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Stimulus and Hormonal Determinants of Flehmen Behavior in Cats

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ABSTRACT

Felids are the main group of animals, other than ungulates, that display flehmen behavior during sociosexual interactions. In ungulates the behavior is evoked most readily by olfactory investigation of urine and vaginal secretions, and is believed to be involved in the transport of fluid-borne chemical stimuli, such as sex pheromones, from the oral cavity to the vomeronasal organ. In this study of cats flehmen was virtually always preceded by nasooral contact with the stimulus material, supporting the notion that flehmen in this species is also involved in the transport of fluid-borne stimuli. As in ungulates, flehmen in cats during heterosexual encounters was found to be displayed by males only. However, the sexual dimorphism was situation specific. In exploring a urine-marked room without another cat present, females also performed flehmen, albeit less frequently than males, and when urine was applied to the nasooral surface, flehmen was evoked equally reliably in females and males. Administration of testosterone propionate to spayed female cats paired with estrogen-treated females markedly increased their tendency to genitally inspect the female partner and subsequently perform flehmen. Thus the sexually dimorphic attributes of flehmen behavior are not only stimulus dependent, but also influenced by concurrent hormone stimulation.

Flehmen is a prominent aspect of sociosexual behavior in a number of mammalian species. The behavior is most readily identifiable in ungulates where the animal curls the upper lip, extends the head in a stereotyped posture, and holds it in this manner for several seconds. The behavior is highly sexually dimorphic in ungulate mating encounters in that it is frequently displayed by males both prior to and subsequent to copulation but virtually never displayed by females (Hart, 1983; Ladewig, Price, and Hart, 1980). Male ungulates typically perform flehmen after they have investigated vaginal secretions or recently voided urine of females. Although the behavior has been observed in certain bats, viverrids, and hyenas (Estes, 1972), as well as marsupials (Gaughwin, 1979), felids are the main group other than ungulates that display this behavior. In cats the behavior is sometimes referred to as the gape response because instead of the lip being curled upwards as in ungulates, the upper lip is elevated and the mouth is held slightly open for several seconds (Fig. 1).

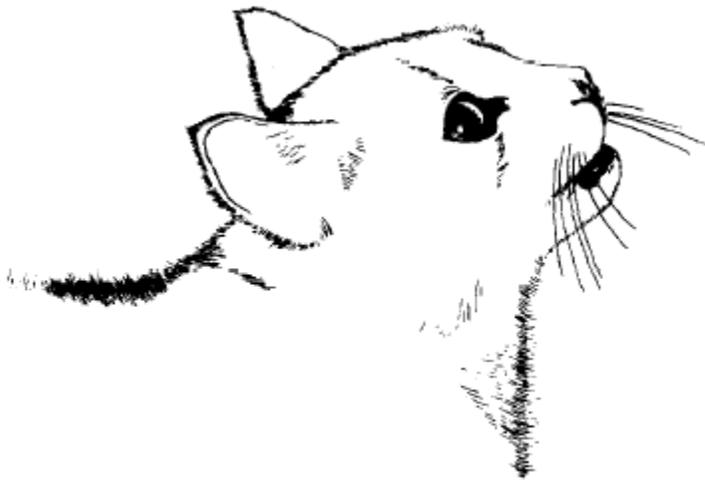


FIG. 1 Typical facial grimace and posture of a cat performing flehmen. The posture and grimace are usually held in this manner for 5–10 sec.

It has been hypothesized for some time that flehmen behavior is involved in the transport of fluid-borne materials from the oral cavity to the vomeronasal organ (VNO) for chemosensory analysis (Estes, 1972; Hart, 1983). There is now considerable direct evidence for this function (Ladewig and Hart, 1980; Melese d'Hospital and Hart, 1985). In felids, as in ungulates, a nasopalatine (incisive) duct runs from the crypts of an incisive papilla just behind the upper incisors to the VNO. The anatomy of the VNO system in cats and the autonomic control of a vasomotor pumping mechanism which presumably pulls material into the VNO during flehmen has been described (Eccles, 1982). Thus there is good reason to believe that in cats the flehmen response is also involved in the transport of fluid material from the oral cavity to the VNO.

One purpose of the present study was to examine some stimulus parameters regarding flehmen behavior in cats, particularly as they relate

to sexually dimorphic aspects of the behavior. Verbeme (1970) has noted that the behavior is primarily a male response that occurs during genital investigation of females by males.

