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Access of Urinary Nonvolatiles to the Mammalian Vomeronasal Organ

Abstract. *Guinea pigs were allowed to investigate urine that contained rhodamine, a nonvolatile fluorescent dye. Guinea pigs given free access to dyed urine exhibited fluorescence in their vomeronasal and septal organs but not on their olfactory epithelium. Fluorescence was not seen when unadulterated urine was presented. Thus compounds of low volatility, which do not reach the olfactory epithelium, may stimulate the vomeronasal system and provide information that is normally not provided by gustation or olfaction.*

Peripheral chemoreception has traditionally been thought to consist of two systems, gustation and olfaction. In air-breathing organisms, these two systems have often been distinguished by the method of stimulus access; that is, by "direct contact between the stimulus source and the receptor sheet (gustation) or migration of the molecules over distance from the stimulus source to the receptor sheet (olfaction)" (*1*). Until recently (*2*), one of the receptor organs in the mammalian nose, the vomeronasal organ (or Jacobson's organ), has been considered a redundant olfactory organ. However, the vomernasal organ may be part of a unique chemosensory system (*3*). The olfactory and vomeronasal systems exhibit a substantial degree of anatomical independence in both the nasal cavity (*3*, *4*) and the central nervous system (*2*, *5*). Although there has been much speculation about the possible functions of the vomeronasal sensory epithelium (*3*), the compounds that normally stimulate this olfactory-like epithelium (*6*) remain unknown (*7*, *8*).

Recent studies indicate that substances that communicate social and sexual information often appear to be of low volatility (*9*, *10*). Consequently, the sensory structure responsible for the detection of these substances is called into question. In this report, we present evidence that the vomeronasal receptor sheet may be stimulated by liquid-borne compounds of low volatility—specifically, substances in conspecific urine that are transported to the organ in a liquid medium. Thus the vomeronasal system is an anatomically distinct system that possesses characteristics of both gustation and olfaction.

When presented with conspecific urine, guinea pigs approach the stimulus and spend considerable time bobbing their heads and investigating, sniffing, and licking it. The amount of time devoted to these activities is influenced by the sex of the urine donor and of the recipient (*11*). We hypothesized that urine is transported to the vomeronasal organ for sensory processing during these investigatory behaviors. To test this hypothesis, we used urine mixed with rhodamine (B or 6G) hydrochloride, a nonvolatile fluorescent dye (*12*). Eighteen healthy guinea pigs were offered conspecific urine with or without the dye (*13*). Thirteen additional guinea pigs were subjected to other experimental manipulations (see Table 1). After a brief exposure to the urine (*14*), each guinea pig was killed with an overdose of pentobarbital. The bilateral vomeronasal organs and, in some cases, septal organs ($N = 4$) (*15*), the olfactory organs ($N = 7$), the external nares ($N = 7$), and the nasopalatine ducts ($N = 8$) were removed, sectioned (*16*), and examined by epifluorescence microscopy. We used two filter sets that are normally employed to visualize fluorescein isothiocyanate (FITC) and rhodamine fluorescence (*17*). No attempt was made to measure the degree of fluorescence.

When viewed with the rhodamine filters, fluorescence was seen in the vomeronasal organ of every guinea pig that had been exposed to rhodamine-dyed urine while awake (Fig. 1, left). Rhodamine fluorescence was absent from the vomeronasal organs of guinea pigs that had been exposed to stimuli that lacked the dye (Fig. 1, right). Also, no rhodamine was seen on the olfactory epithelium of any guinea pig, regardless of exposure condition (*18*). In the cases sampled, rhodamine fluorescence was observed on the septal organ of each guinea pig offered dyed urine. To control for the possibility of passive diffusion of rhodamine into the vomeronasal organ during dissection or sectioning, rhoda-

Table 1. Presence or absence of rhodamine fluorescence in the vomeronasal organ after various experimental treatments.

