

February 1979

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BEHAVIORAL RESPONSES OF PINE VOLE YOUNG AND ADULTS TO
OLFACTORY CUES

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ABSTRACT: Ultrasonic vocalization rates of young pine voles are highest when pups begin to move into and out of nests and exhibit olfactory-directed movement. As olfactory preferences become established and movement is coordinated, a decline in vocalization appears in pine vole. In the presence of home shavings young pine voles emit high rates of vocalization, while rat pups emit calls at a low rate. This contrast may relate to the intensive attachment of young pine voles to the mother in early life.

In preliminary studies of adults, priming either the male or female with the odor of a prospective social partner reduces subsequent aggression. While long-term olfactory exposure can familiarize a vole with an unknown conspecific and reduce conflict, a brief unfamiliar olfactory stimulus to a male can disrupt subsequent interactions of a familiar pair.

INTRODUCTION: Pine voles exhibit neonatal, transitional, and socialization stages of development, each lasting approximately one week. During the neonatal stage the hairless pup is almost always attached to the mother. Its eyes and ears are closed and appear nonfunctional. When detachment occurs, both parents appear agitated and initiate retrieving until the mother is again crouched over the pup. In the absence or death of the mother in the laboratory, the male crouches over young. With two parents, simultaneous retrieving efforts are common which sometimes results in pulling from opposite ends of the pup. Young weanlings from an earlier litter also retrieve and crouch over scattered young.

The transitional stage, when sense organs of pups are becoming fully functional, is still a time of frequent mother-young attachment. Young are not left in the nest. Rather, the mother moves about with young attached. Finally, in the socialization stage, pups exhibit locomotion about the cage without parent contact. At this stage young appear more adventuresome than adults, exploring fresh food items and responding to cage disturbance.

Rodent pups of the murid and cricetid families emit ultrasonic vocalizations and pine voles are no exception. These calls can be detected by a device which transduces them to an audible signal. They also can be tape recorded and physically characterized in a pictorial way.

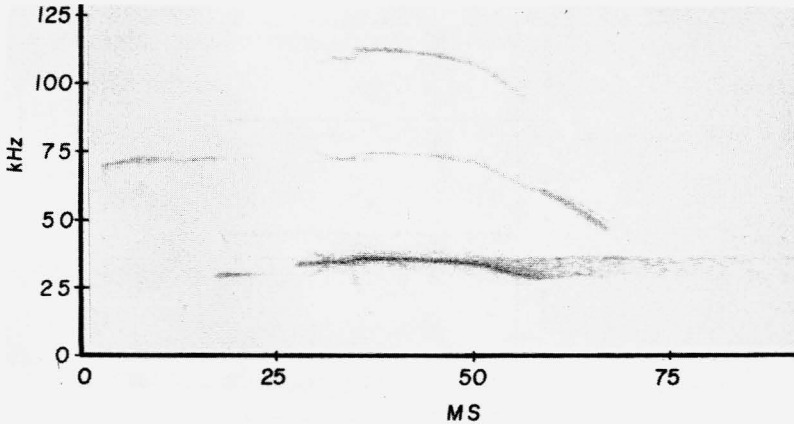


Fig. 1. Sonogram of ultrasonic vocalization produced by a 4 day old pup. Tape recordings were prepared using the following components: a .64 cm Bruel and Kjaer microphone model 4136, with cathode follower; a 100x preamplifier, a Krohn-Hite filter (model 3550) set with bandpass 15-100 kHz; and Lockheed tape recorder (model store 4) set at 30 ips. Vocalizations were then played back at 3.75 ips and analyzed on the Kay Sondgraph (model 7029 A).

Sonographic analysis of calls from pine vole pups shows a slow frequency drift pattern. Figure 1 shows the call of a 4 day old pup in which the primary waveform has a 75 ms duration. The first harmonic exhibits a frequency sweep from 75-52 kHz and the second harmonic a sweep from 115-94 kHz. Harmonics of this call are not simply replicates of the dominant, but exhibit a rapid frequency sweep downward. The simple wave pattern and the frequency range are similar to several other species of Microtus young (1). Studies of other microtines have established that adult rodents hear and behaviorally respond to these calls (2).

