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REVIEW

STRUCTURE AND FUNCTION OF THE VOMERONASAL ORGAN

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Summary

Many animals use their vomeronasal organs to gain direct and specific contact with chemical cues released by congeners and in biological fluids. These cues provide information about the physiological status of the emitter and facilitate or regulate social interactions such as sexual relationships. The present review gives a short description of the discovery of the vomeronasal organ and the pivotal findings of Jacobson. The distribution of the organ and its anatomy in some vertebrates are described. The mechanisms for stimulus entry and egress are discussed, and the findings that led to the appreciation of the vomeronasal organ in mammals as a main chemosensory organ for pheromones mediating reproductive status and inducing sexual behaviour are reported. The anatomical, biochemical and functional properties of the receptor neurones are described.

Key words: vomeronasal organ, mammal, amphibian, olfaction, receptor neurone, physiology, pheromone.

Introduction

In 1813, the Danish anatomist Ludvig Jacobson (1783–1843) described an organ in the nose of mammals that had not been noticed previously. He meticulously observed the many glands of the organ, the dual innervation and the blood supply (Jacobson, 1813). A translation of his original article has been made (Jacobson, 1999). The organ he discovered was renamed organon vomeronasale (Jacobsoni) by *Der Anatomische Gesellschaft* in 1895. Jacobson assumed that the organ was secretory in nature but suspected that it could also be a sensory organ. Today, this organ in mammals is recognised as a chemosensory organ for pheromones.

The original description made by Jacobson (1813, p. 214–216) reads: ‘The organ, according to the investigations I have conducted so far, exists in all mammals. It is located in the foremost part of the nasal cavity, in close contact with the nasal cartilage (septum), on the above-mentioned palatal elongations of the intermaxillary bone. It is so carefully concealed that it has avoided discovery by the very discerning eyes of several anatomists, so that nothing except the opening of its secretory duct has been discovered and described by our great Steno (Niels Steensen 1638–1686). The thing that in particular has hidden this organ from the eyes of the observers is a cartilaginous capsule that surrounds the parts comprising the organ, namely a secretory apparatus, a receptacle, and an exit duct. All these parts are usually enclosed in this capsule, although in some animals one finds that the secretory apparatus extends outside it and lies unattached on the nasal septum or on the side of the nose. This secretory organ is even more remarkable because it has its own large and specific nerves.’ Jacobson considered the vomeronasal organ to be vestigial in adult humans.

Structure of the vomeronasal organ

Fig. 1 is a reproduction of Jacobson’s drawing of the head of a deer (*Cervus* sp.), demonstrating the position of the organ and the nerves running along the nasal septum leading from the vomeronasal organ to the olfactory bulb. He also showed that, in cross section, the elongated organ has a crescent-shaped lumen. It was only with the emergence of new histological techniques in the nineteenth century that it was assumed that the vomeronasal organ was a sensory organ. The chemosensory function of the organ was made evident when Retzius (1894) demonstrated the similarity in morphology between the receptor neurones of the olfactory and the vomeronasal organs in a Golgi preparation of the olfactory placode of a snake embryo (Fig. 6). The primary receptor neurones of the organ have axons that terminate in the vomeronasal bulb (accessory olfactory bulb). In view of the importance of the vomeronasal system among mammals, it seems that the term accessory olfactory bulb is misleading. Thus, we suggest the term vomeronasal bulb.

The sensory cells are situated on the medial concave surface of the vomeronasal cavity (Figs 2, 3). The density of receptor neurones is approximately $92\times10^3\,mm^{-2}$ (Hedgewig, 1980). In

