March, 1987

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Behavioural Processes, 14 (1987) 137–146
Elsevier

RESPONSES TO SNAKE ODORS BY LABORATORY MICE

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(Accepted 10 November 1986)

ABSTRACT

Weldon, P.J., Divita, F.M. and Middendorf, G.A. III., 1986. Responses to snake odors by laboratory mice. Behav. Processes 14: 137–146.

Male and female laboratory mice (Mus musculus; Harlan Sprague Dawley) were tested for reactions to snake odors. In the first experiment, mice were presented with untreated paper on the floor of one side of a test tank and snake-scented or control (water misted) paper on the other side. The scented papers were obtained from rough earth snakes (Virginia striatula), which were fed earthworms, and a rat snake (Elaphe obsoleta), which ate mice. Male mice exhibited no differences in response to the three conditions. Female mice showed no response to the control or earth snake odor, but they deposited significantly more fecal boli on the side of the tank with the rat snake odor than on the blank side. No significant differences in other behaviors, e.g. ambulation, were detected. In the second experiment, female mice were offered food pellets treated with the shed skin extract of the rat snake or with a solvent alone. Less material was bit off and consumed from the snake-scented pellets. The results of both experiments indicate that female mice detect the odors of rat snakes.

KEY WORDS: chemoreception, feeding suppression, predator detection, snake odors

INTRODUCTION

The reactions rodents give to predator odors have been studied mostly using mammals as stimulus animals (Catarelli and Chanel, 1979, Catarelli et al., 1975, Dieterlen, 1959, Stoddard, 1980, Sullivan et al., 1985a, Vernet-Maury et al., 1984, 1986, and others). Snakes also elicit rodent defenses, and some authors suggest that chemical releasers are involved. Richardson (1942), for example, observed wood rats (Neotoma albigula) flee or "thump" their hindfeet on the ground when confronted by snakes. These reactions, he thought, are elicited at least in part by chemicals. Owings and colleagues report that California ground squirrels (Spermophilus beecheyi) emit alarm calls, kick sand, and plug their burrows when snakes are nearby (Coss and Owings, 1978, Hennessy and Owings,

0376-6357/87/$03.50

1979). Hennessy and Owings (1979) suggest that chemicals are among the cues eliciting these or other reactions since squirrels responded more to snakes confined in perforated plastic bags than to snakes in sealed bags, where no odors could escape. Webster (1973) suggested that kangaroo rats (Dipodomys merriami) use their sense of smell to detect rattlesnakes since rats with their olfactory bulbs surgically removed did not display their typical alertness and avoidance during snake encounters.

Chiszar (1975), however, detected no reactions to snakes by laboratory mice (Mus musculus; C57/BL 6J strain). Mice released into an empty arena or into one containing a rattlesnake showed no difference in activity. Similar results were obtained with mice suspended in a glass jar over a rattlesnake eating a mouse; this procedure presumably would have allowed mice to observe the threat posed by snakes.

We have observed that laboratory mice introduced into the home cages of rat snakes (Elaphe spp.) and coachwhips (Masticophis spp.) vibrate their tails and alternately approach and retreat from the snakes. We report here the results of behavioral tests of laboratory mice presented with substrate-borne odors from living snakes or with snake shed skin extracts presented on food pellets. These experiments indicate that mice detect snake chemicals.

EXPERIMENT I: SUBSTRATE PRESENTATION

MATERIALS AND METHODS

Thirty-six Harlan Sprague Dawley mice (Mus musculus; Hsd: [ICR] BR) of each sex (21-30 g) were used as subjects. Mice were housed in groups of 3-7 in 47 x 25 x 13 cm plastic cages and given food (Tekland Mouse/Rat Diet) and water ad libitum. They were placed singly into 29 x 18 x 12 cm plastic tanks and

transferred to a testing room (22°C) where they were kept visually isolated for up to 25 min before testing. Tests were run between 1300-1400 hrs, one subject at a time.

Mice were tested once in one of three stimulus conditions: two stimulus snake species and a control. A male rat snake (Elaphe obsoleta obsoleta x spiloides; snout-vent length = 131 cm) and seven male rough earth snakes (Virginia striatula; lengths = 19-25 cm) provided the stimulus snake chemicals. The rat snake was fed mice. The earth snakes were fed earthworms, which comprise most if not all of their natural diet (Clark, 1964).

Paper laden with snake scent was obtained by confining snakes for 36 hrs to 51 x 26 x 30 cm tanks, the floors of which were lined with wrapping paper lightly misted with distilled water. Five earth snakes were placed at a time into one tank; the rat snake was confined in another. A piece of cardboard was placed over one side of each tank for shelter. In the control condition (prepared control), paper was placed on half of the tank, misted, and allowed to stand for 36 hrs with a piece of cardboard on top.

