

Human Behavioral Assays in the Laboratory: An Alternative Test for Assessing How Rodents Behave Toward Young

GLENN PERRIGO E. LEE BELVIN FREDERICK S. vom SAAL

Infanticide is a behavioral strategy that occurs in many mammals. Laboratory rodents are widely used models to study the psychobiology of this behavior. The humane experimental issue here concerns a standard laboratory assay for infanticidal behavior that can sometimes result in injury to live pups. The authors found a simple, accurate, and humane alternative to this test — one that eliminated injury to live pups and, incidentally, reduced our animal care costs.

Infanticide — defined as the killing of young of one's own species — is an adaptive behavioral strategy that occurs in a variety of organisms (e.g. vom Saal & Howard, 1982; Hausfater & Hrdy, 1984). Field and laboratory studies in recent years have dramatically documented that many mammals — including humans — will deliberately kill another's offspring. Wild populations of African lions and Indian langurs are classic examples of this sort of socioecological violence. When an invading male usurps the territory of another male, one of the first things he does is kill the children of his vanquished rival.

Infanticidal behavior also occurs widely among rodents. Thus, commonly studied laboratory animals such as house mice (*Mus*), rats (*Rattus*), deer mice (*Peromyscus*), gerbils (*Meriones*), hamsters (*Mesocricetus*) and cotton rats (*Sigmidon*) have become benchmark species for understanding the evolutionary and physiological factors that regulate how mammals recognize and treat their own young. Recent studies, particularly in the house mouse (*M. domesticus*), have revealed a wealth of rich, new developmental, hormonal and neural phenomena concerning the biology of infanticide (Labov, et al., 1985; vom Saal, 1985; Kennedy & Elwood, 1988; Mennella & Moltz, 1988; Soroker & Terkel, 1988; Perriog, et al., 1989b; Perriog, et al., 1990). Yet, regardless of these exciting new findings, the study of infanticidal and parental behavior in laboratory rodents has, in fact, been somewhat curtailed in the past few years — not because of the unsettling subject matter — but because live pups are often used to measure whether an animal responds violently or benevolently toward young. This type of test procedure raises obvious ethical questions because of the potential injury to live pups.

“If the male did not attempt to harm the pup, he would most likely groom the pup and retrieve it to his nest . . . ”

In our laboratories at The University of Missouri one of our main interests is the neuroethology and physiology of infanticidal and parental behavior in male mice. 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 (e.g., vom Saal, 1985; Perriog, et al., 1989b). The standard behavioral assay used in previous experiments consisted of quietly placing a 1 to 3 day old newborn pup at one end of a male's home cage. If the male did not attempt to harm the pup, he would most likely groom the

pup and retrieve it to his nest. This “parental” behavior is remarkably similar to that of a lactating female.

In sharp contrast, a pup-killing male typically rattles his tail and immediately lunges at the pup, attempting to kill it with rapid bites to the head and back. This is an acute and dramatic response. Unfortunately, the attack is often so sudden that the experimenter cannot always intervene fast enough to rescue the pup from injury (This procedure, however, has been approved by the University of Missouri Institutional Animal Care Review Board, Protocol #208; and NIH Grant NS20075).

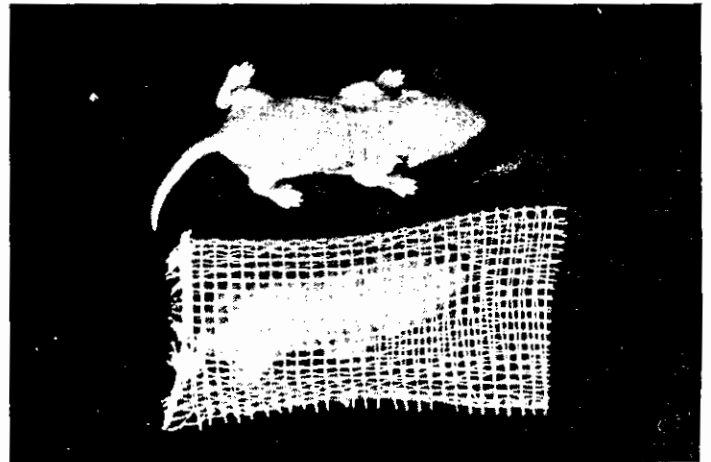


FIGURE 1: A vulnerable pup next to its screen-protected counterpart.

