Bulletin of the Psychonomic Society
1980, Vol. 15(6), 419-421

Responses by young house mice (*Mus musculus*) to odors from stressed vs. nonstressed adult conspecifics

W. J. CARR
E. R. Johnstone Training and Research Center, Bordentown, New Jersey 08505

and

PATRICIA A. ZUNINO and MICHAEL R. LANDAUER
Beaver College, Glenside, Pennsylvania 19038

Mice were reared from birth until testing with their dams, sires, and siblings. In a two-choice odor-preference test, 22 48-day-old mice reliably preferred ($p=.02$) the odor from nonstressed adult males over that from stressed adult males. Twenty-seven 24-day-old mice showed no reliable preference for either test odor. When contrasted with earlier research, the present findings suggest that previous experience with an adult other than the dam may play a role in the development by mice of species-typical responses to socially significant odors. This experiential effect seems more evident in female mice than in males.

In a two-choice preference test involving two odors, adult house mice (*Mus musculus*) of both sexes prefer the odor from nonstressed adult conspecifics over that from stressed adult conspecifics (Carr, Martorano, & Krames, 1970; Carr, Roth, & Amore, 1971; Colyer, 1972). In a two-choice test involving one odor vs. no odor, the odor from nonstressed conspecifics serves as a true attractant (Irwin, 1971), in the sense that adult mice prefer the odor from nonstressed conspecifics over no odor. On the other hand, the odor from stressed conspecifics serves as a true repellent (Irwin, 1971), in the sense that adult mice prefer no odor over the odor from stressed conspecifics (Colyer, 1971; Müller-Velten, 1966; Rottman & Snowdon, 1972; Whittier & McReynolds, 1965). Stressors eliciting this olfactory repellent include defeat in intermale aggression, rough handling by humans, electric shock, and hypertonic saline injections.

The tendency to approach the odor from nonstressed conspecifics may promote aggregation, whereas the tendency to withdraw from the odor from stressed conspecifics may minimize exposure to predators and other dangerous situations. Some evidence suggests that the repellent effect of the stress-induced odor is species-specific. Müller-Velten (1966) reported that house mice are not repelled by the odors from stressed field mice (*Apodemus sylvaticus*) or fat dormice (*Glis glis*).

This research was supported in part by DHEW Research Grant HD 07043. We thank Dianna Taylor for technical assistance. Michael R. Landauer is now at the Department of Pharmacology, Virginia Commonwealth University, Richmond, Virginia 23298. Requests for reprints should be sent to W. J. Carr, E. R. Johnstone Training and Research Center, Bordentown, New Jersey 08505.

The present experiment was designed to explore the ontogeny of the reaction by house mice to the odors from stressed vs. nonstressed adult conspecifics. More precisely, we wished to determine the minimal age at which developing male and female mice react differentially to the two test odors. Our interest in this problem stemmed from the observations by Müller-Velten (1966) of a single wild-caught house mouse. At 12-13 days of age, this mouse was mildly attracted by the odor from a stressed adult male conspecific, but at 18-19 days of age and thereafter, the mouse was repelled by that odor. Although he recognized the need for more data, Müller-Velten tentatively concluded that the repellent effect of the odor from stressed adult conspecifics first appears at about the time of weaning.

Slevin and Carr (Note 1) tested Müller-Velten's (1966) conclusion, using mice aged 12, 19, 37, 64, and 98 days of age. The 98-day-old mice reliably preferred the odor from nonstressed adult males over that from stressed adult males (14-16 weeks old), but those aged 64 days or less showed no reliable preference for either test odor. However, prior to testing, the 98-day-old mice had cohabited with other adult males, some from other litters. On the other hand, the mice aged 64 days or less had cohabited only with their dams and siblings. Considerable evidence now supports the view that, among house mice and other rodents, the reactions to socially significant odors are influenced by exposure to those odors early in life (Alberts & Brunjes, 1978; Brown, 1979; Doty, 1974; Hayashi, 1979; Leon, 1978; Mugford & Nowell, 1972; Rottman & Snowdon, 1972; Brown, Note 2). Therefore, the present experiment was a replication of that performed by Slevin and Carr (Note 1), except that the immature subjects used in the

0090-5054/80/15/0419-03$00.55/0

present experiment had been reared from birth until testing with an adult male (their sire), as well as with their dams and siblings.

