Time and Sex in the Male Mouse: Temporal Regulation of Infanticide and Parental Behavior

*Glent Perrigo, *Lee Belvin, and *HFrederick S. Vom Saal

*Division of Biological Sciences, +Department of Psychology, and 1The John M. Dalton Research Center, University of Missouri, Columbia, Missouri, U.S.A.

Summary: Infanticide is a violent but successful reproductive strategy found in many mammals, particularly rodents. In mole house mice (Mus domesticus and M. musculus), the act of ejaculation provides a reliable neural signal for timing the birth of their offspring. However, a unique chronobiological aspect of this phenomenon is the extraordinary temporal latency that can occur between the stimulus (coital stimulation) and its adaptive neural response (male mice cease killing pups and behave paternalistically toward them instead). Specifically, the inhibition of infanticide is often time delayed for many days after a male ejaculates, but virtually always occurs before or around the time his own offspring would be born 18-20 days later. Furthermore, infanticide spontaneously reemerges 50-20 days after mating. In CT-1 stock male mice this entire behavioral sequence is synchronized with the female's reproductive cycle, and occurs even in the total absence of social cues or changes in environmental or gonadal hormones after mating. When entrained and reared at 22 h light/day (1:11) vs 23 h light/day (3:5:12:3:3) T-cycles, photoperiodic cues appeared to synchronize this dramatic shift in behavior, because a sudden transition from pup killing to paternal care was matched with the number of light/dark cycles experienced after ejaculation rather than the amount of real-time experienced, suggesting a circadian timing link. Housing in continuous light accelerated the postmating transition to parenting, whereas constant dark significantly delayed the transition to parenting, but still occurred by 3 weeks after mating. Most males tend to oscillate between infanticide and parental behavior for several days before locking in to contain parenting, regardless of lighting conditions. Variation in the time delay between ejaculation and the inhibition of infanticide was consistent within young individuals (<10 months of age), but in older males >18 months of age the time interval between ejaculation and parenting was significantly prolonged and attenuated. Another unique aspect of this phenomenon is that variation among individuals in their timing and response to light cues is correlated with phenotypic variation in sex steroid exposure during last fetal development. So far, no simple physiological explanation can account

Received August 8, 1991; accepted with revisions December 12, 1991.

Presented at the 26th International Congress on Chronobiology of the International Society of Chronobiology, June 18-21, 1991, Tel Aviv, Israel.

Address correspondence and reprint requests to Dr. G. Perrigo, Division of Biological Sciences, 105 Life Science Hall, University of Missouri, Columbia, MO 65211 U.S.A.
Infanticide—defined as the killing of conspecific young—is no longer considered maladaptive or sociopathological. It occurs in a wide variety of organisms of both sexes (1–4) and, in most natural situations, infanticide represents a violent but extremely effective reproductive strategy. With regard to male mammals, for example, field and laboratory studies have dramatically documented the reproductive advantages that accrue when an infant-killing male usurps the territory of another male. By killing the offspring of a deceased competitor, an infanticidal male benefits in two ways: first, he eliminates potential genetic and resource competition with his own offspring, and second, once a female's own young are killed, she rapidly ovulates again, typically being remated by the usurper male.

Infanticide is ubiquitous among rodents (5). When a male house mouse (Mus domesticus or M. musculus) encounters a neonate, he either tries to kill it or he does not harm it. Obviously, an effective infanticidal strategy must provide behavioral mechanisms that prevent male mice from accidentally killing their own progeny. Social cues, fetal and adult hormones, and genetic differences are three factors known to modulate a male’s behavior toward pups (6–9). However, there is a fourth and physiologically unique dimension to infanticide: time-dependent changes in a male’s behavior toward pups are triggered specifically by the stimulus of ejaculation during mating (10–12).

**A UNIQUE TIMING PHENOMENON**

It is now extensively documented that male house mice can use the stimulus of coital ejaculation as a reliable neural signal for timing the birth of their offspring (9, 10). 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 3 weeks later. When infanticide ceases, males routinely express parental behavior toward pups similar to that of a newly lactating female. Furthermore, infanticidal behavior spontaneously reemerges after offspring are weaned (10). These timed behavioral changes, which result specifically from coital ejaculation, occur consistently among various house mouse stocks (7–9, 13, 14). This phenomenon also occurs in the Norway rat, Rattus norvegicus (15), and probably in other rodents as well.