Pieces 25 x 26 cm were cut from each of the sheets and taped onto one side of the floor of three 51 x 26 x 30 cm test tanks. Any part of the snake-conditioned paper visibly stained with snake feces was not used. A fresh sheet of unmisted, unscented (blank) paper 25 x 26 cm was taped onto the opposite side of each tank, separated from the snake-scented or prepared control sheets by a center strip about 5 mm wide. Each tank was used for only one of the three treatments and was washed with soap and water and dried after every test.

A mouse was placed at the center of a tank and filmed for 10 min by a video camera placed 70 cm directly overhead. The time mice spent on each side of the tank and the number of times mice crossed the center strip (ambulation score) were recorded for each min by a blind observer. The number of fecal boli and the

presence of urine on each side of the tank were recorded at the end of every test.

The order in which the conditions were presented was counter-balanced in a Latin square design. The side on which the snake-scented or prepared control conditions were presented was reversed with each test.

RESULTS

No significant differences were detected in total time spent on the snake-scented or prepared control side as a function of the sex of the mouse, scent (*Elaphe*, *Virginia*, or prepared control), or the interaction of sex or scent (ANOVA, $p > 0.05$). Neither were significant differences detected during the first minute of observation. The ambulation scores also were not significantly affected by sex of the mouse, scent or their interaction (Table I).

Table I. Side preference and ambulation mean scores (± 1 SD) for male (n=36) and female (n=36) mice for 10 minute observation period. No statistically significant differences were detected.

Condition	Sex	*Elaphe* scent	*Virginia* scent	Control
Time on treated side (sec)	m	318.7 ± 125.3	265.5 ± 111.5	266.2 ± 27.0
	f	329.5 ± 65.3	321.0 ± 85.0	318.4 ± 70.5
Lines crossed	m	35.2 ± 14.9	37.4 ± 17.1	41.1 ± 19.8
	f	30.2 ± 7.3	32.7 ± 17.1	35.3 ± 10.9

No significant differences were detected between scent conditions or sexes in urinations (Table II), however, the number of fecal boli deposited by females on the *Elaphe*-scented side of the tank was significantly greater than that on the blank side (paired t-test, $t = 2.7$, $p < 0.02$). No other significant differences

were detected in the number of boli left by either sex ($p > 0.05$) between snake-scented or prepared control sides vs. blank sides (Table II).

Table II. Number of trials in which urination occurred and means (± 1 SD) for defecations by location for male (n=36) and female (n=36) mice. The blank side contained an unmisted, unscented piece of paper.

Side of tank	Sex	Elaphe	- blank	Virginia	- blank	Control	- blank
Trials during which urination occurred	m	4	5	3	3	5	3
	f	4	4	5	1	5	2
Fecal boli	m	0.7±1.1	1.2±1.3	1.3±1.4	1.5±1.6	1.2±1.4	1.2±1.5
	f	2.0±1.4*	0.8±0.6	1.8±1.0	1.0±1.3	1.0±1.1	1.1±1.3

* $p = 0.02$, $p > 0.05$ for all other comparisons

EXPERIMENT II: FOOD PRESENTATIONS

MATERIALS AND METHODS

Thirty-two female mice (19-27 g) each were presented once with food pellets treated with either snake skin chemicals or a solvent control. Mice were maintained as described in the previous experiment, except that they were isolated and permitted access to only water for 15 hrs before testing. Tests began at 0900 hrs.

The snake chemicals were obtained by extracting a shed skin (15.2 g) of the rat snake for 48 hrs with methylene chloride (CH_2Cl_2) in a Soxhlet apparatus. A total of 18 mg of residue was obtained after the CH_2Cl_2 was removed by rotary evaporation. The residue was air-dried, weighed, and dissolved in CH_2Cl_2 to give a 10 mg/ml solution of snake skin extract.

A pipette was used to apply 1 ml of the skin extract solution to food pellets; control pellets received 1 ml of plain CH_2Cl_2. All food pellets were air-dried (23°C) for 11 hrs to remove the CH_2Cl_2 and then reweighed. Their final weights ranged between 3.02 and 3.58 g.

Mice were placed singly in 31 x 16 x 8 cm clear plastic boxes with lids. Small holes in the upper walls of each box permitted ventilation. Two plastic dishes (diameter = 9 cm; depth = 1.5 cm), each containing a single food pellet, were placed 10 cm apart at opposite ends of the boxes. The food pellets were held by metal clamps wired in place through the bottom of the dish and the floor of the test box.

