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approximately 1 μg per week and of volatile heterocyclic nitrosamines 3 μg per week.

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Ultrasonic vocalisations facilitate sexual behaviour of female rats

ULTRASONIC vocalisations commonly occur during social interactions among rodents. During mating, adult male and female rats (*Rattus norvegicus*) emit brief 50–60 kHz ultrasonic calls[1]; however, the function of these vocalisations is not known. In this study, we demonstrate that these mating calls have a precise function for communication. Specifically, 50-kHz vocalisations elicit sexual activity in female rats. Female rats exhibit a series of solicitation patterns during sexual behaviour, including orientation, darting and ear wiggling. These movements excite the male and enhance the likelihood of mounting, thereby facilitating copulation[2–4]. (Beach has emphasised the importance of solicitation by the female during mating and has suggested the term 'proceptivity' to include the female's behaviour in the initiation and maintenance of copulation.) Although the presence of an intact male is usually a prerequisite for solicitation behaviour, the specific sensory cues which elicit it are not known. Isolated oestrous female rats exposed to ultrasonic vocalisations from a male exhibited a shorter latency to, and higher rate of darting when subsequently placed in a mating situation[5]. Although the auditory cues primed oestrous females to display increased solicitation behaviour when later confronted with an intact male, there was no indication that these ultrasonic vocalisations had a direct function for communication in the induction of these behaviour patterns. The objective of the present report was to determine if ultrasonic mating calls of rats have a direct function for communication during copulation.

Sixteen ovariectomised Long–Evans female rats served as subjects. Fifteen experienced males and 15 experienced female rats were used for transmitting vocal signals. Seven long-term castrated males were used as stimulus animals. The animals were maintained on a 12:12 h light–dark cycle (lights off at 0930). Ultrasonic vocalisations were electronically transmitted from a transmitting cage to a receiving cage. Both were glass-walled aquaria (52 × 26 × 29 cm high). The transmitting cage contained a central divider of wire mesh screen and was covered by a 10-cm thick sheet of polyfoam. The polyfoam was covered by corrugated cardboard within which a hole was made to hold a 0.64-cm Bruel and Kjaer microphone (model 4136). The microphone was placed directly over the male's section of the cage. Vocalisations wers picked up using the microphone, and amplified, filtered and relayed by a transducer driver amplifier to an electrostatic condenser microphone head connected for use as a speaker. The speaker was angled above the receiving cage. The speaker input was monitored on an oscilloscope and a Holgate ultrasonic receiver (bat detector) recorded vocalisations in the receiving cage. The transmitting cage and receiving cage were placed at diagonal corners of a 3 × 3 m room. Illumination was provided by red light above the receiving cage.

Female rats received subcutaneous injections of 50 μg oestradiol benzoate 54 h and 500 μg progesterone 6 h before the tests to induce behavioural oestrus. Testing was carried out during hours 4–8 of the 12-h dark period. Experimental females were placed in the empty receiving cage for a 2-min acclimation period. A castrated male was then introduced into the receiving cage and the stimulus treatments were begun simultaneously. Females were tested for 10 min in the receiving cage with one of the following treatments: (1) transmitted vocalisations from oestrous female and intact male previously given three intromissions (vocalisations, Voc); (2) transmitted sounds from empty cage (control, C); (3) transmitted sound from empty cage to receiving cage containing intact male urine (urine, U). (Urine from intact males was obtained on the test day by placing a male and oestrous female on opposite sides of a wire-mesh divider. The male typically urinated and his urine was collected on gauze squares. The urine-covered gauze was placed in a small wire box on the cage wall which enabled the female to smell the urine but prevented any gustatory contact.); (4) transmitted vocalisations from oestrous female and intact male to receiving cage containing intact male urine (urine + vocalisation, U + Voc). Each female received each treatment at 1-week intervals in a counterbalanced fashion. Darting and lordosis by the female in the receiving cage were manually scored on a pushbutton-activated Rustrak event recorder, as was the occurrence of transmitted vocalisations at 50 and 22 kHz. Darting and lordosis latencies and frequencies were calculated from the strip-chart record.

As shown in Table 1, females exposed to both treatments with vocalisation showed significantly more darting behaviour than those in the non-vocalisation treatments (two-way analysis of variance: $P < 0.01$). Similarly, both vocalisation treatments resulted in significantly shorter latencies to darting compared with control or urine alone treatments (two-way analysis of variance: $P < 0.01$). Table 1 also demonstrates that significantly higher lordosis frequently occurred during the two treatments involving vocalisations compared with non-vocalisation treatments (Friedman analysis of variance: $P < 0.001$). No differences were found between the Voc and Voc + U groups. It is important to note here that lordosis was usually exhibited in response to minimal contact stimulation by the castrated males. Castrated males were never observed to mount the females, and often during the vocalisation treatments a slight ano-genital sniff or nudge would be an adequate stimulus to elicit a lordosis posture. On occasion, lordoses were exhibited without any physical contact from the male.

It was generally observed that vocalisations occurred in an episodic fashion during the 10-min test period. The sexual responses of the female seemed to follow the fluctuations in vocalisation very closely. When transmitted vocalisations slowed or ceased, there was a decrease in the rate of solicitation; accordingly, when vocalisations were re-initiated, darting and lordosis were markedly increased in frequency. In some tests, males in the transmitting cage exhibited a transition from 50- to 22-kHz vocalisations. It was casually observed that at these times the females in the receiving cages immediately increased their rate of darting. A point-biserial correlation[6] showed that there were significantly more darts in tests with (median, 78; $n = 8$) than in those without (median, 21.5; $n = 24$) 22-kHz vocalisations ($P < 0.001$).