METHOD

Subjects

The subjects were 49 house mice (*Mus musculus*) of the C57B1/6J strain, bred in our laboratory from adults obtained from the Jackson Laboratory, Bar Harbor, Maine. Twenty-seven were tested at 24 days of age and 22 at 48 days of age. Each age group consisted of approximately equal numbers of males and females.

From birth until testing, the subjects were maintained as litters (four to eight pups) with their dams and sires in cages measuring 20 x 25 x 25 cm. They were housed in a temperature-controlled room (22°C) on a 12-h light-dark cycle. Charles River Lab Chow checkers and water were available constantly.

Apparatus and Procedure

Each subject received a single 10-min odor preference test, conducted in a rectangular runway (12 x 18 x 64 cm). During the test, the subject could investigate two cardboard cylinders (10 x 15 cm), located at opposite ends of the runway. For 1 h immediately preceding the test, each cylinder had housed an adult male mouse (either stressed or nonstressed) and a small amount of clean pinewood shavings. Just before the test, the adult males were removed from the cylinders, leaving behind urine, feces, and the bedding. Cardboard caps on both ends of the cylinders barred the subject's entrance, but perforations in the caps allowed odor-laden air to pass through the cylinders and into the runway. A cardboard atrium (10 x 10 cm) was attached to each cylinder in a manner illustrated elsewhere (Carr, 1974). The floor of the runway consisted of clean newsprint, which was changed after each subject was tested. New cylinders, caps, and atria were used after every fourth subject.

At the outset of the 10-min test, each subject was placed in the center of the runway, facing neither cylinder. The experimenters recorded the amount of time the subject spent investigating each cylinder, that is, the number of seconds any part of the subject's body (less tail) extended inside the atrium attached to each cylinder.

The donors providing the test odors were 26 male mice (C57B1/6J), aged 14-16 weeks. They had been housed individually for at least 2 weeks prior to testing. On the day of their use, several donors were placed individually in a shock box for 10 min, in which they received 20 1-mA shocks, each shock lasting 5 sec. An equal number of donors spent 10 min in another box in which no animal had ever been shocked. Immediately thereafter, each donor was placed in a fresh cylinder for the hour just before testing. Each donor provided test materials only on one occasion and for no more than four subjects.

Prior to their use in the experiment, the subjects and donors were gentled by daily handling, the subjects were habituated to the runway, and the donors were habituated to the shock box and a cylinder. During testing, a counterbalancing procedure was used to eliminated the effect of a possible position preference on the part of the subjects.

RESULTS AND DISCUSSION

As shown in Table 1, neither the male nor the female subjects that were 24 days old at the time of testing exhibited a reliable preference for either test odor during the 10-min preference test. However, the 48-day-old females reliably preferred the odor from nonstressed males over that from stressed males [Wilcoxon $T(12) = 12$, $p < .05$]. The 48-day-old male subjects exhibited a preference in the same direction that was not statistically significant. When combined into a single group, the 48-day-old males and females reliably preferred the odor from nonstressed adult males [$T(22) = 56$, $p = .02$]. Therefore, we conclude that the repellent effect of the odor from stressed adult conspecifics first appears sometime between weaning and early adulthood. Male and female house mice are capable of reproducing at 40-50 days of age (Asdell, 1964; McKinney & Desjardins, 1972).

The repellent effect of the odor from stressed adult conspecifics may depend on the subjects' social history. Slevin and Carr (Note 1) found that 64-day-old mice that had been reared from birth until testing with only their dams and siblings are indifferent to the odors from stressed vs. nonstressed adult males. The odor-preference testing technique used in the present experiment was virtually identical to that used by Slevin and Carr (Note 1). Yet, the present results indicate that 48-day-old mice that had been reared with an adult male (their sire) as well as with their dams and siblings prefer the odor from a nonstressed adult male over that from a stressed adult male. Brown (1979, Note 2) also reported that previous experience with adults other than their dams plays a role in the development by rodents of species-typical responses to socially significant odors.

