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3. Animals were waxed ventral side up to a platform, the legs were removed, and a small rectangle of cuticle was lifted from the ventral mesothorax. The preparation was then moved to the center of a 0.7 by 2.0 m chamber lined with acoustic foam. A suction electrode was placed over the cut end of the connective, usually just caudal to the prothoracic ganglion. Sound stimuli (30 to 300 msec long; 5-msec rise and fall) were produced by a Tektronix FG501 function generator and a homemade trapezoid generator. We calibrated and analyzed the output of speakers using a quarter-inch B&K microphone (grid off) on a B&K 2209 Impulse SPL meter and a Nicolet MiniUbiquitous FFT spectrum analyzer.
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5. Animals were prepared as described in (3) except that a small square of cuticle was removed to expose the connectives between the meso- and metathoracic ganglia. We used glass microelectrodes filled with 5 percent Lucifer yellow CH in 1.0*M* $LiCl_2$ (resistance: 130 to 180 megohms) and standard recording electronics.
6. The auditory organs of the tettigoniids and gryllids are on the tibia (1). Most insects also have in their legs subgenual organs that respond to substrate vibration and low frequency sound. We found that the mantis subgenual response had thresholds as low as 45 to 50 dB SPL at the best frequency of 700 to 800 Hz. Thresholds exceeded 85 dB SPL above 4 kHz.
7. The tracheal system is an important sound route to the ear in some insects [H. Nocke, *J. Comp. Physiol.* 110, 25 (1975)].
8. The 13 to 16 scolopales were visualized in a related species, *Tenodera aridifolia,* using the Masson-Baker stain [D. Young, *Phil. Trans. R. Soc. Ser. B* 256, 401 (1970)]. They were oriented along the ligament so that their distal attachment is to relatively thick cuticle at the edge of the tympanal depression. It is not clear whether the tracheal sac vibration or motion of tympanum itself is transduced.
9. The maximum time of arrival difference between the tympana would be less than 0.5 μsec. Intensity differences at the two tympana due to sound shadowing at 35 kHz are not possible since they are less than 1/50 wavelength apart (2). A system using phase differences (a pressure difference receiver, for instance) could theoretically provide directional information but normally would be effective only at frequencies below the best hearing range of the mantis [A. Michelsen, *Z. Vergl. Physiol.* 71, 102 (1971)].
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Infantile Experience with Suckling Odors Determines Adult Sexual Behavior in Male Rats

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Because infant rats learn about odors that elicit suckling, and because certain chemosensory cues that help elicit mating behavior in adults are similar to those that elicit suckling, an experiment was undertaken to assess the influence of suckling-associated odors experienced during infancy on adult sexual behavior. Rat pups lived with and suckled dams whose nipple and vaginal odors were altered with citral, a lemon scent. The rats were weaned and never exposed again, until testing, to citral or females. At about 100 days of age, the males were paired in mating tests with a normal sexually receptive female or with a sexually receptive female that had been treated perivaginally with citral immediately before testing. The males ejaculated readily when paired with citral-treated females but were slow to achieve ejaculation when paired with normal females. These findings implicate an infantile experience as a determinant of adult sexual behavior in a mammal.

THE POSSIBLE EFFECT OF EARLY experience on adult sexual responsiveness has long been a subject of debate. Whereas some birds "learn" in infancy certain features typifying the preferred mate (1), such extreme plasticity has not, to our knowledge, been demonstrated in a mammalian species (2). We report here that adult male Norway rats respond sexually to a chemosensory feature of the sexually receptive female that was associated with suckling behavior in infancy.

Chemoreception is crucial to both suckling and mating in rats. Suckling is prevented by anosmia (3) or nipple lavage (4), and mating is severely impaired by anosmia in sexually inexperienced males (5). Certain sulfur compounds affect the expression of both behaviors (6). In the case of suckling, such chemosignals appear to be acquired, as an artificial odor can be substituted for normal suckling stimuli (7).

Functional equivalence between suckling and sexual chemosignals is directly indicated by recent findings regarding "probing," an olfactorily controlled component (8) of suckling behavior in which the pup repeatedly pushes its snout into the dam before attaching to a nipple. Probing is elicited not only by maternal odors but also by the odors of sexually receptive (estrous) females (9). Moreover, this responsiveness, like responsiveness to maternal suckling odors, reflects particular rearing experiences. Specifically, pups reared with dams whose nipple and vaginal odors—odors that normally elicit probing—were altered by daily painting with citral, a lemon scent, probed normal estrous females only rarely, but readily probed estrous females scented with citral (9). In the study reported here, males reared in this manner until weaning, and then isolated from both females and citral until adulthood, mated more readily with citral-scented than with normal estrous females.