The results of this experiment demonstrate that ultrasonic vocalisations are a sufficient cue to increase darting by a female rat in the presence of a castrated male rat. This comple-

Table 1 Proceptive behaviour of female rats in response to ultrasonic vocalisations

Behavioural measure	Treatment group				
	Control	Urine	Vocalisation	Urine + vocalisation	$P <$
Darting (mean ± s.e.m.)	14.3 ± 3.0	16.6 ± 4.3	44.4 ± 8.8	41.0 ± 7.2	0.01*
Lordosis (median)	0.5	0.0	2.5	1.5	0.001†
Dart latency (s) (mean ± s.e.m.)	105 ± 48.8	154 ± 56.4	9.3 ± 2.4	15.3 ± 3.6	0.01*

*Two-way analysis of variance.
†Friedman two-way analysis of variance.

ments our earlier finding that vocalisations prime the female to dart when she is exposed to an intact male[5]. It seems that whether or not a male is present, vocalisations enhance proceptivity of females.

Previously, we have found that urine of males in the presence of vocalisations synergistically primed females to dart in subsequent mating with intact males. In the present study, the presence of male urine did not augment the effect of the transmitted vocalisations in the presence of a castrated male. This may reflect either the difference in the hormonal status of the stimulus male, or alternatively, whether or not the stimuli were presented in the presence of the stimulus male.

This experiment has shown that the threshold to display lordosis is reduced by ultrasonic vocalisations. To our knowledge, this is the first report of non-somatosensory cues augmenting lordosis frequency, an indication of receptivity. Detailed studies have shown how somatosensory stimulation of the female by the male initiates lordosis[7-10]. It would seem, therefore, that ultrasonic vocalisations are also significant sensory inputs which influence the initiation of lordosis.

Oestrous female hamsters exhibit lordosis in response to tape recorded vocalisations of the male[11]. Similarly, partially deafened oestrous hamsters do not normally exhibit lordosis postures in the presence of a male with which they have no actual contact[12]. In mice, vocalisations are correlated with the sexual readiness of the male and the presence of the odour of the female[13]; however, a causal relationship between male vocalisations and the response of the female has not yet been demonstrated in this species. On the basis of the above results and those presented here, it would seem that ultrasonic vocalisations are significant in the integration of mating activity in rodents in general.

In the present study, a changeover in the transmitted vocalisation from 50 to 22 kHz resulted in increased female solicitation behaviour. It has previously been shown that males which shift to 22-kHz vocalisations were most active in mating tests[14,15]. It has also been shown that 22-kHz vocalisations sometimes occur during the copulatory sequence, particularly as ejaculation becomes imminent (ref. 16 and our unpublished observations). This suggests that 22-kHz vocalisations during the copulatory sequence may normally function to facilitate mating. In contrast, 22-kHz vocalisation following ejaculation are generally associated with sexual inactivity[17]. Twenty-two-kHz vocalisations also occur in a variety of other situations including solitary caging[18], and subordination in agonistic encounters[19]. Twenty-two-kHz vocalisations in an agonistic context serve to inhibit attack[20], but in general the functional significance of these sounds is not known. It seems that the functions of 22-kHz vocalisations in social contexts is situation-dependent. Further study will be required to elucidate these functions.

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Ultradian rhythm of plasma noradrenaline in rhesus monkeys

KLEITMAN has postulated that a 'basic rest–activity cycle' exists which is an extension of the rhythmically recurring patterns of sleep cycles in man[1]. Such ultradian rhythms, with a periodicity of approximately 90 min in non-human primates, have been associated with motor activity[2] and feeding behaviour[3]. Additionally, we have reported that plasma cortisol secretion in the rhesus monkey occurred in 90-min cycles which were synchronised for an entire group of monkeys over a 6-mth period, despite individual housing conditions of the animals[4]. Plasma noradrenaline (NA) is a reliable indicator of sympathetic nervous system activity[5], in part because of its short half life of less than 2 min[6]. If Kleitman's hypothesis is correct, one might expect to see a basic 90-min rhythm for plasma NA, even though its short half life would favour the finding of a much faster rhythm. We have found a 90-min rhythm for plasma NA in the rhesus monkey, which was temporally synchronised for the experimental group as a whole. The presence of this rhythm suggests a tonic waxing and waning of sympathetic nervous system activity compatible with Kleitman's hypothesis.

Five adult male rhesus monkeys (*Macacca mulatta*) weighing 6.4–8.0 kg, were housed individually in primate restraining chairs within closed, sound-attenuating booths. Lights were turned on at 0700 and off at 1915. Behaviour was observed through a one-way mirror and feeding was carried out on a restricted 4-h schedule over 1300–1700. Water was available on an *ad libitum* basis. Blood was collected over a 4-h period at 15-min intervals through an intravenous or intra-arterial (monkey 3) catheter which extended outside the booth. Blood was collected (5 ml) in heparinised glass tubes and immediately spun at 2000*g* at 4 °C. Plasma was removed and frozen, and the red cells were resuspended in 5 ml 0.9% saline and re-infused immediately following the next blood drawing. Behavioural ratings were made just before each blood sampling. The state of arousal was noted according to whether the animal was asleep, tranquil, alert or excited. Blood samples were drawn over 0900–1300 in experiment 1 in all five animals and, 11–60 d after this, samples were drawn over 0900–1300 in monkeys 15 and 16, and over 1200–1600 in monkeys 3 and 12 in experiment 2. The latter two monkeys were fed on schedule at 1300 by opening the booth and manually placing the food within. Plasma samples