

A unique neural timing system prevents male mice from harming their own offspring

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Abstract. Infanticide is a behavioural strategy that occurs in many mammals. In male house mice, *Mus domesticus*, the specific stimulus of ejaculation during copulation inhibits male mice from killing pups. An unusual aspect of this phenomenon, however, is the extraordinary latency that occurs between the stimulus (ejaculation) and its adaptive response (male mice cease killing pups and behave 'parentally' toward them). Specifically, the inhibition of infanticidal behaviour is usually delayed for many days after a male ejaculates, but virtually always occurs before or around the time his own sired offspring would be born 18–20 days after mating. CF-1 male mice were maintained at artificially 'fast' or 'slow' daylengths (22 h versus 27 h light/dark cycles) to test one possible hypothesis of how a biological timing system could gauge such a long stimulus–response interval. The results suggest that photoperiodic cues synchronized the shift in behaviour, since a sudden transition from violent to benevolent behaviour 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.

Infanticide, defined as the killing of pre-weanling young, is no longer considered maladaptive or sociopathological. Field and laboratory studies in recent years have shown it to be a violent but extremely effective reproductive strategy occurring in a variety of male mammals (e.g. Hrdy 1979; vom Saal & Howard 1982; Hausfater & Hrdy 1984). When a male house mouse, *Mus domesticus*, encounters a neonate he either tries to kill it or he does not harm it. At issue here are the mechanisms that prevent a male mouse from harming his own offspring. Social cues, fetal and adult hormones, and genetic differences are three factors known to modulate the male's behaviour toward pups (Svare et al. 1984; Perrigo & vom Saal, 1989). There is, however, a fourth dimension to infanticide that appears physiologically unique: time-dependent changes in a male's behaviour toward pups are programmed by an unusual stimulus–response system triggered specifically by ejaculation during mating (vom Saal 1985; Perrigo et al. 1989).

In sexually naive male CF-1 house mice, about 45% of individuals exhibit spontaneous infanticide when they encounter a pup. The stimulus of ejaculation, however, promotes infanticide such that immediately following mating, 90% of CF-1 males will now exhibit infanticide. But, by the time a male's own sired offspring would be born around 3 weeks after mating, infanticide is inhibited in vir-

tually all males. When infanticide ceases, most males express parental behaviour similar to that of lactating females (vom Saal 1985). These timed behavioural changes, which result from ejaculation, are clear-cut and remarkably consistent among both wild and laboratory stocks of house mice (e.g. McCarthy & vom Saal 1986; Kennedy & Elwood 1988; Soroker & Terkel 1988). This phenomenon has also been verified in the Norway rat, *Rattus norvegicus* (Mannella & Moltz 1988). Thus, a profound and adaptive shift in behaviour toward pups has evolved to occur many days after the specific stimulus of ejaculation. As a result, virtually all male mice (and rats) are likely to eliminate a competitor's pups following mating but are inhibited from harming their own progeny during their mate's lactation. In fact, with the exception of mating, this entire behavioural sequence can occur even when a male is isolated from his mate (e.g. vom Saal 1985; Perrigo et al. 1989).

An effective, fail-safe infanticidal strategy in male house mice thus seemed to depend on a behavioural timing mechanism that could operate independently from any post-mating social cues. This prompted us to ask how male mice gauge the time between mating and the birth of pups. We hypothesized that individuals could accomplish this either by (1) measuring the absolute amount of

time after ejaculation, or by (2) using light/dark cycle cues for assessing the amount of time experienced after ejaculation. We suspected the latter hypothesis because photoperiodic variation in nature provides infallible cues for entraining daily (circadian) and seasonal cycles of feeding, breeding, metabolism and movement (e.g. Pittendrigh 1960; Perrigo 1987; Turek & Van Cauter 1988). To test both possibilities, we maintained male house mice at artificially 'fast' (11:11 h light:dark cycle = 22 h) or 'slow' (L:D 13.5:13.5 h = 27 h) day-lengths and characterized their behaviour toward pups between 16 and 25 absolute (24 h) days after mating.

