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Effects of Ultrasonic Vocalizations and Male's Urine on Female Rat Readiness to Mate

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Solitary estrous female rats were presented with ultrasonic vocalizations and/or urine of males to determine their effects on the females' subsequent copulatory behavior. Following exposure to ultrasounds, females exhibited a shorter latency to dart and a higher rate of darting when a male was presented. The vocalization effect was enhanced when male urine was present, but urine alone had no effect. The rate of copulation was also increased by the vocalization priming of the female. For a few individual females, ultrasounds elicited darting in the absence of a male. These results indicate that auditory cues, particularly in association with appropriate olfactory cues, prime estrous females for proceptive behavior. This increases both the likelihood and the rate of sexual performance and, therefore, the probability of successful mating.

During copulation, the female rat (*Rattus norvegicus*) exhibits hopping and darting movements. Typically, these movements immediately precede mounting sequences by the male rat (McClintock, 1974). When sexually naive males are tested with females exhibiting darting, there is a higher probability of copulation occurring than when females showing only the lordotic response are used (Hlinak & Madlafousek, 1972). Thus, hopping and darting may be a form of solicitation that facilitates copulation.

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Darting has been characterized as pre-lordotic behavior by Pfaff and Lewis (1974). At the end of a darting sequence, the female comes to an abrupt halt and assumes a crouching posture, a forward extension of the front legs, a bilaterally symmetric posture, and a skeletal musculature tension that facilitates lordosis (Pfaff, Lewis, Diakow, & Keiner, 1972).

Solicitation patterns such as hopping and darting appear to be affected by experience as well as hormones. Gerall and Dunlap (1973) described the enhancement of solicitation behavior by experience in several mating tests. At later ages sexually experienced females had longer intervals between solicitation bouts and markedly delayed ejaculation by preventing mounting. They achieved greater reproductive success than virgins of similar ages that did not show the control of intromission pacing (Dunlap & Gerall, Note 1).

Immediately before and during mounting sequences, rats produce ultrasonic vocalizations, with the main energy around 50 kHz (Sales, 1972). Both males and females emit pulses in mating situations (Geyer, 1976). High pulse production is associated with the hormonal and behavioral readiness of both the male and the female to copulate.

Specific sensory cues eliciting solicitation have not been identified. The presence of

a male is usually necessary for darting to occur, i.e., females rarely dart when no male is present. We hypothesized that sensory cues associated with a copulating male could serve to prime the female for mating. If this were the case, it would suggest that sensory stimuli of the male may interact with female behavior in a complex way to pace and organize the mating behavior. Specifically, we decided to determine whether vocalizations and/or urine of a male presented to a solitary female would enhance her solicitation behavior during subsequent mating.

Method

Subjects

Sixteen ovariectomized, experienced female rats (Long-Evans strain, Charles River Farms) served as subjects. Twenty experienced male rats served during mating and vocalization-transmission portions of tests. Experience consisted of two mating tests delivered at biweekly intervals when copulation proceeded during a 2-hr period for a maximum of two ejaculations. Additional males, not serving as subjects in this study, were utilized for collection of urine. Likewise, females other than the subjects of this study were used for the experimental conditions with vocalization transmission.

Apparatus

Ultrasonic vocalizations were electronically transmitted from a transmitting cage to a receiving cage. Both cages were glass-walled rectangular enclosures (52 × 26 × 29 cm). A central divider of hardware cloth was attached inside the transmitting cage. The receiving cage had a central Plexiglas divider in which there was an opening (10 × 10 cm) screened with hardware cloth. Before testing began, each cage was covered by a 10-cm-thick sheet of polyfoam, corrugated cardboard, and a 3-kg weight. This served to sound-isolate the cages from each other within the limits of our sound-detection apparatus. The transmitting cage and receiving cage were placed on tables at diagonal corners of a 3 × 3 m room. Illumination was supplied by two 60-W red bulbs placed 30 cm above the top of the receiving cage.

The broadcast system included the following components: .64-cm Brüel and Kjaer microphone (Model 4136), preamplifier (Model 2619), and power supply; a 100× preamplifier; a Krohn-Hite filter (Model 3550) set with bandpass 20–100 kHz; an electrostatic transducer-driver amplifier; and a condenser microphone head connected for use as a speaker. An oscilloscope was hooked-up in parallel with the speaker. In addition, the Holgate bat detector microphone was used to monitor vocalizations in the receiving cage. Microphone specifications by Brüel and Kjaer indicate a flat response through 100 kHz. Oscillator tests of the sys-

tem visually appeared on an oscilloscope to provide a high-fidelity response; however, sonographic comparisons were not made.