One group of male pups (group C, $n = 39$) was reared with dams whose nipple and vaginal areas were painted with citral (0.03 ml per dam per day; Aldrich) beginning 2 days before parturition and continuing until separation from the dam on day 28. A control group (group S, $n = 24$) was reared with dams painted in the same areas with isotonic saline. A second control group (group CB, $n = 16$) was reared with dams whose backs only were citral-scented. CB rats, therefore, experienced the citral odor in association with the dam, but not as a conjunct of suckling. All males were reared in mixed sex litters.

After weaning, the males were housed in groups in a room free of citral until being tested at 90 to 120 days of age. Each male was then observed in his first postweaning encounter with a female. All testing took place in wire mesh hoops 45 cm across by 30 cm high under dim red illumination during the first half of the animals' dark cycle. The animals were tested two at a time (one male per hoop, hoops 60 cm apart) in videotaped sessions. Each male was allowed 10 minutes alone in the arena before a female was gently lowered into it. All the females were in natural estrus, but half of them had been scented perivaginally with citral (0.015 ml)

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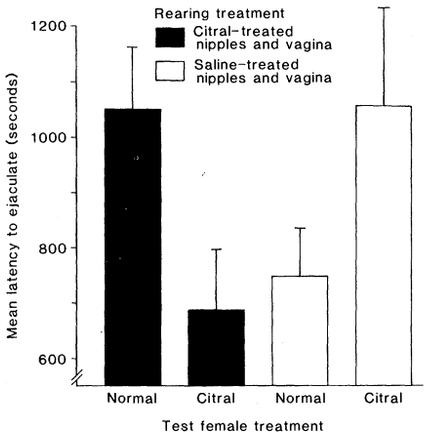

Fig. 1. Latency of C and S males to ejaculate, in their first postweaning heterosexual encounter, with an unscented or citral-scented estrous female. Latencies (means ± standard errors) were measured from the time of introduction of the female.

just before testing. Perivaginal sniffing typically precedes copulation (*10*); even sexually inexperienced males direct their sniffing perivaginally (*11*). No female was used in more than one test.

The major components of male sexual behavior were identified, quantified, and statistically assessed. These components were duration of anogenital sniffing (S); mounting latency (ML); intromission latency (IL); ejaculation latency, as measured from the time of introduction of the female (ELF) and from the first intromission (ELI); interintromission interval (III); mounting frequency (MF); intromission frequency (IF); and postejaculatory interval (PEI). All measures refer to the first ejaculatory series.

Some tests lasted 30 minutes and others 1 hour, but 15-minute and 30-minute cutoffs were imposed on all pre-ejaculatory and ejaculatory latencies, respectively, for the statistical analysis. Data were subjected to a 3 (rearing condition) × 2 (test female treatment) analysis of variance and planned *t*-comparisons.

Different infantile experiences led to different patterns of sexual behavior with respect to latency to ejaculate (Fig. 1). These differences are reflected as an interaction between rearing and test female conditions for both measures of ejaculatory latency. Figure 1 presents only ELF values [$F(2, 73) = 4.198$, $P < 0.02$]; for ELI, $F(2, 73) = 4.443$, $P < 0.02$. Specifically, group C males paired in adulthood with citral-scented females ($n = 19$) ejaculated significantly more quickly than did identically reared males paired with normal females ($n = 20$) [ELF: $t(73) = 2.655$, $P < 0.01$; ELI: $t(73) = 2.874$, $P < 0.01$] (*12*). Group S males tended to show the opposite pattern [ELF: $t(73) = 1.563$, $P < 0.07$] (Fig. 1). Group C males paired with normal estrous females were slower to achieve ejaculation than were group S males paired with such females [ELF: $t(73) = 2.092$, $P < 0.05$; ELI: $t(73) = 1.767$, $P < 0.05$]. Finally, CB males mated with either type of female with equal readiness. Their latencies to ejaculate with normal and citral-treated females were, for ELF, 814 ± 173 and 800 ± 172 seconds (means ± standard errors), respectively, and, for ELI, 607 ± 230 and 718 ± 225 seconds.