DEVELOPMENT OF OLFACTORY AND ULTRASONIC RESPONSES: Recently, I have studied responses of developing pine voles to various olfactory stimuli. Individual pups were tested in a clean opaque shoe-box cage with the length grid-marked into four sections. Soiled shavings from the home nest were in one extreme quadrant and clean shavings in the other extreme quadrant. A clean screen floor was placed over the shavings, 2cm above the cage floor. Individual pups were given three min tests. Behavior was manually recorded on an Esterline-Angus event recorder. Measures included: ultrasonic vocalizations, with a maximum score of 2.5 pulses/sec.; cage grid location; grooming. Ultrasounds were detected at broadband setting using the QMC detector. The vocalization frequency with peak energy was verified at the end of each test by using the narrow band setting of the detector. A preference percentage was extracted from the record, based on the number of seconds spent above home shavings, divided by the total number of seconds at either extreme end of the cage.

Data are presented in Figure 2. Scores were pooled in 5 day age brackets for all measures. Each age bracket included 12-18 pups, except days 1-5 when pup removal is life-threatening. No individual pup was tested more than three times.

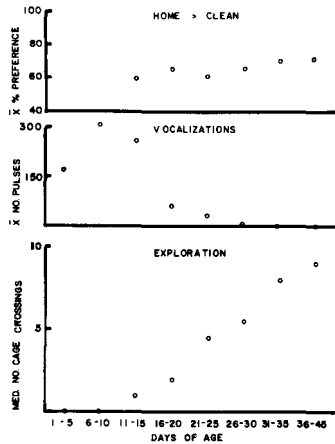


Fig. 2. Olfactory preference, cage exploration, and ultrasonic vocalizations in developing pine vole young. (Six pups aged 0-5 days were tested. Other age groups each included 12-18 pups).

Vole pups emitted an increasing number of pulses from birth through 11-15 days of age, then showed a significant decline at 16-20 days of age (t -test: $p < .005$). The number of pulses decreased further in pups aged 21-25 days and pulses were absent in a majority of animals.

Few pups exhibited movement to another end of the cage before 10 days of age. At 11-15 days of age pups moved about the cage, and the mean number of cage crossings fluctuated little through 30 days of age. A majority of 16-20 day pups moved to both ends of the cage during the 3 min test whereas in day 11-15 young a majority moved only to one end of the cage.

Pups given this motor task did not exhibit an olfactory preference before ten days of age. At 11-15 days of age 60% of the preference was for home shavings ($p = ns$). At 16-20 days of age pups first significantly preferred home to clean shavings. This preference was retained through all ages of pups tested.

The age of 16-20 days marked both the initial occurrence of olfactory directed movement and a major decline in ultrasonic vocalization. These results suggest a well-coordinated behavioral synchrony, including high levels of ultrasonic vocalizations through the period of initial olfactory exploration. Once the locomotor skills are developed, vocalizing sharply declines. Available data suggest a similar pattern in the development of mouse, rat, hamster, and spiny mouse (see review, 3). A relationship between the decline in vocalization and the increase in motor development has also been suggested in studies of the gerbil (4).

In a second experiment, I tested young voles with a single bedding type to determine whether familiar odors influence production of vocalizations by pups. Ten pups aged 6-18 days were tested at 5-6 day intervals in counterbalanced order with the following shavings: clean; soiled home; soiled unfamiliar. An additional 11 naive pups, aged 19-33 days were each tested with clean and soiled home shavings in counterbalanced order. Table 1 shows vocalizations produced during a 3 min test with different bedding conditions. At both age ranges tested, more ultrasonic pulses were emitted in the presence of soiled bedding than with clean bedding (ANOVA and t -tests: $p < .05$).

Table 1. Ultrasonic vocalization as a function of cage bedding in pine vole young. (Number of pulses/3 min).

<u>Age of Pups</u>	<u>N</u>	<u>Clean Shavings</u>	<u>Home Shavings</u>	<u>Unfamiliar Soiled Shavings</u>
6 - 18 days	10			
\bar{x}		16.7	229.7	173.4
S.E.		5.8	53.7	55.3
19 - 33 days	12			
\bar{x}		.25	14.55	
S.E.		.25	6.31	

In the young rat, vocalizations increase in the absence of nest odors (5) whereas the opposite occurs in the pine vole. For the rat pup, the nest is likely to have other pups which offer warmth. For the pine vole pup, the mother herself is a portable nest, and the nest site is unlikely to be occupied in her absence. The contrasting vocalization responses to home nest odors by young rats and pine voles may relate to these differences in their natural history.