Treatment	Sample	Rhodamine fluorescence
Removal from home cage	2 males	No
Contact with female urine	3 males	No
Contact with female urine mixed with rhodamine (*20*)	7 males	Yes, bilaterally
Contact with male urine mixed with rhodamine (*20*)	8 females	Yes, bilaterally*
Contact with female urine mixed with rhodamine after unilateral nasal closure†	4 males	No, ipsilateral to closure; yes, contralateral to closure
Contact with female urine mixed with rhodamine while wearing Plexiglas nasal tube (*19*)	2 males	No, ipsilateral to tube; yes, contralateral to tube
Contact with drinking water mixed with rhodamine	2 males	Yes in one male (bilaterally); no in the other
Water mixed with rhodamine flushed through mouth of anesthetized animal	1 female, 1 male	No
Male urine mixed with rhodamine flushed through mouth of anesthetized animal	1 female	No

*One female did not contact the urine. Rhodamine was not seen in the mouth or on the rhinarium, nor was rhodamine fluorescence seen in the vomeronasal organ. †The right naris was closed for two males; the left for the other two.

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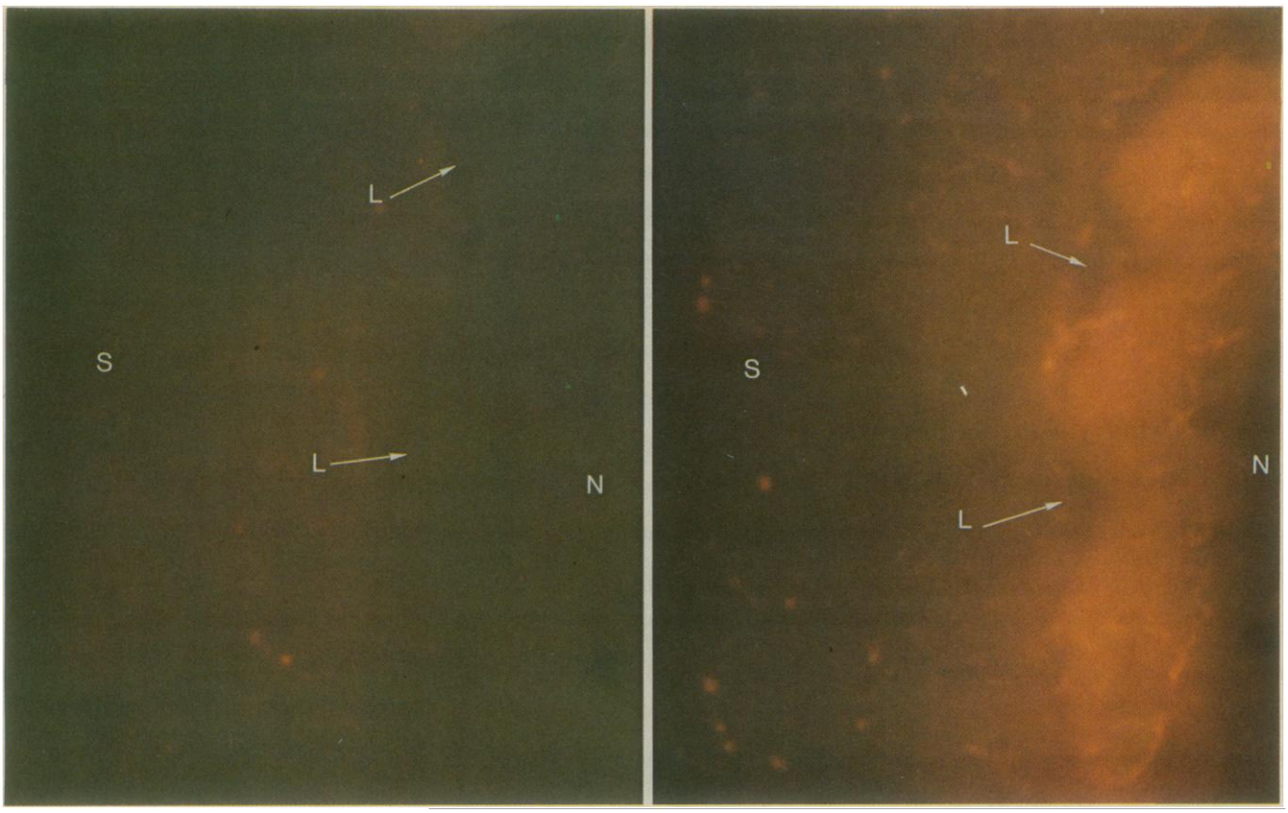