The second purpose was to explore hormonal determinants of the behavior. In sheep, at least, manipulation of the perinatal hormone environment alters the display of flehmen along the lines of other male sex-typical behavior (Clarke, 1977) and castration of adult male goats reduces the display of the behavior as it does male copulatory attempts (Hart and Jones, 1975). However, there are some indications of differences in the neural control of flehmen and other male sexual behaviors in that in male goats medial preoptic-hypothalamic lesions which impair male copulatory responses (as in all male mammals studied) do not reduce the frequency of display of flehmen in sexual encounters (Hart, 1986). Thus, one might expect some differences between hormonal control of flehmen versus other aspects of male sexual behavior. The question chosen for exploration here was if flehmen behavior in female cats could be potentiated during sexual encounters by administration of testosterone.

EXPERIMENT 1

In this experiment some basic parameters of flehmen in cats were examined with regard to sexual dimorphism of the behavior and the degree to which nasooral contact with the material is made prior to the display of flehmen. Both male and female cats were observed in two situations. One situation was during a series of mating tests with gonadally intact or castrated, testosterone-treated males, paired with sexually receptive females. The second situation was during a structured test in which the same cats were placed individually in a test chamber, the walls of which had been marked in two places with cat urine.

Methods

Subjects. The male subjects were 10 adult cats that were gonadally intact, or castrated and maintained on replacement testosterone with silastic subcutaneous implants containing crystalline testosterone in amounts found previously sufficient to maintain sexual behavior (Hart and Leedy, 1983). Female subjects were 11 spayed females given two injections of 0.2 mg estradiol cypionate (ECP), 48 hr apart (sufficient to induce sexual receptivity).

Flehmen during mating tests. In each test both the male and female were subjects and data for both subjects were recorded simultaneously. Subjects were given a mean of nine tests (range 6-10). The tests were conducted in an insulated 2 x 2m test chamber with a one-way glass window for viewing. An exhaust fan provided a sound-masking noise. The duration of each test was 15 min and, in addition to the occurrence of mating, the display of flehmen by either the male or female to the anogenital area of the other cat was recorded. To make certain that the flehmen data were taken from sexually active animals, only those tests were used in which copulations occurred.

Flehmen to cat urine on chamber walls. The same subjects and test chamber were used as in tests for flehmen during mating behavior. Because of the angle of view, it was not always possible to see if nasooral contact was made, so these instances were disregarded in tabulation of data on nasooral contact. Each male or female subject was placed in the chamber to which 1.5 ml of cat urine had been applied by syringe to each of 2 walls about 0.3 m from floor level. This is the approximate height of urine deposition when a cat sprays urine. Urine was collected from donors by manual bladder expression and was frozen until needed. Each subject was given two tests, each 10 min in duration, conducted at 7- to 10-day intervals. The first test was with urine from spayed female cats and the second with urine from a castrated male maintained on replacement testosterone (see above). The behavioral measures taken were number of flehmen responses, whether or not flehmen was preceded by olfactory investigation, and whether or not nasooral contact with the urine spots occurred during the olfactory investigation. Data were also tabulated as to whether the flehmen responses were directed towards the (1) male urine, (2) the female urine, and (3) the unidentified stimuli of the test chamber.

Results and Discussion

The performance of flehmen was clearly sexually dimorphic during sexual encounters between males and females inasmuch as 8 of the 10 males, but none of the 11 females, were observed to display the behavior to their sexual partner at least once in mating tests ($P < 0.01$; Table 1). Males in which the behavior was observed at least once performed flehmen about one time per mating test.

Sexual dimorphism in flehmen performance was not so pronounced when subjects were allowed to explore the test chamber with no other cat present inasmuch as 90% of males and 64% of females performed flehmen at least once. The difference between number of males and females performing flehmen at least once did not reach significance ($P > 0.1$, Fisher exact probability test). However, performing males exhibited a frequency of flehmen that was three times that of performing females. This was a significant difference ($P < 0.05$; Table 1).

When flehmen toward areas of unidentified stimuli was disregarded, males performed flehmen about equally to urine of females and males, whereas females performed flehmen more often when the urine was from males. The behavior was too infrequent to examine the significance of this preference, however (Table 1).