Close-fitting holes in the foam and cardboard ceiling held the Brüel and Kjaer microphone above the broadcast cage and the Holgate microphone above the receiving cage. The speaker was placed on the floor of the unoccupied half of the receiving cage at a 3-cm distance from the screen doorway.

Female behavior patterns in the receiving cage were manually recorded on two push-button Rustrak event recorders by two observers. Durations of the following items were scored: grooming, movement about the cage, nose to screen doorway, sniffing as indicated by vibrissae movements, transmitted vocalizations at 50 kHz and at 22 kHz, and vocalizations by the female in the receiving cage. Episodes of urination and darting were also scored.

Transmitted vocalizations were detected both on the oscilloscope and by the Holgate receiver, whereas pulses originating in the receiving cage were detected by the Holgate without being visible on the oscilloscope. During the pretest and posttest period the sound-transmitting system was not connected, so all vocalizations in the receiving cage were necessarily produced by the female. During the test period, transmitted vocalizations were easily identified, but vocalizations by the female in the receiving cage were masked during periods of rapid transmitted vocalization. For this reason, data concerning vocalizations by the experimental female could not be analyzed.

Additional glass-walled rectangular cages (52 × 26 × 29 cm) served as mating cages. During mating tests, the following behaviors were manually scored by the observer on a Rustrak strip-chart event recorder: mount—mount with pelvic thrusts; and intromission—mount with pelvic thrusts followed by a deep thrust and a rapid dismount, usually followed by genital grooming. The following measures were extracted from the strip-chart record: mount latency (ML)—time elapsed from the entrance of the female to the first copulatory attempt; intromission latency (IL)—time elapsed from the entrance of the female to the first intromission; and mounting rate—total mounts and intromissions per minute of mating test. Darting by the female was also scored during the postvocalization mating tests except during the first four control tests.

Procedure

When required, estrus was induced by sc injections of 30 µg of estradiol benzoate and 500 µg of progesterone 54 and 6 hr, respectively, before the time of mating tests. All tests were made during Hours 2–6 of the 12-hr dark period. Each experimental female was tested alone in the receiving cage with each of four treatments presented in counterbalanced order: (a) transmitted vocalizations from an estrous female and a male that was previously given three intromissions (vocalizations: VOC); (b) transmitted sounds from a solitary nonestrous female (control: C); (c) transmitted vocalizations from an estrous female and a male to a receiving cage containing male's urine (urine + vocalizations: U + VOC); and (d) no sound transmission to a receiving cage

Table 1
Effect of Sensory Treatments to Female Rats
on Behaviors Emitted with No Male Present

Parameter	Treatment			
	C	U	VOC	U + VOC
Nose-to-screen duration	90	150	115	160***
Movement or sniff duration	145	105	155	110**
Groom latency	165	150	240	250
Urination latency	300+	75	135	90*

Note. Data are medians, expressed in seconds. C = control; U = urine of male; VOC = vocalizations; U + VOC = urine and vocalizations. Data were analyzed with the Friedman two-way analysis of variance (3 *df*).

* $p < .02$.

** $p < .01$.

*** $p < .001$.

containing male's urine (urine: U). Urine from males was obtained each day of testing immediately prior to the test. A stud male and an estrous female were placed on opposite sides of the transmitting cage for 5 min. The male typically urinated. His urine was then collected on gauze squares, picked up with forceps, and wrapped in plastic wrap until required. The procedure was repeated with an additional pair of rats. Before the tests with urine, the urine-covered gauze squares were placed on waxed paper adjacent to the speaker in the receiving cage. Thus the urine was present in the cage throughout the test.

Rats were placed in an adjacent room when not being tested. The receiving cage was cleaned with 70% ethanol prior to each test. Immediately before each test, the appropriate rat(s), if any, was placed in the transmitting cage. The experimental female rat was then placed in the receiving cage.