Fig. 1. Fluorescence photomicrographs of a portion of the lumen and surrounding epithelium of the vomeronasal organ (*17*). (Left) Fluorescence in a male that had contacted rhodamine-dyed female urine. Note the presence of rhodamine, especially in the lateral, nonsensory epithelium. (Right) A similar section from a male that was not exposed to a rhodamine-dyed stimulus. Note the absence of fluorescence. The apparent affinity of rhodamine for the nonsensory epithelium was observed in all cases; the reason for this affinity is unclear. Rhodamine is a lipophilic dye, and differences in lipid content may exist between the sensory and nonsensory epithelia. The route taken by the rhodamine to the epithelium was not through the vasculature lateral to the nonsensory epithelium because (i) no vessels contained rhodamine fluorescence and (ii) the superficial rather than deep epithelial tissues contained most of the dye. *S*, sensory epithelium; *L*, lumen; *N*, nonsensory epithelium (×110).

mine-containing fluids were flushed through the mouths of anesthetized guinea pigs (see Table 1). With one exception (in which a surgical instrument penetrated the caudal portion of the organ unilaterally), rhodamine fluorescence was not seen in the vomeronasal organ.

We also explored the route by which rhodamine entered the vomeronasal organ. When the dye was present in the vomeronasal organ, it was also observed in the nasopalatine ducts (possibly as a result of licking the stimulus), on the septal organ, and on the ventral surface of the ipsilateral external naris. Closure of an external naris with cyanoacrylate glue prior to the presentation of dyed urine resulted in unilateral fluorescence: rhodamine was present in the vomeronasal organ ipsilateral to the open naris (see Table 1). Also, rhodamine fluorescence was not observed in the vomeronasal organ ipsilateral to a naris that was fitted with a Plexiglas tube (*19*) designed to allow a patent airway while diverting airflow. The tube also prevented the animal from licking its naris and inhibited contact between naris and urine, thus disrupting normal functioning of the rhinarium during urine investigation. These results (see Table 1) suggest that some nonvolatiles reach the vomeronasal organ of the guinea pig through the external nares. Stimuli may be deposited on the rhinarium after direct contact between the nose and the stimulus or after the animal licks the stimulus and then the rhinarium.

The nasopalatine duct also appears to influence the filling of the vomeronasal organ. In several experiments, partial disruption of the unilateral nasopalatine duct appeared to diminish the amount of rhodamine fluorescence in the ipsilateral vomeronasal organ (*20*). Perhaps patency of the nasopalatine ducts facilitates flow of substances through the nares and thence to the vomeronasal opening. The distinctive head bobbing of guinea pigs during chemosensory investigation may also serve to move substances to the vomeronasal opening (*11*, *21*).

Chemical studies have indicated that the substances in guinea pig urine that transmit species and sexual identity are molecules of low volatility (*9*). The data reported here provide evidence for a route by which such molecules could reach a receptor sheet.

Direct contact with conspecific chemical substances is common in many other organisms (*22*). Such contact may serve to initiate transport of biologically significant substances of low volatility to the vomeronasal organ (*23*). However, since dye fluorescence was seen in the vomeronasal organ of at least one guinea pig that had consumed dyed drinking water (Table 1), nonurinary compounds may reach the vomeronasal epithelium. Perhaps the sensory functions of the vomeronasal organ encompass more than the mediation of sexual and social information (*24*).