TABLE 1
Performance of Flehmen by Male and Female Cats during Tests of Mating Behavior
and to Urine Applied to Walls of Test Chamber

Performance of flehmen		
To sexual partner during mating tests		
	Males (<i>N</i> = 10)	Females (<i>N</i> = 11)
No. of subjects performing	8 (80%)*	0 (0%)
Mean no. responses per test for subjects performing	1.1**	0
To urine-marked walls in test chamber		
	Males (<i>N</i> = 10)	Females (<i>N</i> = 11)
No. of subjects performing	9 (90%)	7 (64%)
Mean no. flehmen responses		
To female urine	1.3	0.1
To male urine	1.4	0.6
To all stimuli	3.3**	1.1
Mean no. olfactory investigations	4.7	2.2
Olfactory investigations with contact	(98%)	(50%)
Flehmen responses with contact	(100%)	(100%)

* Differs significantly from females ($P < 0.01$, Fisher exact probability test, one-tailed).

** Differs significantly from females ($P < 0.05$, Mann-Whitney *U* test, one-tailed).

Since the cats' approaches to a flat wall gave the best view of contact with the stimulus material, observations of single subjects in the test chamber were used to address the issue of whether nasooral contact with the stimulus preceded flehmen. Accordingly, in the test chamber, males were seen to make nasal or nasooral contact with the surface when investigating urine 98% of the time and females made such contact only half of the time. However, when flehmen occurred, it was, in both sexes, always preceded by nasooral contact.

EXPERIMENT 2

The results of Experiment 1 suggested that the sexually dimorphic character of flehmen lies on a continuum. Although females did not exhibit flehmen to sexual partners, they often performed flehmen when allowed to explore urine marks when alone in the test chamber. It was also observed that nasooral contact with the stimulus always preceded flehmen. In this second experiment, the behavioral continuum between males and females was pursued further by observing responses of male and female subjects when nasooral contact with urine was experimentally induced. To accomplish this, a small sponge soaked in urine was applied to the subject's nose.

Methods

Subjects. Ten gonadally intact male and 10 gonadally intact female cats served as subjects. These subjects lived in pens of about 15 cats and were from a breeding colony maintained on an artificial daylight cycle. Females were not in estrus when tested.

Tests for elicitation of flehmen. A subject was removed from the colony pen and placed in an individual cage for about 2 min. It was then gently restrained while a 1 x 2 cm sponge applicator, soaked with cat urine, was pressed gently against its nose. Some urine undoubtedly entered into the nasal cavity. Latency to flehmen and duration of flehmen were recorded. If no flehmen was displayed within 2 min, the test was terminated. For the first set of tests urine obtained by direct bladder tap from a gonadally intact male was used. For the second test, 7 days later, urine obtained in a similar manner from a young (6 months) nonestrous female was used.

Results and Discussion

Flehmen was evoked from most males and females (70-90%) by nasooral application of either male or female urine (Table 2). As shown in Table 2, measures of percentage of animals responding, latency to flehmen and duration of flehmen did not differ between the two sexes. Thus, in contrast to the occurrence of sexual dimorphism in the display of flehmen in mating tests and exploration of the test chamber with no other cat present, there was no such dimorphism when urine was directly applied to the surface of the nose.

TABLE 2
Behavioral Measures of Flehmen in Male and Female Cats following Nasooral Application of Male and Female Urine

Subjects	N	% Subjects performing flehmen		Mean (\pm SEM) latency (sec) to flehmen*		Mean (\pm SEM) duration (sec) of flehmen*	
		Male urine	Female urine	Male urine	Female urine	Male urine	Female urine
Males	10	80	80	4.3 \pm 0.4	4.3 \pm 0.3	11.2 \pm 2.0	9.9 \pm 1.2
Females	10	90	70	3.5 \pm 0.4	3.3 \pm 0.4	14.2 \pm 1.5	9.1 \pm 1.7

* For responders only.

EXPERIMENT 3

The finding of Experiment 2 that flehmen could be as readily evoked from female as male cats with the appropriate stimulus presentation prompted an examination of whether testosterone treatment of females would induce them to display flehmen when tested (as males) in sexual encounters with estrogen-treated females. After it was evident that testosterone

propionate (TP) injections markedly increased the tendency of females to genitally inspect and flehmen to estrogen-treated females, the question arose as to whether this increase was due to TP treatment or to a greater opportunity for the females to investigate their sexual partner. The latter possibility arose because in normal male-female sexual encounters the sexual prowess of the male often seems to preclude an opportunity for the female to investigate and flehmen to him. Two control groups were used to address this possibility. One group was spayed female cats given estrogen (ECP) treatment and paired with gonadally intact, but sexually inactive, males, and the second group was spayed female cats given no hormonal treatment and paired with estrogen-treated females.