METHODS

Animals and Experimental Housing

One hundred virgin CF-1 stock male house mice (6 months of age) were housed individually in polypropylene cages measuring 28 × 18 × 12 cm with aspen shaving bedding and provided with food (Purina Mouse Chow) and water ad libitum. The cages were placed in light-tight, ventilated, plywood boxes measuring 1.8 × 0.4 × 0.4 m, illuminated inside with a 15-W fluorescent lamp and maintained initially at a standard L:D 12:12 h cycle.

Assessment of Infanticidal Behaviour

As described earlier, when a male house mouse encounters a neonate, he either attempts to kill it or he does not harm it. These are clear-cut and unambiguous responses. At the time of this experiment, our standard protocol for assessing a male's behaviour consisted of placing a pup 1–5 days old at one end of the male's home cage farthest from his nest. Neither the sex, age (1–10 days of age) nor paternity of the pup has any influence on the male's infanticidal behaviour (vom Saal 1985; Perrigo et al. 1989).

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 and, in previous studies, the male was allowed to kill the pup. This testing procedure was approved by the University of Missouri Institutional Animal Care Review Board (Animal Protocol reference no. 208) and NIH Grant NS20075 to FVS. However, we have observed

repeatedly that virtually all CF-1 male mice who attack and bite pups will continue doing so until the pup is dead or fatally wounded. So, for humane reasons, we did not allow infanticidal males to kill pups in this experiment. Instead, we made every attempt to intervene and rescue the pup from attack as quickly as possible (see also Perrigo et al., in press, for a recently verified humane and injury-free assay for infanticidal behaviour).

If, however, a male does not attack and attempt to kill the pup, the pup is left in the male's cage for 30 min. Most males who are not infanticidal exhibit 'parental' behaviour. Parental males groom the pup about the head and genitals and retrieve it to their nest where they hover over the pup and keep it warm. There is also a small subset of males (10–15%) who routinely 'ignore' pups, neither harming them nor exhibiting true parental behaviour (vom Saal 1985; Perrigo et al. 1989). While a majority of our males who did not harm pups did, in fact, exhibit parental behaviour, our testing procedure required lifting each male's cage out of the plywood box after 30 min. This cage disturbance did not allow us to assess true parental behaviour with certainty; thus, for the purpose of this experiment, behaviour was scored by recording whether the male immediately attacked and attempted to kill the pup (infanticidal), or, if over a 30-min period the pup was totally unharmed.

Entrainment of Locomotor Activity to Fast Versus Slow Light/Dark Cycles

Starting at the initial L:D 12:12 h cycle, 50 males in each group were adapted slowly over a 25-day period to either the 22-h fast day cycle (11:11 h L:D) or the 27-h slow day cycle (L:D 13.5:13.5 h) by respectively decreasing or increasing the length of each group's light and dark exposure by 2.5–3.5 min each day. The 22-h versus 27-h treatments were chosen to yield a maximum separation between the two groups while still maintaining animals well within their physiological limits of circadian entrainment to abnormal light/dark cycles. To monitor the entrainment process, eight randomly chosen males from each group were housed in cages with a running wheel attached to an Esterline Angus chart recorder. Day-by-day assessment of wheel-running patterns showed that seven out of eight males in each group obtained activity rhythms matched to the 22- or 27-h period of their final light/dark cycle (examples in Fig. 1). Furthermore, we verified entrainment by shifting these

