

Determining the sex of neonatal mice (*Mus musculus*)

JILL E. SCHNEIDER, CHARLES J. WYSOCKI,
JOHN NYBY, and GLAYDE WHITNEY

Florida State University, Tallahassee, Florida 32306

Several laboratory investigators have routinely sexed neonatal mice (e.g., Barraclough & Leatham, 1954; Cowley & Wise, 1972; Drickamer, 1974; Haggett & Werboff, 1968; Kennedy & Brown, 1970; Machado, 1957; Vom Saal, Gandelman, & Svare, 1976), but their reports seldom describe the methods used. Moreover, the vast majority of research reports employing manipulations of neonatal mice utilize procedures that suggest an inability to make neonatal gender assessments. For example, the most common published neonatal manipulation involves androgen administration to females. The typical method for neonatally androgenizing females is to treat all newly born animals in a litter with androgen. The control subjects, neonatally treated with the oil vehicle, come from other litters. Female subjects are subsequently separated from males at weaning, when sex determinations are made more easily.

The ability to sex neonatal mice is quite useful. Aside from obvious benefits for studies involving neonatal gonadectomy and/or hormone administration, this ability permits a much greater degree of experimental control. For example, if neonatal gender assessments can be made, error variance due to litter effects may be reduced by selective culling of litters and by counterbalanced designs (across treatment groups by split litter). These two techniques are especially relevant when a small number of litters provide the experimental subjects.

METHOD FOR SEXING NEONATAL MICE

The method used in our laboratory does not differ in principle from that for sexing adult mice and involves a greater anogenital distance in males than females. The mean anogenital distance from the caudal aspect

of the genital area to the rostral aspect of the anus, as measured by a comparator, in newly born Day 1 males (mean = $1.9 \pm .1$ mm) is generally greater than twice that of females (mean = $.8 \pm .1$ mm). Nonalbino males can often be discriminated by what appears as a small darkened spot between the anogenital openings. Note that sophisticated instrumentation is unnecessary for making neonatal sex determinations, and with some practice such determinations can be made easily with the naked eye. We have validated this visual method by individually sexing over 50 mice from inbred strains and hybrid genotypes (C57BL/6J, AKR/J, Reciprocal F₁). In all cases, gender assessments made on the day of birth agreed perfectly with assessments on the day of weaning.

REFERENCES

- BARRACLOUGH, C. A., & LEATHAM, J. H. Infertility induced in mice by a single injection of testosterone propionate. *Proceedings of the Society for Experimental Biology and Medicine*, 1954, **85**, 673-674.
- COWLEY, J. J., & WISE, D. R. Some effects of mouse urine on neonatal growth and reproduction. *Animal Behaviour*, 1972, **20**, 499-506.
- DRICKAMER, L. C. Contact stimulation, androgenized females and accelerated sexual maturation in female mice. *Behavioral Biology*, 1974, **12**, 101-110.
- HAGGETT, B. N., & WERBOFF, J. Temperature reduction in neonatal mice: Effects on later behavior. *Developmental Psychobiology*, 1968, **1**, 257-265.
- KENNEDY, J. M., & BROWN, K. Effects of male odor during infancy on the maturation, behavior and reproduction of female mice. *Developmental Psychobiology*, 1970, **3**, 179-189.
- MACHADO, A. B. M. Sex recognition in newborn mice. *Revista Brasileira de Biologia*, 1957, **17**, 521-523.
- NAGY, Z. M., & MISANIN, J. R. Straight-alley escape behavior in infant mice: Effect of shock intensity. *Developmental Psychobiology*, 1973, **6**, 399-409.
- VOM SAAL, F., GANDELMAN, R., & SVARE, B. Aggression by perinatally gonadectomized male mice administered testosterone propionate when adult and the reestablishment of such behavior in adult nonperinatally androgenized females previously administered TP. *Physiology & Behavior*, 1976, **17**, 53-58.

(Received for publication June 28, 1977;
accepted November 4, 1977.)