CF-1 stock male mice (Mus domesticus) are a superb model for studying the behavioral events triggered by ejaculation. There are four distinct phases to the CF-1 male’s behavioral cycle toward pups: (a) In virgin males, ~50% of all individuals spontaneously kill pups, whereas the other 50% parent them; (b) ejaculation intensifies pup-killing behavior such that virtually all males will attack and ill pups during part or most of their mate's pregnancy; (c) by the time pups are born 18–20 days later, infanticide ceases and males behave parentally during their mate’s lactation; (d) the
remergence of infanticide occurs between 50 and 60 days after mating and coincides with weaning. Remarkably, this entire behavioral cycle occurs even when a CF-1 male is kept totally isolated from his mate and deprived of any social cues after copulation (10,11). We are unaware of any other similar neural phenomenon evolved in mammals where such dramatic shifts in adaptive behavior are timed to occur so many days—indeed weeks—after a specific stimulus such as ejaculation.

The remainder of this article briefly reviews our preliminary findings about the nature of the ejaculatory trigger and the resulting time-delayed behavioral changes in CF-1 males. We have monitored the timing of pup-killing and parenting strategies when males were housed and mated at various light/dark regimens, including free-running rhythm conditions of constant light and constant dark. We have also correlated individual variation in the neural timing of these behaviors with hormonal events that occur during late fetal development. Finally, we have examined the effects of both remating and aging on the timing of these strategies. In all, our research has yielded a variety of exciting chronobiological phenomena.

**ASSESSING INFANTICIDAL AND PARENTAL BEHAVIOR IN MALE MICE**

An infanticidal male mouse will approach a pup, rat its tail, and suddenly lunges at and attempts to kill the pup with rapid bites to its head and back. In dramatic contrast, a parental male gently retrieves a pup to his nest where it grooms it and keeps it warm. These are clear-cut, unambiguous behaviors. We assess a CF-1 male’s behavior by sliding a 1- to 3-day-old newborn inside a 4- to 5-cm tube made of 1.5-mm wire mesh screen; the protected pup is then placed in the male’s home cage. When an infanticidal CF-1 male encounters a screen-protected pup, he typically attacks and repeatedly bites at the screen, but without harming the neonate (16). If a male does not show any intent to harm the pup, he is then tested with an unprotected pup in order to verify his parental behavior. Neither the sex, age, nor relatedness of the pup appear to have any discernible influence on a male’s tendency to exhibit infanticide or parental behavior. Thus, a male’s reaction toward a newborn pup is a generalized, nonspecific response with intrinsic equalization serving as the specific neural trigger that initiates the behavioral changes described earlier.

**HOW DO MATED MALES KEEP TRACK OF TIME?**

The unusually prolonged sequence of behavioral changes triggered by the ejaculation prompted us to ask how male mice gauge the passage of time after mating. Because CF-1 males exhibit this response in local social isolation, this implied the presence of a unique neural timing system modulating these behaviors. We postulated that mixed males could measure time either by (a) assessing the amount of absolute (real) time passing after ejaculation, or (b) assessing the number of light/dark cycles experienced after ejaculation. In natural situations, photoperiodic variation provides reliable temporal entrainment cues, so we suspected the second hypothesis. To test both possibilities, we used an experimental paradigm that allowed us to distinguish between absolute time (as measured in 24-h days) versus the number of light/dark cycles experienced (17).
Fast versus Slow Time

One hundred adult virgin CF-1 males were housed in light-dark boxes illuminated inside with a 15-W fluorescent lamp (initial light:dark cycle 12 h:12 h). Half were slowly adapted to an ambiently Fast (22-h) daylength (light:dark 11:11), whereas the other half were adapted to an artificially Slow (27-h) daylength (light:dark 3:5:1:5). Entrainment to each cycle was verified by monitoring the activity patterns of eight sentinel males kept in testing wheel cages interfaced to an event recorder. All experimental males were mated with an estrous-primed female at the time of lights on, tested for inaction, and then retested between 16 and 25 absolute (24-h) days after mating. 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 (12 light/dark cycles) after mating, whereas all of the slow day males were retested at 20 absolute days (18 light/dark cycles) and all were retested at 24.8 absolute days (22 light/dark cycles) after mating. Because, F-1 pups are born 19 days after mating, our experimental objective was to bracket the time when pups are born by comparing both groups directly at 20 absolute days after mating, while also controlling for the same number of light/dark cycles experienced (18 vs. 22 cycles). The rationale behind this paradigm is visualized in the upper portion of Fig. 1. The lower portion of Fig. 1 shows the postmating inhibition of inaction graphed in two complementary perspectives: first, in relation to the number of absolute (24-h) days experienced after mating, and second, in relation to the number of light/dark cycles experienced after mating. At 20 absolute days after mating there was a significant difference in the proportion of inactional males in the fast versus slow day groups (13% vs. 61% antiscopic, respectively; p < 0.005). In sharp contrast, there was no difference in the proportion of inactional males when both groups were matched for experiencing the same number of light/dark cycles. Viewed side-by-side, the graphs in Fig. 1 suggest that photoperiodic cycles synchronized the dramatic shift in behavior, because the transition from pup-keeping to parenting matched the number of light/dark cycles experienced after ejaculation rather than the amount of absolute time experiences.