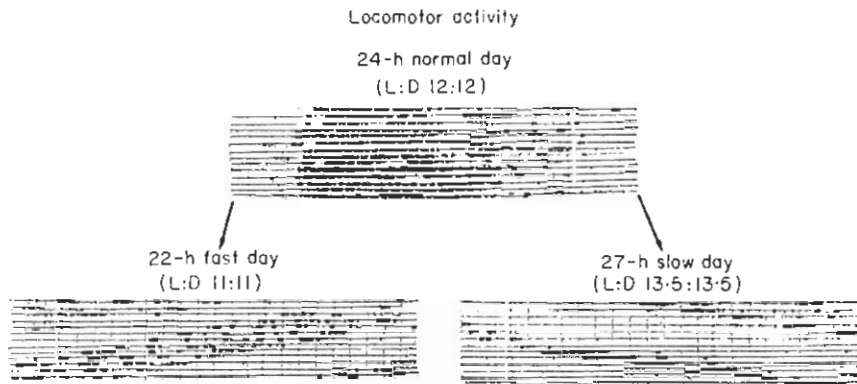


Figure 1. Daily locomotor patterns in a representative fast day male (left) and slow day male (right). 24-h strips from an event recorder are pasted consecutively over several days. The dark areas in each strip represent when the animal was active on a running wheel. All individuals began their entrainment at a normal L:D 12:12 h cycle; the top picture represents a typical activity pattern of a standard 24-h day.

males to constant darkness at the completion of this phase of the experiment; indeed, the respective 22- or 27-h cycle of wheel-running activity persisted among these males for several days afterward.

Mating and Testing Procedure

Most of the remaining males in each light/dark treatment were allowed to copulate by pairing them with an oestrous CF-1 female for 4 h at the end of the dark phase of the light/dark cycle. Ejaculation was confirmed by the presence of a vaginal plug. One day later, each male who mated was screened for infanticidal behaviour by quietly placing a 1-day-old pup in his home cage. All testing with pups occurred just after lights on and males who were not infanticidal on this initial screening test were discarded from the experiment (about 15% in both treatments, which replicates findings from previous studies: vom Saal 1985; Perrigo et al. 1989).

Each male who was infanticidal the day after mating was then retested with a pup between 16 and 25 absolute (24 h) days after mating. Specifically, fast day males were retested at either 16.3 absolute days (18 light/dark cycles; $N=15$) or at 20 absolute days (22 light/dark cycles; $N=23$) after mating. Slow day males were retested at either 20 absolute days (18 light/dark cycles; $N=19$) or at 24.8 absolute days (22 light/dark cycles; $N=13$) after mating. Our objective was to compare both groups at 20 absolute days after mating and also control for the equivalent number of light/dark cycles experienced (18 versus 22 cycles).

RESULTS

Figure 2 shows the post-mating inhibition of infanticide graphed in two complementary perspectives: first, in relation to the number of absolute (24 h) 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 that photoperiodic cues influenced the sudden change in a male's behaviour. At 20 absolute (24 h) days after mating there was a significant difference between the number of infanticidal males in the fast and slow day groups (13 versus 63%, respectively; $\chi^2=9.3$, $df=1$, $P<0.005$). In direct contrast, no differences were noted in the frequency of infanticide with both groups matched for experiencing the same number of light/dark cycles. Furthermore, a sharp transition in behaviour occurred between 18 and 22 light/dark cycles after mating in both the fast day ($\chi^2=5.3$, $df=1$, $P<0.05$) and slow day ($\chi^2=3.5$, $df=1$, $P<0.07$) groups. Although the latter result was not statistically significant, the marginal probability level here did not warrant the potential sacrifice of more animals. So, for humane reasons, we terminated the experiment.

DISCUSSION

The data in Fig. 2 suggest a unique behavioural mechanism that assessed the occurrence of light/dark cycles and somehow influenced a dramatic