Observations were made for a 1-min pretest without

Table 2
Effects of Sensory Treatments to Female Rats
on Solicitation in Subsequent Copulation

Solicitation measure	Treatment			
	C ^a (<i>n</i> = 11)	U (<i>n</i> = 15)	VOC (<i>n</i> = 15)	U + VOC (<i>n</i> = 15)
Mdn dart latency (in sec)	40	40	16	3*
% tests with darts preceding first mount	33	53	50	73
Mdn darts/minute	1.1	3.3	5.7	15.3**
% tests with darts	70	73	93	93

Note. C = control; U = urine of male; VOC = vocalizations; U + VOC = urine and vocalizations.

^a Darts were not scored during the first four control tests. For this reason, the control treatment was omitted from these statistical analyses. Data were analyzed with the Friedman two-way analysis of variance (2*df*).

* $p < .05$.

** $p < .01$.

sound transmission. When required, the sound system was then connected for a 5-min test period and disconnected for a 1-min posttest. A male from an adjacent room was placed in the mating cage immediately after the 5-min test period, which provided him at least 30 sec of acclimation in the cage.

At the end of the posttest period the female was moved from the receiving cage to the mating cage. Mating was allowed to proceed through only three intromissions, after which the test was terminated. A maximum of 5 min was allowed for the mating test. Males each participated in four mating tests with females that had received each of the four treatments. Assigned pairings were different on each test, and order of treatment was counterbalanced.

Data were analyzed with the nonparametric Friedman two-way analysis of variance. This permitted evaluation of contrasts in scores for each male and for each female under varying treatment conditions.

Results

Noncopulatory Behavior

The nose-to-screen duration in the receiving cage shown by the female significantly differed with treatment (Table 1). The two treatments with urine yielded higher nose-to-screen durations than did the control or vocalization treatment. Conversely, the time spent either moving or sniffing was greater in the control and vocalization conditions than in the conditions with urine. Urination latency of females was shortest in treatments including male urine.

Solicitation by the Female

Darting by the female seldom occurred in response to vocalizations. Solitary females exposed to the control or the urine condition never darted. One female darted each time vocalizations were presented. Two other females darted during a test with vocalization.

During the mating portion of the test, the median latency to dart significantly differed in the three treatment conditions (Table 2). Since darting during mating was not scored on the first four control tests, the control treatment was omitted from these statistical analyses.

The count of darts/minute of mating also significantly differed with treatment. The rate of 15.3 darts/min following the urine +

Table 3
Effects of Sensory Pretreatments to Female Rats on Subsequent Temporal Copulatory Parameters of the Male

Copulatory measure	Treatment			
	C	U	VOC	U + VOC
<i>Mdn</i> ML (in sec)	22	8	11	5
<i>Mdn</i> IL (in sec)	140	60	30	15
<i>Mdn</i> mounts/minute	2.4	2.6	2.2	5.1**
<i>Mdn</i> of 3 intromissions including IL (in sec)	232	245	195	95*
No. failing to display (N = 15)				
M	1	3	0	0
I	3	4	2	0
3 intromissions	6	5	3	0

Note. C = control; U = urine of male; VOC = vocalizations; U + VOC = urine and vocalizations; ML = mount latency; IL = intromission latency; M = mount; I = intromission. Data were analyzed by the Friedman two-way analysis of variance (3 *df*).

* $p < .01$.

** $p < .001$.

vocalizations treatment far exceeded the rates following either the vocalization treatment (5.7 darts/min) or the urine treatment (3.3 darts/min). In addition, the vocalization treatments resulted in a higher percentage of tests with darting.

Relation of Sensory Treatments to Copulation by the Male

The temporal parameters of male sexual behavior when mating with females that had received one of the four treatments are shown in Table 3. The ML did not significantly differ with the four treatments, nor did IL. On the other hand, the rate of mounting by the male was significantly affected by the female's treatment. The urine + vocalization treatment resulted in a higher rate of mounts/minute than any of the other treatments. Although females darted extensively following a urine + vocalizations treatment, they frequently did not show lordosis until numerous mounts had occurred. This lack of lordosis appeared to delay intromission on numerous tests. In a few tests, the receptivity of the female matched the rapid mounting rate of the male. In four mating tests following the

urine + vocalizations treatment, the first male mount was an intromission and in each case was preceded by darting. In no other treatment was the male seen to intromit on the first mount. This may reflect a priming of the female for exhibiting the lordotic reflex.

The time required to achieve three intromissions differed across the four treatments (Friedman, $p < .01$). When the female was treated with urine + vocalizations, the time was half as long as in any of the other treatments. In all 15 tests with urine + vocalizations the male achieved three intromissions. The other treatments each had some tests in which three intromissions did not occur: control = 6, vocalization = 3, and urine = 5.

