate the possibility of "masking" in constant light.

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 showed 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; $p > .50$). Thus, testing in either light or dark did not seem to influence how a male behaves toward pups.

Observing the transition at 12 hour intervals and the repeatability of the timing phenomenon within individuals

The following experiment asked whether the effect of first mating and the timing of inhibition observed in Figure 6 is repeatable within individuals. Six males who showed rapid transition times from infanticidal to parental behavior were thus selected from each of the three treatments in Figure 6 and mated for the second time at 100 days after their first mating. The first test with a pup occurred immediately after ejaculation was confirmed.

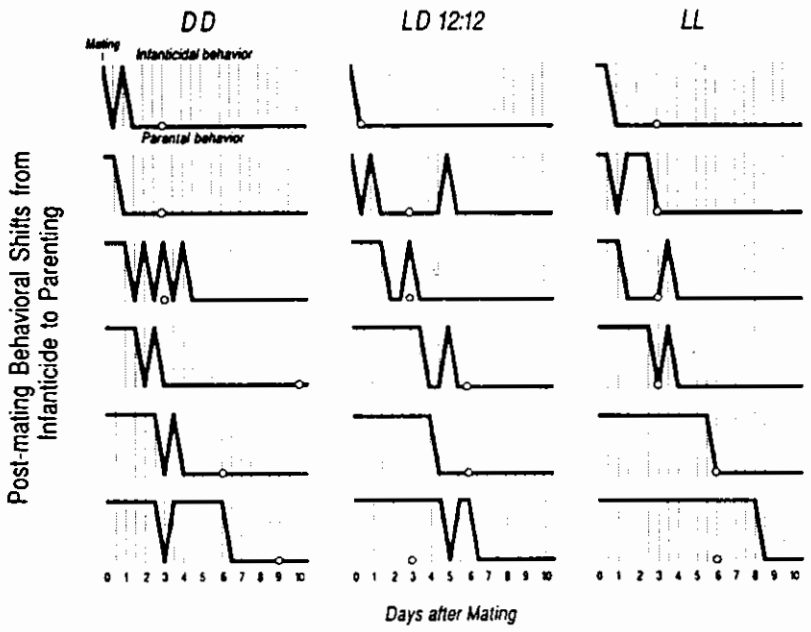

Figure 8 Day-to-day oscillations in behavior toward pups in individual males (six from each treatment) who were re-mated at 9 months of age and tested for infanticide every 12 hours (each division = 12 hrs of real time) for 12 days (the scale ends at 12 days for clarity). For comparison purposes, a white dot indicates the day when parental behavior was first noted (as measured at 3-day intervals) when males were mated for the first time at 6 months of age.(Redrawn from Perrigo, *et al.*, 1991).

Unlike their first mating, however, in which retests occurred at three day intervals, each retest in this experiment occurred instead at 12 hr intervals until the end of Day 12. Thus, all subsequent retests in the L:D 12:12 room occurred at the time of lights off (2400 hrs) and at the time of lights on (1200 hrs). Tests in the DD and LL rooms were done at the same absolute time.

As shown in Figure 8, 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 fluctuations, sometimes periodic, in their behavior toward pups before

"locking in" to consistent parenting. The white dot shown in Figure 8 indicates the test day after their first mating (from Figure 6) when parental behavior was first noted in each male. Since these males were tested at different intervals (every 12 hrs versus every three days), 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 toward pups is "reset", ejaculation can trigger a new cycle of infanticidal inhibition, one that appears programmed to last about the same length of time within an individual.

Aging: A more prolonged and attenuated response

The males kept in the L:D 12:12 condition (from Figure 6) 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 (*i.e.*, 6 months versus 18 months of age). All surviving males (18 out of the original 28) were mated and then tested every three days for 30 days (same procedure as earlier). As shown in Figure 9, the transition to parental behavior after mating was significantly more prolonged when the males were a year older. In fact, five of the older males (28%) never ceased killing pups. With regard to individual variation, the scatterplot in Figure 10 shows there was no individual correlation (as suggested by the previous experiment) between the time elapsed between mating and parenting when each male was a full year older ($p > .35$). In general then, the neural timing mechanism triggered by ejaculation appears to attenuate with age.