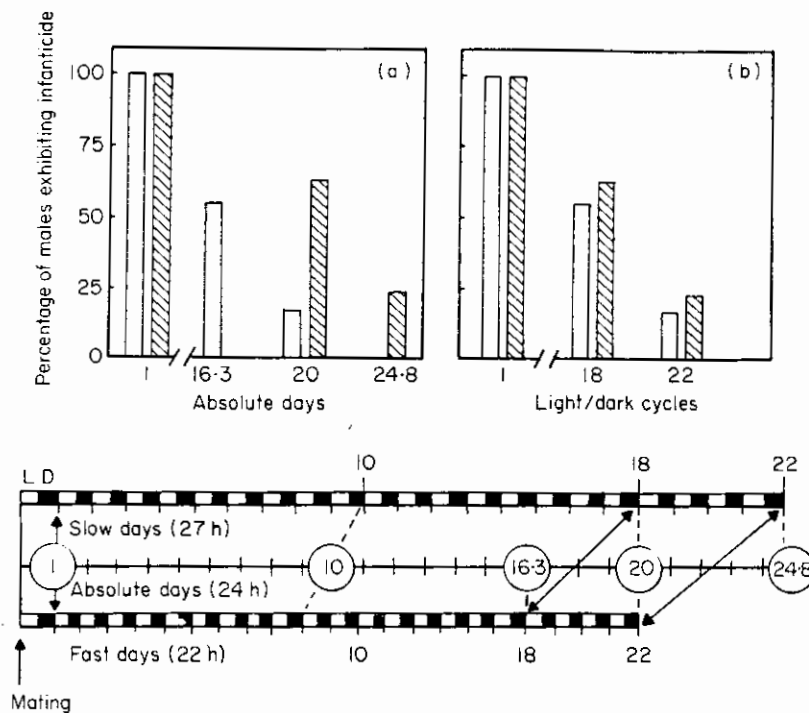


Figure 2. The percentage of male mice maintained at fast (22 h) or slow (27 h) days exhibiting infanticidal behaviour when graphed in relation to absolute (24 h) days (a) versus the number of light/dark cycles experienced after ejaculation (b). Since males in both groups were screened for infanticide at one day after mating, both groups started the experiment as 100% infanticidal (about 85% of all mated males in both groups) □: fast day; ▨: slow day. A timeline below the graphs illustrates the relationship between absolute time and the increasing desynchronization of light/dark cycles experienced by both groups during the experiment. The alternating dark bars on the fast and slow time scales represent the dark phase of the repeating light/dark cycles. By 20 absolute days after mating the fast day males had experienced four more light/dark cycles than the slow days males.

behavioural change around the time pups are normally born. Rats maintained at normal 24-h days (L:D 12:12 h) also show a precipitous drop in infanticidal behaviour between 18 and 20 days after mating (Mennella & Moltz 1988). And, in CF-1 male mice maintained in constant darkness, we found that out of 27 males who were infanticidal immediately after mating, 18 (68%) ceased killing pups by 21 days after mating (unpublished data). The latter finding suggests an underlying circadian timing system, since the transition from infanticidal to parental behaviour occurred in the absence of light/dark cues. We prefer caution in the specific interpretation of these results, however, because most CF-1 male mice maintained at L:D 12:12 h typically cease exhibiting infanticide within 12–14 days after mating (vom Saal 1985; Perrigo et al. 1989).

The fact that a dramatic shift from infanticidal to parental behaviour in male house mice parallels the

timing of pregnancy in females is in itself interesting. Pregnant female house mice also kill pups up until the time of parturition (Jakubowski & Terkel 1982; McCarthy & vom Saal 1985). Thus, both sexes exhibit infanticide and, as a consequence of mating, appear to share a common suite of parental behaviours expressed when pups are born. But female house mice rely on cues from developing fetuses and do not use photoperiodic cues to gauge the length of pregnancy (Lanman & Seidman 1977; Davis & Menaker 1981). Even when entrained to extreme light/dark cycles mimicking a 20- or 28-h day, female house mice still give birth the same number of absolute days after insemination (Davis & Menaker 1981). Our experiment thus suggests that male mice have evolved a novel timekeeping solution for synchronizing their behaviour toward pups with the duration of a mate's pregnancy.

Some photoperiodically mediated phenomena in rodents, such as the pre-ovulatory surge in LH

(luteinizing hormone) and, hence, the organization of oestrous cycles, regularly occur at 4–5 day multiples of daily light/dark cycles (Alleva et al. 1968; Fitzgerald & Zucker 1976). But these events are mediated by cyclic changes in the secretion of pituitary and gonadal hormones. In sharp contrast, the transition from infanticidal to parental behaviour after mating occurs in male mice even when their gonads or pituitary are removed (Perrigo et al. 1989). Thus, our present results suggest a neural mechanism which somehow used repeating light/dark variation as a timing cue for promoting an adaptive shift in behaviour. The prolonged timespan between ejaculation and the inhibition of infanticide (and onset of parental behaviour) seems to redefine the possible time-dependent relationships between a stimulus and its response.

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