Citral was not acting as a general sexual excitant during testing, since it failed to lower ejaculatory latencies for either control group. Neither was citral's effect among group C adults due to its association, in a general way, with the dam during infancy: CB males—which were also reared by citral-scented mothers—mated as quickly with either type of female. Rather, we believe that these findings are best explained as follows: males mate more readily with females bearing a "suckling" odor, that is, an odor experienced on the dam during infancy which was specifically associated with suckling.

This interpretation is consistent with the results of our earlier study (*9*), in which group C infants probed only estrous females scented with citral. Just as saline-reared infants readily probed normal estrous females, saline-reared adults mated readily with such females. And, just as CB infants—which experienced normal nipple odors during the intimacy of suckling against a background of citral odor—probed both normal and citral-scented estrous females with equal readiness, they here mated as quickly with either type of female. We interpret the results for CB animals as reflecting responsiveness to the normal estrous scent that was not deterred by the citral odor, which was not novel to these animals (Table 1) (*13*).

Table 1. Pre- and postejaculatory measures for C, S, and CB rats in mating tests with normal and citral-treated females. Values are means ± standard errors in seconds except for MF and IF measures.

Measure	Rearing condition	Test female condition	
		Normal	Citral-treated
ML	C	236 ± 60	157 ± 56
	S	75 ± 17	225 ± 98
	CB	60 ± 30	146 ± 38
IL	C	326 ± 76	191 ± 60
	S	93 ± 25	281 ± 109
	CB	218 ± 128	152 ± 38
MF	C	12.2 ± 2.6	7.0 ± 1.3
	S	5.2 ± 1.1	4.9 ± 1.7
	CB	8.0 ± 1.6	7.3 ± 2.9
IF	C	11.1 ± 0.9	9.2 ± 0.8
	S	11.0 ± 1.4	8.8 ± 0.6
	CB	8.7 ± 1.8	10.0 ± 2.0
III	C	87 ± 13	56 ± 7
	S	74 ± 12	84 ± 23
	CB	66 ± 17	65 ± 15
PEI	C	329 ± 12	298 ± 10
	S	347 ± 26	318 ± 27
	CB	348 ± 20	321 ± 20
S	C	25 ± 10	6 ± 1
	S	16 ± 5	7 ± 5
	CB	46 ± 19	2 ± 1

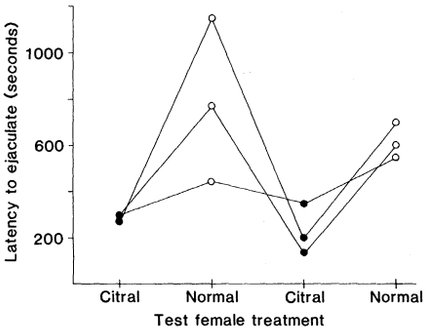

Fig. 2. Latencies to ejaculate (ELF), in four consecutive biweekly tests, of three "fast" C males. These males were paired in their first mating test with citral-scented females and selected for subsequent testing on the basis of their short ejaculatory latency in that test. Vaginal treament was alternated over the four tests.

To evaluate any continued effects on mating of the group C rearing experience, we selected the three experimental males that had mated most quickly with citral-scented females and tested each of them three more times at biweekly intervals. Vaginal treatment was alternated between normal and citral-scented. All tests lasted 1 hour. For each male, latencies to ejaculate were longer with normal females (Fig. 2). The probability that this particular binary pattern, of all six long latencies derived from one grouping and all six short latencies produced by the second grouping, would occur by chance is less than 0.002. Thus the effects of infantile experience on adult copulatory readiness hold for at least four matings with normal and citral-scented females.

These findings suggest that, at least for this mammal, the degree to which a feminine feature is sexually arousing to adult males can be established in the context of suckling.