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

The results report...
with a 15-W safel red light that remained on continuously in all three rooms. Our purpose here was to see whether male mice would exhibit an appropriate mating pattern in the absence of entraining light-dark cycles and, if so, whether males kept in different free-run conditions—and therefore expected to exhibit different circadian periods (Aschoff's rule: see 19,20)—would undergo the transition from pup-killing to parenting at different times after mating. As shown in Fig. 2, all three groups displayed a temporal pattern of behavioral changes well synchronized with the reproductive cycle of a female mouse. Almost all males were infanticidal immediately after ejaculation; however, the time interval between mating and parenting was significantly prolonged in DD males when compared with their LL and LD 12:12 counterparts. Significance differences (p ≤ 0.05) in the frequency of infanticide between DD males and other groups occurred during the first 15 days after mating, but disappeared by the time pups were born. Differences
reemerged at 27, 60, and 90 days after mating; in fact, nearly 80% of the DD males became infanticide again after pups were weaned, whereas only 40–50% of the LL and LD 12:12 males did so.

Eight to 10 representative LL and DD males were also monitored in running wheels to see whether any correlation existed between the period of an individual's free-run (as measured by activity onset) and the timing of postpartum behavioral changes (10). Our results were consistent with Aschoff's rule; thus, the circadian activity cycle of DD males ($r = 24.1 \pm 0.1$ h) was $-1$ h shorter than the cycle of LL males ($r = 25.2 \pm 0.2$ h; $p < 0.0001$). Within the LL males, however, there was a significant positive correlation between $r$ and the number of days elapsed between mating and the expression of parental behavior ($r^2 = 0.75; p < 0.05$). In contrast, no such correlation existed in DD animals ($r^2 = 0.16, p > 0.25$).

A Circadian Paradox?

As to how this behavioral transition is neurally timed remains perplexing. The results from Fig. 1, where mated males were compared at 22-h versus 27-h T-cycles, suggest a circadian-based timing function. However, the results presented here seem inconsistent with a simple circadian day-counting mechanism. The sample of DD males had an average free-run of $-24$ h ($r = 24.1$ h), which was the same as the 24-h period of the LD 12:12 males. Yet, when compared with the LD 12:12 group, the transition to parental behavior in DD males was significantly delayed, whereas the postweaning reemergence of infanticide was significantly accelerated. In contrast, among LL males, the inhibition of infanticide occurred more rapidly; in fact, the LD 12:12 and LL groups both showed a similar pattern of temporal changes despite the LL males averaging a 25.2-h free-run. Based on a circadian day-counting hypothesis, one would have predicted that LL males either should have taken longer to undergo the postmating transition to parental behavior or, at the very least, they should not have differed so dramatically from the DD males. However, this experiment still does not allow us to eliminate whether some sort of masking may have occurred, nor do these results completely rule out the unlikely possibility of a covert coordinating rhythm that free-runs independently from activity/rest cycles.
FIG. 3. The temporal pattern of behavioral changes observed when males within each treatment group are grouped in relation to the behavioral phenotype of the infant (infantidal vs parental), which is consistent with the infantidal phenotype during late fetal development (see text). Males in each group were mated according to the day after mating when they expressed their first bout of parental behavior. Mann-Whitney U tests showed significant differences between the two phenotypes in LL (p < 0.001) and light-dark 12:12 (p < 0.05); whereas in the absence of light (DD), there were no significant differences (p > 0.40).

Phenotypic Differences in Response to Light Cues: Fetal Hormonal Programming of Individual Timing Variation

The previous experiment showed another unique aspect of individual timing variation. As noted earlier, we have always found that in any random sample of virgin CF-1 males, ~50% spontaneously kill pups whereas the other 50% exhibit parental behavior (10,11). In males such as house mice that produce large litters, fetuses are positioned randomly in the uterine horns. This exposes each fetus to varying sex steroid concentrations depending on whether it develops next to some or opposite sex fetuses (2). As a result, an individual's intrauterine position has been correlated with a profound range of variation among reproductive, morphological, and behavioral characteristics expressed when both sexes are adults, including adult-infant interactions (22). Likewise, past experiments have established that CF-1 males who develop between two male fetuses—and are therefore exposed to higher testosterone concentrations—are significantly more likely to exhibit parental behavior both before and after mating than are males who developed between two female fetuses (12,23-25). Infantidal and intermale aggression are thus inversely correlated in CF-1 males (12,22).