So, for obvious humane reasons, we decided to search for an alternative procedure that eliminated the risk of injury to pups. We soon found a simple, but successful solution. Instead of using an exposed and unprotected pup, we simply placed the live pup within a tube made of 1.5-mm² wire mesh screen (1/16 inch window or fly screen found in any hardware store). A tube 4 to 5 cm long and 1.5 cm in diameter is large enough to slide a 1 to 3 day old neonate comfortably inside (Figure 1). The pup is now secure and completely buffered from attack. When an infanticidal male encounters a protected pup, he still attacks and violently bites at the screen, but without injuring the pup (the original methodology, data, and experimental verification of this test can be found in: Perriog, et al., 1989a). Thus, once we establish that a male is not going to harm a newborn, our next step is to introduce an unprotected pup and observe whether the male actually retrieves the pup and incubates it in his nest.

While our new behavioral assay takes a little more time and observer patience, the advantages are obvious. A pup enclosed within a wire screen is usually quiescent and does not at all act distressed or disoriented — and pups can now be safely returned to their own mothers after testing. This also benefits us by substantially reducing the number of pup-producing pairs needed in our mouse colony (the bottom line for cost savings, too!). Best of all, the humane experimental issues are immediately defused whenever seminar audiences are shown a slide of a pup safely encased within a protective screen. The audience now focuses on the real issues of biological importance instead.

We do not, however, know how well this technique might work in

rodents other than house mice. And results from another laboratory suggests that a "screen test" may not work so well with female house mice. But despite a few minor drawbacks, we encourage the development and use of such alternatives. With a little patience and creative effort, the compassionate scientist can usually find a better way for getting accurate behavioral information humanely.

REFERENCES:

1. This work was, in part, supported by NSF Grant BNS 8813375 to G.P.
2. Hausfater, G. & Hrdy, S.B., eds. (1984). *Infanticide: Comparative and Evolutionary Perspectives*. Chicago, Aldine Publishing.
3. Kennedy, H. & Elwood, R. (1988). Strain differences in the inhibition of infanticide in male mice (*Mus musculus*). *Behav. Neur. Biol.* 50. pp. 349-353.
4. Labov, J., Huck, V., Elwood, R., & Brooks, R. (1985). Current problems in the study of infanticidal behavior of rodents. *Quart. Rev. Biol.* 60. pp. 1-20.
5. Mennella, J. & Moltz, H. (1988). Infanticide in rats: Male strategy and female counter-strategy. *Physiology and Behavior.* 42. pp. 19-31.
6. Perrigo, G., Belvin, L., Bryant, W.C. & vom Saal, F.S. (1989a). The use of live pups in a humane, injury-free test for infanticidal behavior in male mice. *Animal Behaviour.* 38. pp. 897-898.
7. Perrigo, G., Bryant, W.D. & vom Saal, F.S. (1989b). Fetal, hormonal and experiential factors influencing the mating-induced regulation of infanticide in male house mice. *Physiology and Behavior.* 46. pp. 121-128.
8. Perrigo, G., Bryant, W.C. & vom Saal, F.S. (1990). A unique neural timing mechanism prevents male mice from harming their own offspring. *Animal Behavior.* 39. pp. 535-539.
9. Soroker, V. & Terkel, J. (1988). Changes in incidence of infanticidal and parental responses during the reproductive cycle in male and female wild mice (*Mus musculus*). *Animal Behavior.* 36. pp. 1275-1281.
10. vom Saal, F.S. (1985). Time-contingent changes in infanticide and parental behavior induced by ejaculation in male mice. *Physiology and Behavior.* 34. pp. 7-15.



Glenn Perrigo

Ph.D.
Division of Biological Sciences
The University of Missouri-Columbia

Glenn Perrigo received his Ph.D. in Zoology from the University of Texas at Austin in 1986. He is currently a Research Assistant Professor in the Division of Biological Sciences at The University of Missouri-Columbia. He has broad research interests in mammalian reproductive physiology, peripubertal development and sex behavior, and the distribution and behavioral ecology of neotropical birds. In his free time, he has a passion for exploring the forests and highlands of South and Central America.

E. Lee Belvin

E. Lee Belvin is a former star quarterback and 1987 graduate of East Prairie High School in Missouri. He is currently a University of Missouri Curator's Scholar majoring in biology. Lee was awarded a Hughes Undergraduate Internship in Biological Sciences in March of 1990. He is an excellent photographer and will enter medical school in the fall of 1991.

Frederick S. vom Saal

Ph.D.
Division of Biological Sciences
The University of Missouri-Columbia

Frederick S. vom Saal received his Ph.D. in neurobiology and behavior from Rutgers University in 1976 and is currently a Professor in the Division of Biological Sciences at the University of Missouri-Columbia. His major research interest concerns the influence of steroids on tissue differentiation during fetal life, with particular emphasis on the development of the brain and thus behavior. His other interests include flying, playing the piano and guitar, and a variety of sports.