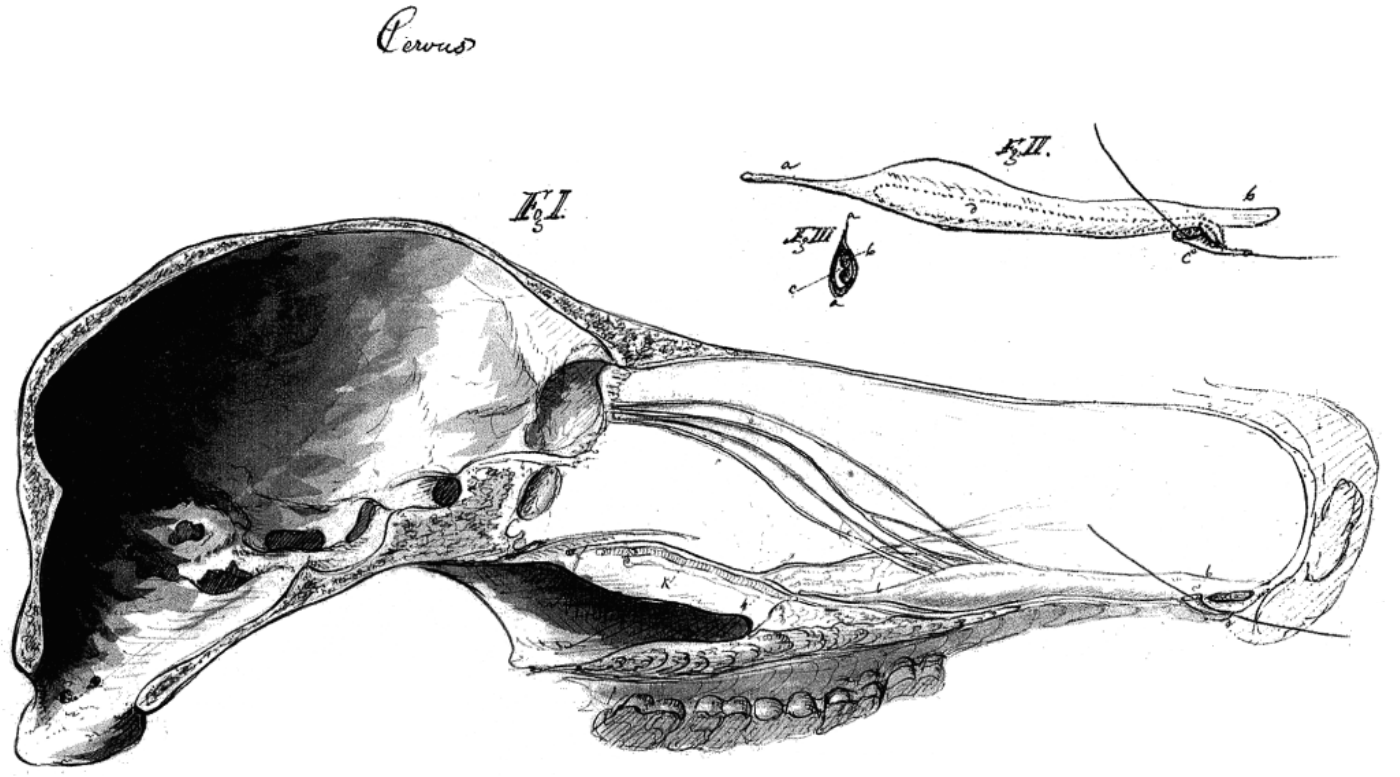


Fig. 1. Drawing of the medial aspect of a head of a deer, *Cervus* sp. (FgI). Note the nerves running from the vomeronasal organ to the olfactory bulb; the canalis incecivus is indicated by a straw. FgII is a drawing of an isolated organ; the lumen is outlined by a stippled line. FgIII is a cross section of the organ. The contrast of the drawing has been enhanced to facilitate reproduction. Figures are not to scale. From Jacobson's unpublished work at the Agricultural University in Copenhagen.

mice, the total number of receptor cells increases between 1 and 4 months of age, after which the number decreases (Wilson and Raisman, 1980).

The lateral convex surface of the organ is lined with non-sensory ciliated cells. The tissue beneath this part of the organ has a cavernous appearance and has been called a mushroom body, eminentia fungiformis (von Mihalkovics, 1899). The numerous glands found at the dorsal and ventral aspect of the lumen, with small secretory ducts ending in the lumen of the organ, were described by Jacobson (1813) and later by Vaccarezza *et al.* (1981).