We controlled for this phenotypic variation in fetal hormone exposure by testing all virgin males for their behavior toward a pup. Thus, males in each of the three groups of 30 males were allocated such that half were infantidal and half were parental before mating. With regard to their premating behavior, ejaculation triggered infantidal behavior in almost all males who were originally parental and as shown in Fig. 3, pretested parental males also underwent the postmating transition back to parenting significantly faster than their pretested infantidal counterparts.
but only in the LL and LD 12:12 conditions. The presence of light in the LL and LD 12:12 treatments seemed to accelerate the photoperiodic inhibitions of infanticide among parental phenotypes, whereas in the DD group there were no significant timing differences between parental or infanticidal phenotypes (11). This intriguing result raises the possibility that variations in timing strategies and differential responses to light cues are programmed hormonally via sex steroids during early development. In a similar vein, we have recently noted differences in the first run period of house mice derived from different in utero positions (G. Perrigo, unpublished observations).

Transition Patterns, Individual Timing Consistency, and the Effects of Aging

In a follow-up experiment, we obtained a more precise picture of the photic transition patterns of mice by assessing their behavior toward pups at much shorter time intervals after mating (every 12 h), but we also examined whether variation in time measurement after mating is preserved within an individual (11). Thus, 12 males from each treatment in the previous experiment who showed relatively rapid transition times from infanticidal to parental behavior after mating were retested at 9 months of age (13 months after their first mating). Unlike their first mating, however, mice in which retests occurred at 3-day intervals each test now occurred every 12 h at the time of lights on (12:00 h) and lights off (24:00 h) in the LD 12:12 room. Tests in the DD and LL rooms were also conducted at the same absolute time. This resulted in all three groups being tested at either exactly (LD 12:12) or, because of the free-runs in DD and LL mice, at ~12 h differences in their circadian phase. It should be explicitly emphasized here that despite the day-by-day phase drift expected among LL and DD mice, past experiments have shown that in untrained CF-1 males there are no differences in the frequency of infanticidal and parental behavior, regardless of when they are tested during their circadian phase (11).

As depicted in Fig. 4, a clear transition from pup-killing to parenting behavior sometimes occurred in as little as 12 h, but only in five of the 75 males tested. In contrast and regardless of their light treatment, most males (13 of 18) showed day-to-
day fluctuations in their behavior toward pups before looking in to consistent parenting. These results suggest that the transition phase from infantilic to parenting is a time of considerable behavioral variation and instability, with most males exhibiting oscillations in their reaction to pups (11). In fact, some of the behavioral flip-flops in Fig. 4 look suspiciously periodic.

With regard to individual consistency, the white marker dots shown with each male in Fig. 4 indicate the test day after their first mating 3 months earlier, when each individual expressed parental behavior. Because these males were retested at different intervals (every 3 days vs. every 12 h), no legitimate statistical comparison is possible here. Nevertheless, the visual results agree remarkably well, timely, from one individual to another. This suggests that after a behavioral cycle is reset, ejaculation will reinstate a new cycle of infantilic inhibition—one that appears programmed to last about the same length of time within a young individual (11). In older males (>18 months of age), however, this temporal reversibility disappears. As shown in Fig. 5, the time interval between mating and the expression of parental behavior was significantly delayed and attenuated in older males when compared with their response when young (6 months of age).

OVERVIEW AND GENERAL DISCUSSION

A Proposed Neuroethological Model

Our past and present research has suggested the following physiological model (1,24). As illustrated by Fig. 6, the intense sympathetic stimulus of coital ejaculation greatly amplifies a male's motivation to kill pups; infantilic phenotypes remain infantilic whereas virtually all parental phenotypes become infantilic immediately after mating. It is not surprising that ejaculation triggers such an immediate pup-killing reaction. Female house mice exhibit a strong postpartum estrus within 24 h after parturition; thus, if a virgin male copulates with a newly lactating female, he will maximize his reproductive advantages by immediately seeking out and destroying her litter.