OLFACTORY INFLUENCES ON SOCIAL BEHAVIOR IN ADULTS: Pine vole adults exhibit a much richer behavioral repertoire than young, including naso-nasal contact, naso-anal sniffing, sonic vocalizations, more complex ultrasonic vocalizations, and conspicuous aggression. At Monell several years ago, Beauchamp and Rogers initiated a study of social behavior of pine voles, especially effects of gender, isolation and familiarity. From this study we learned that familiarity resulted in less aggression and more affiliation following isolation periods of less than one day, but the effect of familiarity could not be detected following isolation of three or more days. The general sex difference was greater aggression by the female, often following repeated approaches by the male.

Recently, in a series of experiments, I have tested effects of priming an individual vole with odor from a conspecific, then observing social behavior between the vole and a partner who is either a cagemate or an unfamiliar vole. In the first experiment, I provided male voles with a brief exposure to urine of an estrous female, and then observed social behavior of the male and his familiar female cagemate. Twelve male-female pairs were each given 2 tests in counterbalanced repeated measure design. They were first observed for a 1 min pre-test of baseline social behavior. Males were then removed from the home cage and placed in a partitioned cage with or without estrous urine and containing a diestrous female on the opposite side. (In the estrous urine collection an estrous female was allowed to spend 30 min in the cage before the diestrous female was placed there). After 5 min in the divided cage, the male was returned to his home cage and social behavior was monitored for a 1 min post-test.

Attacks were significantly increased following treatment of the male with estrous urine (Table 2 - t-test: $p < .025$). The incidence of varied social behaviors, such as naso-nasal sniffing, naso-anal sniffing,

ultrasonic vocalizing, and attack also increased following exposure of the male to estrous urine as compared to its absence (Chi-square: $p < .01$). This result shows that exposing a male to odor of estrous urine increases subsequent social interactions between the familiar pair.

Table 2. Social interactions between familiar male-female pairs of pine voles before and after exposure of the male to a diestrous female with and without urine of an estrous female.

	Estrous urine		Control	
	Pre-test	Post-test	Pre-test	Post-test
Attack frequency				
N	1/12	6/12	2/12	1/12
\bar{X}	.08	1.91*	.33	.33*
S.E.	.08	.71	.10	.33
Naso-anal duration				
N	1/12	7/12	0/12	2/12
\bar{X}	.25	7.64**	0	2.67**
S.E.	.25	2.44	0	1.81
Social interaction types				
N	6/12	12/12	4/12	6/12
\bar{X}	.73	2.36***	.58	.92***

* t-test: 10 df, $p < .025$

** t-test: 10 df, $p < .1$ (ns)

*** Chi square: 1 df, $p < .01$

In a second experiment, I asked whether familiar male-female pairs were affected by a brief exposure of either vole to urine of an unfamiliar male. In 12 male-female pairs, social behavior baselines were again measured in 1 min pre-tests. In a counterbalanced design, either the male or female was then moved for 3 min to a partitioned cage with or without male urine. Social behavior of the familiar pair was then observed in a 1 min post-test.

An ANOVA statistical test showed a sex x treatment interaction ($p < .01$) for attack (Table 3). The short exposure to urine of a novel male had opposite effects for the two sexes. When the female was returned to the familiar partner there were fewer attacks, while attacks increased after the male had been primed with urine from an unfamiliar male.

Table 3. Social interactions between familiar male-female pairs following exposure of one partner to urine of an unfamiliar male.

	<u>Female</u>		<u>Male</u>	
	<u>Control</u>	<u>Urine</u>	<u>Control</u>	<u>Urine</u>
Attack frequency				
\bar{X}	1.75	.67	.33	2.00
S.E.	.68	.36	.26	.81

ANOVA: sex x treatment interaction, 1/22 df, $p < .01$.