Methods

For the first set of tests 15 spayed adult female cats were initially treated with subcutaneous injections of TP, 5 mg/kg every second day. During this time they were maintained in individual cages. Starting after 14 days of TP treatment they were given two tests of 15 min with spayed female cats in which estrus had been induced by two injections of ECP as in Experiment 1. The occurrence of genital investigation and flehmen were recorded on two tests given at 3- to 4-day intervals. Three weeks after the termination of these tests and TP injections, the same subjects were administered 2 injections of 0.2 mg ECP 48 hr apart and given two tests, at 3- to 7-day intervals with a sexually inactive but gonadally intact male beginning 48 hr after the second injection. Observations on genital investigation of the male by the female subjects and flehmen to any part of his body were recorded. The data for the above tests had been collected (but not published) as part of another study.

For the third set of tests, eight different spayed female cats that had received no hormonal treatment for at least 3 weeks were tested with a stimulus female that had been given injections of ECP as in the above procedure. Observations of performance of genital investigation and flehmen to the stimulus females were recorded.

Results and Discussion

In Table 3 are presented the results of the three sets of tests. Treatment of females with TP was the only procedure that brought out genital inspection and performance of flehmen to a sexual partner. The differences between TP-treated females and the same females given estrogen but tested with a sexually inactive male, or the females given no hormone but tested with estrogen-treated females, were significant ($P < 0.05$, Table 3). Thus, it appears as though testosterone treatment increases a female's predisposition toward genital investigation of her partner (female) and the occurrence of flehmen results as a consequence of this investigation.

TABLE 3
Percentage of Female Cats Displaying Genital Inspection and Flehmen toward a Sexual Partner after Different Hormonal Treatments

Subjects	<i>N</i>	Genital investigation	Genital investigation with flehmen
TP treatment: tested with females	15	80	53
ECP treatment: tested with sexually inactive male	15	0*	0*
No hormone: tested with females	8	0**	0**

* Differs significantly from TP treatment ($P < 0.05$ sign test, one-tailed for related samples).

** Differs significantly from TP treatment ($P < 0.05$ Fisher exact probability, one-tailed).

GENERAL DISCUSSION

The results reveal some major similarities between cats and ungulates in the stimulus control of flehmen. These experiments on cats also suggest some new perspectives on flehmen behavior and VNO function that might be explored in some ungulate species. As in goats (Ladewig et al., 1980), cats generally make nasooral contact with the stimulus source before performing flehmen. This observation is consistent with the notion that flehmen is involved in the transport of fluid-borne stimuli from the oral cavity to the VNO.

In the context of sexual encounters between male and female cats, performance of flehmen to a sexual partner is highly sexually dimorphic; females virtually never perform flehmen. This is exactly what is observed in ungulates (Hart, 1983). However, in cats the dimorphism was found to be linked to the type of stimulus. When no other cat was present, 64% of females displayed flehmen in the test chamber to urine marks, albeit at a significantly lower frequency than the 90% of males that performed flehmen to the urine marks. Thus, the sexual dimorphism in flehmen behavior is reduced when solitary male or female cats explore urine marks. The dimorphism disappeared entirely when the urine stimulus was applied directly to the nasooral surface. It appears as though the sexual dimorphism in the expression of flehmen in normal cats reflects a differential predisposition of males and females to approach and make nasooral contact with the stimulus material, rather than a differential level of flehmen probability in response to presence of urine stimuli. Experiment 3 documented that testosterone treatment of females markedly increased genital inspection and the display of flehmen in females to a sexual partner during mating tests, a situation in which they virtually never perform flehmen without testosterone treatment. Thus, the sexually dimorphic attributes of flehmen behavior, while stimulus-dependent, are under hormonal control in the most sexually dimorphic situation. The degree of sexual dimorphism of flehmen as a function of manner of

stimulus presentation and the potentiation of genital investigation with flehmen by testosterone treatment may well occur in ungulates, but has not been systematically explored.

It would appear as though flehmen behavior is mediated by a somewhat different neuronal system than that which governs male sexual behavior. This notion is supported by the observation that male cats receiving medial preoptic-anterior hypothalamic lesions prepubertally, and which have marked impairment of male copulatory behavior, have a sparing of flehmen behavior (Leedy and Hart, 1986). Similarly in adult male goats, lesions of the same forebrain area, which markedly reduce ejaculatory performance, do not have any influence on the display of flehmen behavior (Hart, 1986).

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