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12. The difference in ejaculation latency between the two groups of C males was not due to their mating with females that were differentially receptive. On a four-point scale that evaluated the intensity of female sexual behavior during the first 5 minutes of the mating test, citral-scented females scored 1.7 ± 0.2 (mean ± standard error) whereas untreated females scored 2.0 ± 0.2.
13. While mount and intromission latencies reflect essentially the same pattern shown by the ejaculation latencies, these differences are not statistically significant. Frequency and interval measures did not reflect developmental treatments. The only nonejaculatory measure that attained statistical significance was duration of anogenital sniffing. Males mating with citral-scented females sniffed less, regardless of rearing condition [$F(65) = 6.704$, $P < 0.05$]. The biological significance, if any, of this difference is not known. Possibly the fresh citral odor was slightly irritating to the males.
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Detection of Papillomavirus DNA in Human Semen

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Human papillomavirus DNA has been detected in the semen of three patients, two of whom have severe chronic wart disease. These data support the contention that sexual transmission of human papillomavirus DNA could occur via semen, a possibility suggested by epidemiological data on the sexual transmission of human papillomavirus.

SEVERAL HUMAN PAPILLOMAVIRUS (HPV) DNA genomes have been shown to be physically associated with various types of benign, premalignant, and malignant lesions of the anogenital tract (*1–8*). Epidemiological evidence has suggested a correlation between some male penile cancers and female partner cervical carcinomas (*9*) and has indicated that male sexual partners of women with various benign or premalignant cervical lesions were at high risk for having penile lesions (*10*). Because the incidence of HPV in the male is far lower than in the female (*9, 10*), there are questions about the possible modes of sexual transmission of HPV. In an attempt to determine whether semen can serve as a reservoir for HPV DNA or HPV virus, we have used the Southern blot hybridization procedure to examine semen from several patients exhibiting severe chronic HPV infections. We have found HPV-5 or HPV-2 DNA in semen from three of the patients, an indication that sexual transmission of HPV may occur directly through the delivery of HPV-containing semen.

In our ongoing studies on the nature of severe chronic and recurring papillomavirus-associated diseases, we have concentrated on two groups of patients. (i) Patients with epidermodysplasia verruciformis (EV) disease have a chronic and familial condition characterized by flat warts and pigmented papules which in one out of three patients will progress to squamous cell carcinoma in sun-exposed areas of the skin (*11, 12*). (ii) Veterinarians and meat-handlers have a high incidence of wart disease and frequent recurrence after treatment (*13, 14*). We extracted the total DNA from semen samples of three patients from each of these groups and analyzed it by the Southern blot hybridization technique, using nick-translated probes of molecularly cloned HPV DNA's (*15–17*). The sensitivity of these analyses as determined by reconstruction experiments indicated that less than 0.06 genome copy per diploid cell equivalent could be detected for homologous HPV types and about 0.6 copy per cell for heterologous HPV types detected under conditions of reduced stringency.

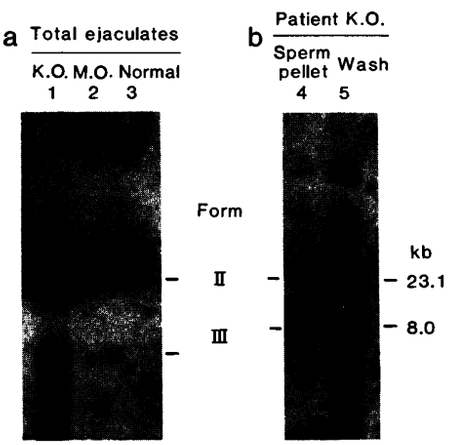

Fig. 1. Detection of HPV-5 in the semen of two patients. (a) Fresh or frozen semen from an EV patient (K.O.), lane 1, his son (M.O.), lane 2, and a normal donor, lane 3, were treated with a mixture of 1 percent sodium dodecyl sulfate and Pronase (500 mg/ml) overnight at 37°C. Donors wore gloves to prevent possible contamination of samples. After extraction with phenol, phenol:chloroform (1:1 by volume), and chloroform, the ethanol-concentrated nucleic acids were treated with ribonuclease A and extracted as above. Total cellular DNA (about 15 μg) was analyzed by electrophoresis in agarose gels, transferred to nitrocellulose, and hybridized under stringent conditions with a ^{32}P-labeled HPV-5 probe. Form II and form III represent the positions of nicked, double-stranded or linear, double-stranded native HPV DNA, respectively. (b) The semen of patient K.O., obtained about 5 months after the sample analyzed in (a), was pelleted and washed three times with isotonic saline. Both the sperm pellet, lane 4, and pooled supernatant washes, lane 5, were extracted for DNA as above. Size of linear HPV DNA and markers are given in kilobases (kb).

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