

Short Communications

The Use of Live Pups in a Humane, Injury-free Test for Infanticidal Behaviour in Male Mice

Rodents such as house mice, *Mus domesticus* and *M. musculus*, and the Norway rat, *Rattus norvegicus*, are important laboratory models for testing evolutionary predictions about the social, ecological hormonal and neural factors modulating infanticidal and parental behaviour in male mammals (vom Saal & Howard 1982; Svare et al. 1984; vom Saal 1985; Elwood 1986; McCarthy & vom Saal 1986; Mennella & Moltz 1988; Kennedy & Elwood 1988; Soroker & Terkel 1988; Perrigo & vom Saal, in press). However, escalating public and scientific concern about the moral and ethical dilemmas of animal experimentation has already begun to inhibit research in the behavioural sciences. This is especially true in the study of infanticide, where live pups are still used to assay behaviour.

At present, a standard procedure to test a male mouse's behaviour towards pups consists of placing a newborn at one end of his home cage. If the male does not attempt to harm the pup, he will most probably behave 'parentally'. By definition, a parental male retrieves the pup to his nest where he incubates it and keeps it warm (e.g. vom Saal 1985; Elwood 1986). In sharp contrast, an 'infanticidal' male who encounters a pup will typically rattle his tail and suddenly lunge at and attempt to kill the pup with rapid bites to the head and back (e.g. Perrigo & vom Saal, in press). This is an acute and dramatic response. The attack is often so sudden that the experimenter cannot intervene and the injury of neonates in this type of test situation is sometimes unavoidable. Because of our concern about the ethical aspects of conducting these experiments, we have developed a humane method of testing for infanticidal behaviour that eliminates any risk of injury to live pups.

Our new behavioural assay consists of only a minor modification to the above procedure. Instead of using an exposed and unprotected pup, we simply place the pup within a tube made of 1.5-mm² wire-mesh window/fly screen. A tube measuring 6 cm long and 1.5 cm in diameter is large enough to slide a 1-3 day-old neonate comfortably inside and the ends of the tube can be pinched so that a pup is secure and completely buffered from attack. We validated this technique in the following experiment.

Twenty virgin CF-1 stock male house mice, *M. Domesticus* (4-5 months of age), from our colony were assigned randomly to one of two groups

($N=10$ /group). Both groups were tested twice for their behaviour toward a newborn pup: once with an exposed and 'unprotected' 1-day-old pup, and once with a 'protected' 1-day-old pup placed within the wire-mesh tube. The only difference between the groups was a reversal of test order (i.e. unprotected-protected versus protected-unprotected). Our objective was to determine whether we could accurately predict the behaviour of a male toward an unprotected pup based on his behaviour exhibited toward a protected pup encased within the wire screen. The two tests occurred 1 h apart, and two observers were used. The two observers switched to the opposite group for the second test to eliminate any potential bias as a result of knowing the outcome of the first test.

As shown in Table 1, regardless of testing order, 90% of the predictions based on a male's behaviour toward the protected pup matched with his behaviour toward the unprotected pup. The number of males who changed their behaviour when tested with protected versus unprotected pups (two out of 20) was analysed using the McNemar test for significance of changes (Siegel 1956), which revealed that the males did not behave differently toward the protected and unprotected pups ($\chi^2=0.5$, $df=1$, $P>0.4$). As determined with the unprotected pup, there were seven infanticidal and three parental males in each group. For the 18 males whose behavioural status was predicted correctly, both observers noted two clear-cut and stereotypic behavioural patterns during their test with the protected pup. Among the six parental males (as assessed with the unprotected pup), four males tried to retrieve the pup by dragging the wire screen to their nest, whereas the remaining two males attempted to lift and carry the wire screen toward their nest. (We have since eliminated retrieval problems by using a shorter (3-4 cm), and hence, lighter screen tube in subsequent experiments; parental males will indeed retrieve and incubate a pup encased within a less bulky tube than the prototype used here.) In contrast to the parental males, 12 out of 14 infanticidal males (as assessed with the unprotected pup) attacked and bit at the wire screen (no parental males did this). The two infanticidal males who were incorrectly labelled as parental did not attempt to harm or parent the protected pup. Nevertheless, these behaviours mimic the reactions we normally see when a male encounters an unprotected neonate, since 10-20% of male mice tested with unprotected pups will ignore the pup (neither kill nor parent it) on one test but will kill or parent a pup on a subsequent test

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(Received 11 November 1988; revised 6 February 1989; MS. number: A5324)

The Use of Li Test for Infa

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Our new behavioral modification. Instead of using we simply placed 1.5-mm² wire-mesh measuring 6 cm enough to slide inside and the e that a pup is seen attack. We valid experiment.

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