Ejaculation also activates the time-delayed inhibition process. The neural sub-
strategy's governing infanticide 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 in
Fig. 6), then parental behavior can emerge. Specifically, the inhibition of infanticide
seems to mask the expression of parental behavior. Also implicit in the Fig. 6
scheme is that when a male is exposed to a pup at or near his behavioral threshold (i.e.,
the transition zone) during the inhibition process, then any one of three behaviors
can occur: infanticide (K), parenting (P), or ignoring the pup. Ignoring is a
neutral behavior in which males neither harm nor remove pups to their nest (12,24).
Although this last behavior occurs infrequently, CF-1 males tend to ignore pups
mainly during the unstable transition phase between infanticide and parenting (11).
Finally, variation in the slope (time course) and threshold of a CF-1 male's behav-
ioral inhibition appear programmed to a large extent by hormonal events related to
his social position, although light cues, genetic differences, and social factors (e.g.,
stress in female cues) all seem to interact in shaping this decay process. In fact,
there are always a few CF-1 mice (~10-15%) that simply do not respond to the
stimulus of ejaculation; some males always remain parental and some always kill
pups and, as described earlier, these behavioral phenotypes are correlated with intra-
uterine position. Among those few individuals in which mating per se does not seem
to inhibit pup-killing, the stimulus of ejaculation and female cohabitation are proba-
bly both required in order to inhibit infanticide (7,8,26). With regard to aging, the
time interval between mating and parenting was consistent in individual young
males (6-9 months of age, Fig. 8), but in older males (18 months of age) this time
interval was significantly longer (Fig. 5); in fact, 28% of the older males never ceased
killing pups. This suggests that the aging causes a shift in response thresholds and
changes the male's sensitivity to redundant female cues (9,11).

Male versus Female: Different Systems for Time Measurement

Infanticide is also a fundamental component of the reproductive strategy of female
house mice; pregnant females routinely kill pups up to the time of parturition (3,18).
Thus, both sexes exhibit infanticide and share a common suite of parental behaviors.
which, over the

pups. Once an

zone in

infanticide in the Fig. 6

threshold

of three beha-

ing as a

their nest (12,24).

ignoring pups

and parenting (11).

-1 male's behav-

events related to

ratial factors (e.g.,

in process. In fact,

respond to the

some always kill

related with intra-

when does not seem

ation are proba-

aid to aging, the

individual young

of age) this time

er never ceased

thresholds and

growth in

females (20). The

behavior.

of female

emerges (18).

behavioral behav-

ior.

CONCLUSIONS

Finally, we have made no attempt to explain how infanticidal behavior spontaneously reemerges in male mice 2 months after mating. In general, the presence of light seemed to inhibit infanticide during this phase, too (11). No simple physiological explanation can account for these time-delayed responses, and little more can be said here except that our recent experiments suggest both parallels and paradoxes with widely studied behavioral and reproductive timing processes (34,35). Ablation of the
suprachiasmatic nucleus, exposure to high-intensity constant light (both of which disrupt circadian rhythms), or maintenance at skeleton photoperiods are several potential experiments that could distinguish whether light has a photoperiodic, direct, or some other prorular regulatory role in the timing of this entire behavioral cycle.

There is a final caveat here, too. House mice are known for their enormous reproductive, behavioral, and photoperiodic flexibility (28-36,37). They breed vigorously in a wide variety of feral situations, including circumstances where photoperiodic cues may be irregular or even nonexistent (e.g., building interiors and cars). Likewise, the study of infanticide among various wild and laboratory house mouse stocks has shown a variety of independent and/or multiple caging mechanisms evolved to inhibit infanticide in male mice: ejaculation, female cohabitation, and social subordination (8,9,14,15,38,39). The proper timing of infanticide and parental behavior is at the core of a male's reproductive success; thus, from an ecological standpoint, one should not be surprised by the evolution of redundant, backup inhibitory systems operating in the absence of typical temporal or social cues. Given the house mouse's behavioral flexibility, neither can we be certain whether the same or independent inhibitory mechanisms were responding to the various experimental treatments imposed here. However, Ewellood (40) noted that in some male house mice, one day of female cohabitation elevates male parental behavior 2 weeks later, suggesting that ejaculation and cohabitation may operate via the same timing mechanism. Be regardless of how these behavioral shifts are neurally timed, the prolonged time interval between ejaculation and the inhibition of infanticide (and onset of parental behavior) seems to redefine the range of potential time-dependent relationships between a stimulus and its response.

Acknowledgments: This work was supported by NSF Grants IOS 8813375 to G.P. and DCB 8518094 to F.S., and a Higher Biomedical Undergraduate Research Internship to L.B.

REFERENCES

4. Perrigo G. Breeding and feeding strategies in deer mice and house mice when females are challenged to work for their food. Animal Behav 1987;36:529-36.

Obstet Res Vol 9, No 6, 1991


17. Perrigo G., Bryant WC., vom Saal FS. A unique neural timing mechanism prevents male mice from learning their mates' aggressive, from mice 1989;39:535-49.


Chromosomes 1973;5:472.