OVERVIEW AND CONCLUSIONS

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 (Svare, 1981; McCarthy and vom Saal, 1985; Perrigo *et al.*, 1990). 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 (Huck *et al.*, 1982; Labov *et al.*, 1985; vom Saal, 1985; Elwood, 1986), it is now extensively documented that multiple, redundant mechanisms

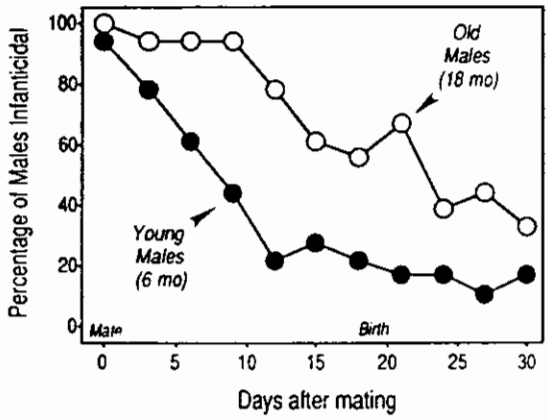

Figure 9 Males kept in L:D 12:12 condition remained individually housed until 18 months of age, at which time the 18 (out of 28) survivors were again mated and re-tested for infanticide every three days until 30 days after mating. The graph demonstrates that the transition time from infanticide to parenting becomes substantially prolonged by aging. (Redrawn from Perrigo, *et al.*, 1991).

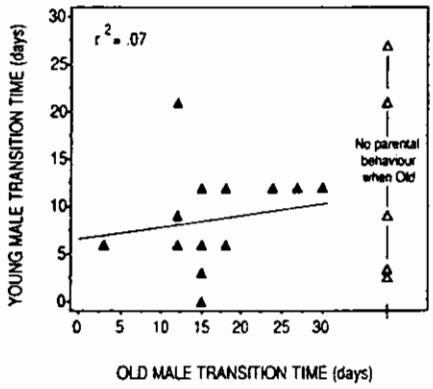

Figure 10 The neural timing mechanism also attenuates with age. There was no correlation in transition time, defined here as the test day after mating when an infanticidal male exhibited his first bout of parental behavior, among individual males (closed triangles) when they were mated when Young (6 months old) versus when they were Old (18 months old); $r^2 = .07$, $p > .35$. Since five of the Old males did not cease killing pups and, hence, did not exhibit parental behavior after 30 days of testing, they are shown separately (open triangles) on the right side of graph.

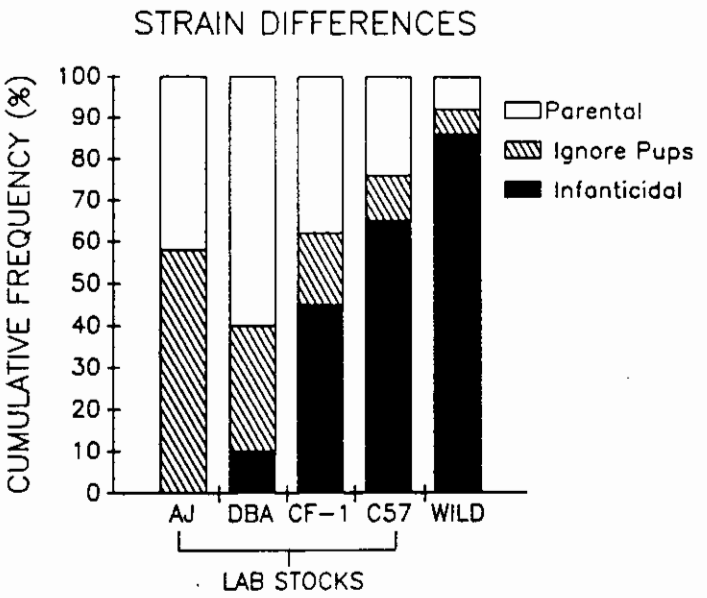

Figure 11 The genetic variation in background frequencies of spontaneous infanticidal and noninfanticidal behavior (parenters and ignorers) among virgin male house mice from four laboratory stocks and one wild stock.

have indeed evolved to inhibit pup-killing behavior in male mice (*e.g.* see Elwood, Parmigiani, this volume). As a general rule, these behavioral polymorphisms stem from the amplification of genetic differences among the myriad of inbred domestic and wild stocks used in the study of infanticide (*e.g.* see Figure 11; Perrigo and vom Saal, 1989; Palanza and Parmigiani,1991: Parmigiani *et al.*, this book). While house mice originated in the Old World, they can now be found living worldwide in an amazing variety of feral and commensal habitats, mainly because of their enormous behavioral and reproductive flexibility (Bronson, 1979; Berry, 1981; Perrigo, 1990). Thus, the degree to which these multiple inhibitory mechanisms are expressed may reflect subtle differences in the socioecology and deme structures of original founder stocks.