Each mouse in the experimental group was presented with a snake-scented food pellet in one dish and a CH_2Cl_2 treated pellet in the other. Control animals received a CH_2Cl_2 treated pellet in both dishes. All pellets were weighed prior to testing. Mice were left in the boxes for 7 hrs. The mice were then removed and the remaining food in each dish was weighed. Weights were taken of the main part of the food pellet (if present) and of the smaller particles remaining in the dishes. The total food gnawed off was estimated by subtracting the weight of the main remaining pellet from the original pellet weight. The total food consumed was obtained by substracting the weight of the smaller particles in the plastic dishes from the amount gnawed.

Each test box was washed and dried after every test. Fresh plastic dishes were used for every test.

Table III. Means (± 1 SD) for food consumed and food gnawed for control-control trials and for control-snake extract trials (n = 16 for all trials).

Trial	Control - Control		Control - Snake Extract	
Food gnawed (g)	1.02 ± 0.84	1.05 ± 0.47	1.63 ± 0.73	0.46 ± 0.51*
Food consumed (g)	0.66 ± 0.53	0.78 ± 0.39	1.14 ± 0.43	0.27 ± 0.30*

* $p < 0.01$, $p > 0.05$ for control-control comparisons

RESULTS

Mice showed no significant difference in pellet preference between two control-treated pellets (t-test, $t = 0.09$, $p > 0.05$), however, they gnawed significantly less food from the Elaphe-scented food pellet when it was paired with a control pellet ($t = 5.00$, $p < 0.01$, Table III). Similarly, mice did not differentiate with regard to amount of food consumed between control treated pellets ($t = 0.55$, $p > 0.05$), but ate significantly less of the snake-scented pellet ($t = 5.70$, $p < 0.01$, Table III).

DISCUSSION

Price (1984) discusses the popular notion that adaptive traits degenerate during domestication, and he concludes that nearly all behavioral differences between wild and domestic stock are quantitative, with shifting thresholds for the release of behaviors determined by genotypic changes, the captive environment or both. The extent to which domestic rodents differ from their wild counterparts in reacting to predator scents is not known, but it is clear that some domestic strains do respond. Reports of Wistar rats' reactions to carnivore

fecal scents, for example, indicate that strong "stress" or "fear" responses are given to fox (Vulpes vulpes) fecal odors (Catarelli and Chanel, 1979, Catarelli et al., 1975; Vernet-Maury et al., 1968 and others). Chemicals eliciting these reactions have been identified (Vernet-Maury et al., 1984).

Our results suggest that Harlan Sprague Dawley mice perceive snake odors, although only some of the measures scored support this conclusion. In the first experiment, female mice deposited more fecal boli on the Elaphe-scented side of the test tanks. Heightened defecation scores generally are thought to reflect "fear" in rodents (c.f., Archer, 1973). Rat snakes certainly are a greater threat to rodents in the field than are earth snakes. Increased fear, then, may be an appropriate response vis-a-vis Elaphe spp. However, we suspend judgement on whether the observed reactions are defensive or contribute to survivorship until tests are performed.

It is unclear why females and not males in our first experiment showed defecation responses to Elaphe scent. Males may not detect the snake odors or, alternatively, they may not respond to snakes as do females. Henderson (1967) found sex differences in defecation scores of mice during open field tests, but his results indicate that males deposit more fecal boli. The possibility that there exist sex differences in perception of and response to snake odors should be investigated.

In the second experiment, the reduced amount of food gnawed off and consumed from the Elaphe-scented pellets indicate that female mice perceive snake odors. Kobayashi and Watanabe (1986) report that Siberian chipmunks (Eutamias sibiricus asiaticus) gnaw on dead snakes and apply the carcass odors to their fur, apparently to render themselves less recognizable to predators dependent upon chemoreception, e.g., snakes. Snake skin chemicals, as well as cloacal and fecal substances, elicit the scent application behaviors. Snake skin chemicals also

elicit behavioral responses in interactions between snake-eating snakes and their ophidian prey (Weldon, 1982; Weldon and Burghardt, 1979; Weldon and Schell, 1984).

The deterrent effect of predator chemicals on feeding has been shown with deer (e.g., Sullivan et al., 1985b; Müller-Schwarze, 1972) and rabbits (Sullivan, et al., 1985a) responding to carnivore scents. Our results indicate that snakes also may be useful stimulus animals to include in such experiments. Studies of the reactions of wild rodents to snake odors are needed to determine whether these chemicals or those of other predators could be used in rodent control.

ACKNOWLEDEGMENTS

This study was done while P.J.W. was supported by a grant from the Whitehall Foundation and G.A.M. was supported by a faculty research grant from Howard University.

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