Discussion

Noncopulatory Behavior

The presence of male urine appeared to increase the female's nose-to-screen duration, which suggests a strong attractant effect of male urine. Previous studies have demonstrated that estrous females prefer intact male odors to those of castrates (Carr, 1965), and the female preputial gland has been implicated as a source of attractive odor (Gawienowski, Orsulak, Stacewicz-Sapuntzakis, & Joseph, 1975). Our data also showed urination by the female when male urine was present. In a related finding, Brown (Note 2) reported that male rats urinate on objects odorized with either male or female urine more often than on nonodorized objects.

Vocalizations exerted a slight increase in time spent with nose to screen. They may have elicited searching for the male once it became evident he was not at the screen, as indicated by the highest movement duration. This suggests that vocalizations may indeed function to facilitate social contact.

Solicitation by the Female

Much of the work on female sexual behavior has, understandably, focused on the

lordotic reflex (e.g., Diakow, 1975; Komisaruk, 1971; Pfaff & Lewis, 1974; Pfaff et al., 1972). The fact that lordosis can be elicited in the absence of a male by manual stimulation has facilitated the clarification of the somatosensory cues provoking it. Since solicitation tendencies in female rats occur only occasionally if the female is not allowed contact with the male (Beach, Note 3), fewer observations of darting have been reported. Recently, Beach (1976) emphasized the importance of solicitation patterns of females and suggested the term "proceptivity" to describe the female's initiative in establishing or maintaining sexual interaction.

The results of this experiment show that for some small proportion of females, rapid ultrasonic pulses from a male and female rat are sufficient cues to elicit darting. Darting was never observed in the absence of vocalizations in this experiment. No additional information is available concerning why only a few females sometimes exhibit darting when vocalizations are transmitted. However, individual female differences in darting are not surprising. It is widely recognized that individual males widely differ in the temporal parameters of copulation performance and that these differences tend to be retained over time. It appears that females vary in their threshold to dart, just as males vary in their readiness threshold to mount.

Ultrasonic vocalizations have been shown to affect female sexual behavior. Floody (Floody & Pfaff, 1977; Floody, Note 4) showed that the female hamster exhibited lordosis in response to tape-recorded male vocalizations. Beach (1976; Note 3) extended these findings by showing that deafened females did not usually exhibit lordosis in response to a male in an enclosure. The females did, however, occasionally exhibit lordosis when tactile contact with an intact anesthetized male was provided.

The results reported here show that the female rat's response to transmitted vocalizations is less dramatic than that of the female hamster. Vocalizations alone only rarely elicited darting; however, the incidences of darting were increased in subsequent copulatory encounters. This short-term change in responsiveness suggests that auditory cues primed darting responses.

Relation of Sensory Treatments to Copulation by the Male

As described earlier, female proceptivity is enhanced by sexual experience. Proceptivity, in turn, improves the likelihood that sexually naive males will mate (Hlinak & Madlafousek, 1972) and facilitates copulation with experienced males. Tiefer (1969) observed copulation in males that were each presented five females. She found that females which solicited males received the most intromissions. Another study showed that when males are castrated, the decline of copulatory behavior is slower for males tested with proceptive females (Madlafousek, Hlinak, & Beran, 1976).

These data may explain an observation by Clemens (1969) in *Peromyscus maniculatus gambeli*. He noted that males had a shorter latency to mount when the female was first allowed to copulate with another male than when she was fresh. Perhaps her courtship patterns increased as a result of the priming from another male (Doty, 1974). It is common practice in many laboratories to provide female rats with a few intromissions from stud males prior to using them in sex tests. The results of the present studies suggest that the proceptivity of the female may be drastically altered by such a priming procedure.

The data presented in this experiment show that sexual performance of the male rat can be accelerated by presenting specific sensory cues to the female immediately prior to the mating test. Vocalizations presented to the female result in an increase of the copulatory rate of the male. In the presence of vocalizations, the odor of male urine appears to synergistically affect the female, as evidenced in subsequent copulation. These data suggest that darts of the female are the stimuli that facilitate male copulation.

When presented in the absence of vocalizations, urine was found to have a negligible effect on female sexual behavior. However, urine presented with vocalizations markedly enhanced solicitation. The contrasting effect of urine with and without vocalizations suggests that the vocalizations may be a sufficient cue to define a copulatory context.

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