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: Marshal and Sage 1981). Wild male

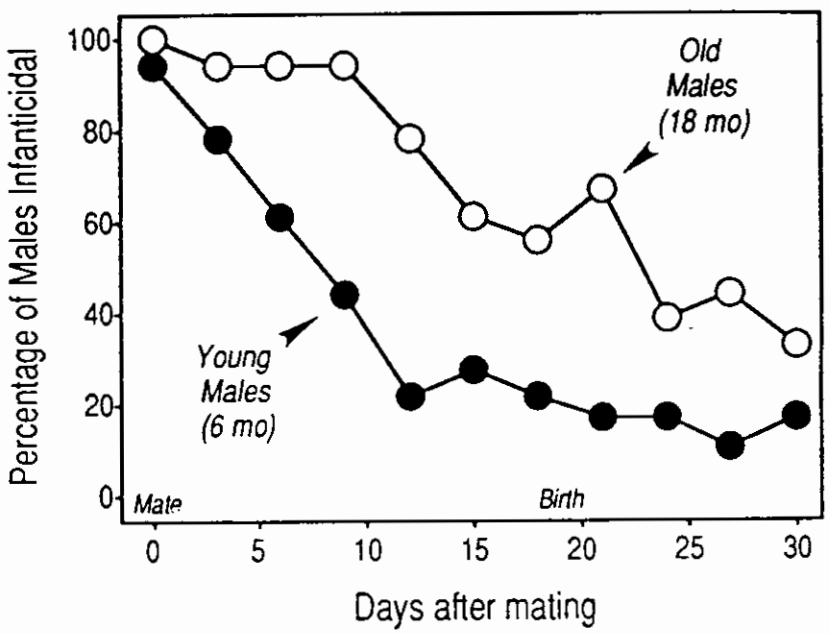

Figure 12 The response pattern of 19 individual virgin male wild stock house mice (five months of age) who were maintained at LD 12:12 and tested for their behavior toward a newborn pup (KILL=infanticidal; P=parental; Ign=ignored pup) immediately after mating (Day 0), and at Days 7, 14, 21, 60 and 90 after mating.

M. domesticus trapped in Alberta, Canada behave similar to CF-1 males. As shown in Figure 12, their pattern of infanticide inhibition after ejaculation in L:D 12:12, including the expression of parental behavior and re-emergence of infanticide 60–90 days later, resembles that shown in Figure 6; likewise, 20+ days of cohabitation with pregnant females does not inhibit infanticide either, although neither does the birth of pups in a wild male's home cage (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 (Soroker and Terkel, 1988). And 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 soiled cage bedding of a pregnant female. The latter results suggests the evolution of female counter-strategies to defend their litters from infanticidal attack (Mennella and Moltz, 1988; see also Parmigiani *et al.*, 1988; Elwood and Kennedy, 1990).

As demonstrated earlier, however, 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 clearly seemed to inhibit infanticide (Figure 2). Interestingly, the data from Figure 2 also suggest that when a virgin male is present during the birth of pups, infanticide is inhibited to such a degree that even the ensuing postpartum mating will not trigger his typical pup-killing behavior. It cannot be discerned, however, whether this reflects a female counter-strategy or represents yet another redundant mechanism to prevent a male from accidentally killing his own offspring.

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 environment for a nocturnal species such as the house mouse. On the other hand, house mice in the laboratory routinely show a great deal more flexibility in their daily activity patterns than do other nocturnal rodents (Perrigo, 1987,1990), nor is their reproduction under photoperiodic control (Bronson, 1979; Berry, 1981).