These social behavior studies suggest the following effects of conspecific urine odor on interaction of familiar male-female pairs: 1) exposing males to novel odor of an estrous female or a male results in increased social interaction with a familiar partner, including attack; 2) exposing females to odor of an unfamiliar male reduces attacks.

Table 4. Social interactions between unfamiliar male-female pairs after priming the female with either clean shavings or shavings soiled by the male.

	<u>Two hour priming</u>	
	<u>Soiled shavings</u>	<u>Clean shavings</u>
Sonic vocalizations *		
N	7/9	9/9
\bar{X}	14.22	53.11
S.E.	5.48	18.45
Attack frequency **	<u>Eighteen hour priming</u>	
N	4/6	6/6
\bar{X}	5.00	14.67
S.E.	2.77	2.57

* Paired t-test: 8 df, $p < .025$

** Paired t-test: 5 df, $p < .05$

In the last two experiments, I gathered preliminary data on the effects of olfactory priming on interactions between unfamiliar pairs. I observed responses of nine females to unfamiliar males, when females

were first primed with either clean shavings or shavings from the cage of the prospective male partner. Order of shaving treatment was counter-balanced. A two hour exposure to soiled shavings did not alter attack frequency, although sonic vocalizations were higher following exposure to clean than to soiled shavings (Table 4, t-test: $p < .025$). When another group of individually housed females was tested following an 18 hr exposure to shavings, significantly fewer attacks occurred when females had been primed first with odor from the males' cages (t-test: $p < .05$). This experiment shows that olfactory familiarity of the female with the male can reduce aggression.

Table 5. Social interactions between unfamiliar pairs when a male or a female is placed with a male following exposure to his shavings or clean shavings.

	Female		Male	
	Clean	Soiled	Clean	Soiled
Attack frequency				
\bar{X}	7.0 ^d	3.9 ^e	5.1	2.9
S.E.	1.7	1.5	2.1	.9
Male partner attack frequency ^a				
\bar{X}	2.9 ^d	.7 ^e	3.1	1.6
S.E.	.9	.4	1.1	.8
Ultrasonic voc. latency ^b				
\bar{X} sec.	183.3	175.6	71.0	202.7
S.E.	55.8	50.3	39.5	46.3
Sonic vocalizations				
\bar{X}	7.1	8.1	19.0	7.1
S.E.	3.2	5.0	9.3	3.1
Naso-anal ^c				
\bar{X} sec.	2.3	.7	25.0	14.6
S.E.	1.7	.7	8.9	11.3
Male partner anal				
\bar{X} sec.	7.9	8.9	17.4	20.0
	5.9	5.1	5.4	13.9

ANOVA: 1/12 df, $p < .05$

a treatment

b sex x treatment interaction

c sex

d,e paired t-test: 6 df, $p < .05$

Finally, individually housed males (n = 7) and females (n = 7) were socially paired following a priming exposure to either clean or soiled shavings from a male who was a prospective social partner. Sex differences were especially evident in naso-anal investigation (Table 5, ANOVA: 1/12 df, $p < .05$). The non-experimental male partners of subjects exhibited fewer attacks when they were paired with voles who had received the olfactory priming treatment (ANOVA: 1/12 df, $p < .05$). A sex by treatment interaction was found in ultrasonic vocalization latency (ANOVA: 1/12 df, $p < .05$). Comparison of attacks by females and their male partners showed more aggression by females regardless of the treatment received by the female (paired t-tests: 6 df, $p < .05$). The sex difference in aggression is consistent with the earlier study done at the Monell Center (6).

Taken together, these studies of social behavior in unfamiliar pairs following olfactory priming show that olfactory familiarity of either the male or female reduces subsequent aggression. While long-term olfactory exposure can familiarize a vole with an unknown conspecific and reduce conflict, a brief unfamiliar olfactory stimulus to a male can disrupt subsequent interactions of a familiar pair.

Studies of responses to odors verify that, like other rodents, pine voles are influenced in their social responses by recent olfactory stimulation. Further understanding of behavioral responses to conspecific odors could indirectly suggest strategies for modifying aggression and affiliation among pine voles.

ACKNOWLEDGMENTS: It is a pleasure to thank Leslie Y. Shimabukuro for laboratory assistance. The U.S. Fish and Wildlife Service supported this project.

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