The reliable preference exhibited by 48-day-old females (but not 48-day-old males) for the odor from nonstressed adult males is congruent with the view expressed by Doty (1974) that early olfactory experience may be a more important determinant of the female rodent's response to socially significant odors than of the male's response to such odors. Alternatively,

Table 1
Mean Time (in Seconds) Spent by Young Mice Investigating Odors from Shocked vs. Nonshocked Adult Conspecifics

	24-Day-Old Subjects					48-Day-Old Subjects				
		Male Odor		Mean Difference Score	Preference Ratio		Male Odor		Mean Difference Score	Preference Ratio
	N	Nonshocked	Shocked			N	Nonshocked	Shocked		
Males	12	159	216	−57	3/9	10	239	190	+49	7/3
Females	15	161	188	−27	7/8	10	240	152	+88*	11/1
Both	27	160	200	−40	10/17	22	240	170	+70**	18/4

Note–Preference ratio is defined as the number of mice preferring the nonshocked male odor divided by the number preferring the shocked male odor. $*p < .05$. $**p < .02$.

sexually mature female mice may simply react more discriminatively than males to the odors from stressed vs. nonstressed males, both males being potential sex partners for the females. Likewise, mature males may react more discriminatively than females to the odors from stressed vs. nonstressed females.

REFERENCE NOTES

1. Slevin, A., & Carr, W. J. *The development of the "fear scent" response in house mice.* Unpublished manuscript, 1973. (Available from W. J. Carr, E. R. Johnstone Training and Research Center, Bordentown, New Jersey 08505.)

2. Brown, R. E. *Rearing environment and the generalization of odour preferences in infant rats.* Paper presented at the meeting of the Animal Behavior Society, Tulane University, New Orleans, Louisiana, June 1979.

REFERENCES

ALBERTS, J. R., & BRUNJES, P. C. Ontogeny of thermal and olfactory determinants of huddling in the rat. *Journal of Comparative and Physiological Psychology*, 1978, **92**, 897-906.

ASDELL, S. A. *Patterns of mammalian reproduction* (2nd ed.). Ithaca, N.Y: Cornell University Press, 1964.

BROWN, R. E. Mammalian social odors: A critical review. In J. S. Rosenblatt, R. A. Hinde, C. Beer, & M.-C. Busnel (Eds.), *Advances in the study of behavior* (Vol. 10). New York: Academic Press, 1979.

CARR, W. J. Pheromonal sex attractants in the Norway rat. In L. Krames, P. Pliner, & T. Alloway (Eds.), *Advances in the study of communication and affect* (Vol. 3). New York: Plenum Press, 1974.

CARR, W. J., MARTORANO, R. D., & KRAMES, L. Responses of mice to odors associated with stress. *Journal of Comparative and Physiological Psychology*, 1970, **71**, 223-228.

CARR, W. J., ROTH, P., & AMORE, M. Responses of male mice to odors from stress vs nonstressed males and females. *Psychonomic Science*, 1971, **25**, 275-276.

COLYER, S. W. Valence of stress and nonstress indexing odors and the effectiveness of post stress odor emission in mice. (Doctoral dissertation, Temple University, 1971). *Dissertation Abstracts International*, 1972, **32**, 7336B. (University Microfilms No. 72-17685)

DOTY, R. L. A cry for the liberation of the female rodent: Courtship and copulation in rodentia. *Psychological Bulletin*, 1974, **81**, 159-172.

HAYASHI, S. A role of female preputial glands in social behavior in mice. *Physiology & Behavior*, 1979, **23**, 967-969.

IRWIN, F. W. *Intentional behavior and motivation.* Philadelphia: Lippincott, 1971.

LEON, M. Filial responsiveness to olfactory cues in the laboratory rat. In J. S. Rosenblatt, R. A. Hinde, C. Beer, & M.-C. Busnel (Eds.), *Advances in the study of behavior* (Vol. 8). New York: Academic Press, 1978.

MCKINNEY, T. D., & DESJARDINS, C. Androgens, fighting and mating during postnatal maturation in male house mice. *Biology of Reproduction*, 1972, **7**, 112.

MUGFORD, R. A., & NOWELL, N. W. Paternal stimulation during infancy: Effects upon aggression and open-field performance of mice. *Journal of Comparative and Physiological Psychology*, 1972, **79**, 30-36.

MÜLLER-VELTEN, H. Über den Angstgeruch bei der Hausmaus (*Mus musculus* L.) *Zeitschrift für Vergleichende Physiologie*, 1966, **52**, 401-429.

ROTTMAN, S. J., & SNOWDON, C. T. Demonstration and analysis of an alarm pheromone in mice. *Journal of Comparative and Physiological Psychology*, 1972, **81**, 483-490.

WHITTIER, J. L., & MCREYNOLDS, P. Persisting odours as a biasing factor in open-field research with mice. *Canadian Journal of Psychology*, 1965, **19**, 224-230.

(Received for publication April 24, 1980.)