Regardless of these potential arguments, Figures 6 and 7 still revealed that the stimulus of light clearly influenced the timing of behavioral changes following ejaculation in house mice. Photoperiodic (L:D 12:12) and constant light (LL) dramatically accelerated the inhibition of infanticide and emergence of parental behavior following ejaculation, but mainly in those males who had displayed parental behavior when they were pretested as virgins. 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.

As mentioned earlier, 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 (vom Saal, 1983;

Perrigo *et al.*, 1989a; see also Samuels *et al.*, 1981). This is known as the intrauterine position phenomenon (vom Saal, 1989) 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 therefore exposed to differential sex steroid concentrations depending on whether they develop next to same or opposite sex siblings. 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 (vom Saal, 1989).

The broad range of timing variation shown among all three treatment groups in Figure 6 matches with the phenotypic variation in infanticide predicted by the intrauterine position model (Perrigo *et al.*, 1989a; Perrigo and vom Saal, 1989). 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 represents about 10-15% of the population, and the data from Figure 6 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 (see also Elwood and Ostermeyer, 1984). In fact, we have obtained preliminary evidence for this by examining the five Old males from Figure 9 who were still infanticidal at 30 days after mating. When cohabited with a pregnant female, four out of five of these mated males did indeed express parental behavior within 14 days of cohabitation (unpublished observation).

A Proposed Neuroethological Model: The Programming of Individual Behavioral Thresholds

Figure 13 is a scheme depicting the transition from infanticide to parental behavior following ejaculation in CF-1 males. As depicted in the top half of Figure 13, the intense sympathetic stimulus of ejaculation triggers a sudden and dramatic behavioral change, which thus intensifies a male's motivation to kill pups. Implicit in this prediction is that the act of ejaculation is a neural "supercharge" that immediately drives most parental CF-1 males well above their threshold for infanticidal behavior (verified in Figures 7 and 8). From an ecological standpoint, it is not surprising that ejaculation

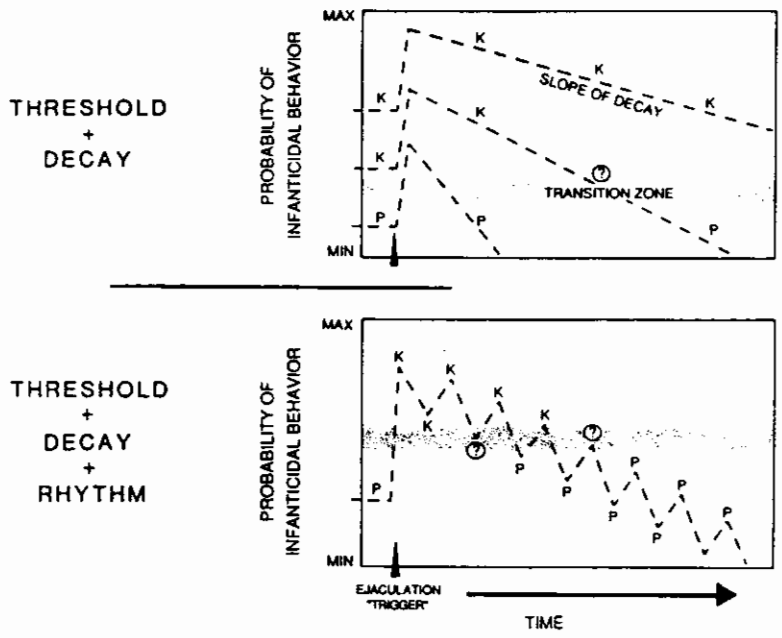

Figure 13 A potential scheme for understanding how the post-ejaculatory inhibition of infanticide might operates in CF-1 stock males. As shown in the top half of the figure (Threshold + Decay), males who behave parentally (P) before they mate are stimulated by the act of ejaculation to exhibit infanticide, while 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. The bottom half of this figure (Threshold + Decay + Rhythm), while purely speculative, depicts the potential behavioral results if a rhythmic (oscillating) behavioral decay is superimposed on the inhibition process (see text).

triggers such an immediate pup-killing reaction. Female house mice exhibit a strong postpartum estrus within 24 hours after parturition; thus, if a virgin male copulates with a newly lactating female he will increase his reproductive success by immediately seeking out and destroying her litter.