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13. The concentration of rhodamine was approximately 1 percent in 2 to 4 ml of urine. Portions of urine (approximately 1 ml) were drawn from each urine-containing vial.
14. Male guinea pigs were allowed access to female urine on three glass plates (7.5 by 15.0 cm) for 4 minutes per plate, with a 30-second pause between plates. For females, the male urine was placed on the rim of their food bowl or presented on cotton swabs saturated with the dyed urine. The females were allowed access to the stimulus for less than 5 minutes. We have no evidence that the different modes of stimulus presentation influenced the results.
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17. Broadband excitation of about 450 to 490 nm, with emission wavelength $\gtrsim$ 515 nm, was obtained with a Leitz Orthoplan fitted with an I2 filter combination (FITC). A Leitz M2 filter combination yielded a maximum excitation wavelength of about 546 nm, with a half-bandwidth of 14 nm and emission at $\gtrsim$ 580 nm (rhodamine). At other times, a Zeiss Standard was fitted with filters BP 450-490, FT 510, LP 520 for broadband excitation of 450 to 490 nm and emission at $\gtrsim$ 520 nm (FITC); or with BP 546/12, FT 580, LP 590 for a maximum excitation wavelength of about 546 nm, with half-bandwidth of 12 nm and emission at $\gtrsim$ 590 nm. When viewed with the FITC filters, the vomeronasal organ of each subject, regardless of experimental manipulation, exhibited considerable autofluorescence. Without preliminary treatment of the tissue, the medial sensory epithelium contained a scattered array of colored points not seen in the lateral respiratory-like (nonsensory) epithelium. Also, a band of elastin lateral to the respiratory-like epithelium exhibited an intense green fluorescence. The source of this autofluorescence is not known.
18. With the rhodamine filters, autofluorescence was seen in the deep cartilagenous and bony tissues of the olfactory organ (but not the neuroepithelium) of every guinea pig surveyed, regardless of the experimental group assignment.
19. A Plexiglas tube (outside diameter, 10.0 mm; inside diameter, 6.5 mm) was cut to an approximate length of 7.0 mm and sealed at one end with a flat sheet of Plexiglas. A 1.5 by 2.5 mm hole was punched in the tube near the closed end. The open end of the tube was affixed to the rhinarium with glue.
20. Prior to the presentation of dyed urine, six females and one male had undergone one of a variety of surgical attempts to block the nasopalatine duct unilaterally. None of the attempts were a complete success. Patency, albeit slight in some cases, was observed in each instance. However, in the male and in four of the females, the gross amount of rhodamine fluorescence was less in the vomeronasal organ ipsilateral to the damaged duct than in the organ contralateral to the damage.
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Filter-Mediated Color Vision with One Visual Pigment

Abstract. *The compound eye of the grasshopper* Phlaeoba *has alternating bands that appear clear or brown. Electroretinograms recorded from the individual bands have different action spectra: The spectrum of the clear band peaks at 525 nanometers and that of the brown band at 545 nanometers. Spectrally selective whole-eye adaptation with light of either long or short wavelength yields identical action spectra. This evidence suggests that this eye has only one visual pigment, whose spectrum is altered in the brown bands by a screening pigment. In behavioral tests of spontaneous choices between stimuli that appear green to the normal human and those that appear red, the green stimuli are preferred even when the relative intensity is varied by 0.9 log units around the equal-brightness level (determined by the electroretinogram). When some red light is mixed with the green light, the preference for the mixture is less than for the green light alone, even though the mixture is more intense. True color vision therefore seems to exist. Painting the bands shows that behavioral color vision requires the presence of both types. These data suggest that* Phlaeoba *has true color vision mediated by one visual pigment and suitable optical filters.*

True color vision requires at least two receptor types with different action spectra. The receptor action spectra usually differ because they contain different visual pigments (*1*). However, a receptor's spectrum is also modified by ocular screening materials that selectively filter incident light (*2*) and thereby modify color vision. The colored oil droplets found in some birds and the corneal filters found in some flies have therefore been thought to play a role in color vision (*3*). But direct experimental evidence is lacking, and there has been some controversy (*4*) about the significance of these filters. Nevertheless, it is possible for true color vision to exist, even in organisms that have only a single visual pigment, if suitable optical filters exist. We now report data from the grasshopper *Phlaeoba* sp. (native to Hong Kong; common name, "kan chow" or "choose nest"); these data provide what is, to our knowledge, the first evidence for filter-mediated true color vision. Three lines of evidence lead to this conclusion. (i) Physiological studies show that the eye of *Phlaeoba* is organized for filter-mediated color vision; (ii) behavioral studies show that *Phlaeoba* exhibits true color vision; and (iii) this behavior depends on the integrity of the optical filter system.

The compound eye is strikingly divided into brown and clear bands (*5*) by screening pigments (Fig. 1). Such screens modify the action spectra of other insect and invertebrate eyes (*2, 6, 7*); their functions are to shield the eye from strong light and to improve visual acuity. Electroretinographic (ERG) action

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