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 (*i.e.* the Transition Zone in Figure 13), then parental responses can emerge. When CF-1 males are hypophysectomized, which eliminates the pituitary hormone prolactin, a facilitator of parental behavior in females, they are significantly less likely to display parental behavior after mating and thus tend to ignore pups (Perrigo *et al.*, 1989a). Since hypophysectomy does not prevent the post-ejaculatory inhibition of infanticide, when viewed together, these findings suggest that the inhibition of infanticide and the occurrence of parental behavior are independent phenomena.

As implied by the previous section, 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 ejaculation and parenting was relatively constant in individual young males (6 to 9 months of age), while in old males (18 months of age) there was a more prolonged inhibition of infanticide and an increased proportion of males who failed to show parental behavior following ejaculation. 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.

Also implicit in the Figure 13 model is that when a male "ignores" a pup, he is at or near his behavioral threshold (Transition Zone) during the inhibition process. Thus, if testing with a pup occurs during this transition period, then any one of three behavioral states could be observed: Infanticide (K), ignoring, or parenting (P). The evidence for this comes from Figure 8. By testing males at 12 hr 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 this transition period. While purely speculative, the bottom half of Figure 13 depicts the potential behavioral results when an oscillating behavioral decay is superimposed on the inhibition process. This type of mechanism is one possibility that could explain the unusual behavioral fluctuations shown in Figure 8.

Is There a Circadian Timing Mechanism?: Pros and Cons

How this behavioral transition is neurally timed still remains perplexing. When we compared the timing of the transition from infanticide to parental behavior in CF-1 males entrained to 22 hr daylengths (L:D

11:11) versus 27 hr daylengths (L:D 13.5:13.5), the results clearly suggested that ejaculation triggers a neural timing system that "counts" photocycles. Thus, the objective in testing animals in conditions of constant dark and constant light was to determine how males would undergo the postmating transition from infanticide to parental behavior in the absence of a light/dark cycle. The males who were given a running wheel in constant dark (DD) showed a free-run of almost 24 hr (t = 24.1 hr). When tested at 18 days (or activity cycles) after ejaculation, 39% of all males housed in DD exhibited infanticide. This result, however, is not statistically different from the proportion of males housed in 22 hr or 27 hr days that killed pups when tested at 18 activity cycles (Perrigo *et al.*, 1990). On one hand, these findings are not inconsistent with the hypothesis that males can use an endogenous circadian system to "count" cycles following ejaculation.

But on the other hand, males kept in the free-running condition of constant, relatively low intensity light (LL) did indeed appear to measure the passage of time after mating differently than DD males. The inhibition of infanticide after ejaculation occurred more rapidly when males were housed in LL; in fact, the L:D 12:12 and LL groups both showed the same pattern in transition times. It must also be emphasized here that males in the L:D 12:12 condition do indeed exhibit a 24 hr activity/rest cycle under the lighting conditions provided in this experiment (unpublished observation). These findings are thus inconsistent with an entrainable "day-counting" mechanism, since the period (τ) of free-running activity for the males who were given running wheels in LL was 25.2 hrs. This suggests that males in LL 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. It must be explicitly emphasized, however, that within the context of this experiment, it not possible to eliminate whether a circadian timing system could have been "masked" by exposure to constant conditions (*e.g.* see Aschoff and von Goetz, 1988; Binkley, 1990). The process of masking occurs when an internal rhythm may be present, but is obscured (masked) by other direct effects; in this case, the propensity for nocturnal house mice to run on an activity wheel more often in darkness, and vice versa, to run less frequently when subjected to constant light.

Finally, we have made no attempt to explain how infanticidal behavior spontaneously re-emerges 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 stud-

ied behavioral and reproductive timing processes (*e.g.* Silver and Bittman, 1984; Daan, 1987). Ablation of the Suprachiasmatic Nucleus, exposure to high intensity constant light (both of which can disrupt circadian rhythms by rendering animals arrhythmic), or maintenance at skeleton photoperiods are several potential experiments that could distinguish whether light has a photoperiodic, direct, or some other regulatory role in the timing of this entire behavioral cycle.

In conclusion, and regardless of how such a neural timing system operates, the two to three week timespan often intervening between ejaculation and the inhibition of infanticide — plus the spontaneous re-emergence of infanticide after pups are weaned — clearly seems to redefine the possible temporal and behavioral relationships between a neural stimulus and its response.

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