Distribution of the vomeronasal organ

Mammals

Jacobson's (1813) original description was based mainly on domesticated animals (cat, cow, dog, goat, horse, pig and sheep), but he also described the organ in other mammals, such as tiger, camel, buffalo, deer and seal. The presence of this organ has been confirmed in most mammals (for reviews, see von Mihalkovics, 1899; Pearlman, 1934), and it is also present in marsupials (Wohrmann-Repenning, 1984). The vomeronasal organ is well developed in some primates, such as *Nycticebus tardigradus* (Lemuroidea) and the platyrrhine *Cebus capucinus*, but is reduced or absent in the catarrhine *Macaca mulatta* (Jordan, 1972; Stark, 1975).

Humans

The opinions concerning the presence and functioning of the vomeronasal organ in humans are controversial. The vomeronasal cavities appear early in human foetuses. At 12–23 weeks, the vomeronasal cavity is lined by a smooth pseudostratified epithelium with neurone-specific enolase-positive cells that look like olfactory receptors. At 36 weeks, the cavity is lined by a respiratory epithelium and there is no evidence of any receptor-like cells (Boehm and Gasser, 1993). As in other mammals, the vomeronasal system plays a role in the migration of luteinizing-hormone-releasing hormone (LHRH) neurones from the olfactory placode towards the brain during the development of the embryo (Kjaer and Fischer-Hansen, 1996; Schwanzel-Fukuda *et al.* 1996). Cells immunoreactive for LHRH are detected in the vomeronasal organ at 8–12 gestational weeks and later (at 12–19 weeks) along the nerve fascicles arising from the vomeronasal organ. No LHRH-positive cells can be seen close to the vomeronasal organ in foetuses older than 19 weeks (Kjaer and Hansen, 1996).

The vomeronasal organ appears rudimentary in new-born humans, as first pointed out by Kölliker (1877). It is located in the antero-inferior part of the nasal septum. Jordan (1973) failed to find sensory cells or nerve fibres in the ciliated stratified epithelium lining the vomeronasal duct. Histological examination of the nasal septum revealed the presence of vomeronasal cavities in approximately 70 % of adults (Johnson

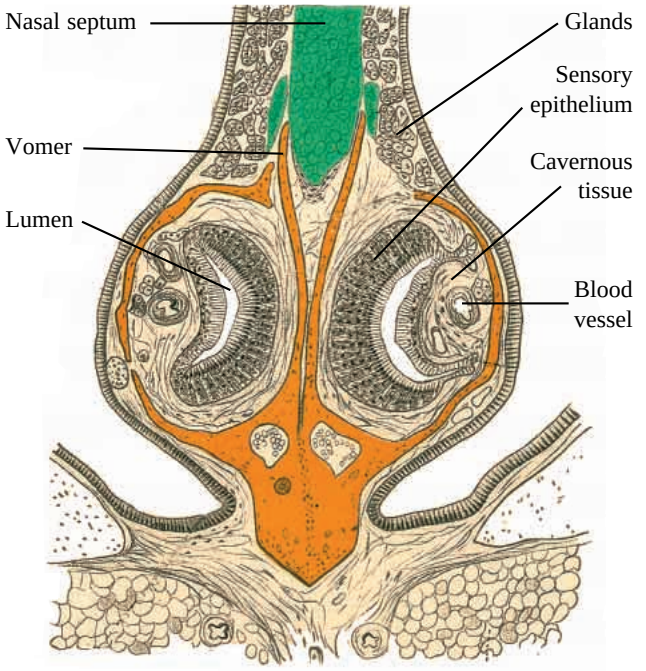


Fig. 2. Cross section of the ventral region of the nasal septum of a mouse, at the level of the middle of the vomeronasal organ. The organ is encapsulated in the vomer. The sensory epithelium is on the medial wall. Note the large blood vessels running in the mushroom body on the lateral side of the vomeronasal lumen. From von Mihalkovics (1899).

et al. 1985). In contrast to the situation in other mammals, the organ is not supported by a rigid tube of bone or cartilage. There is no erectile tissue around the cavities to draw in the stimulus (see below). It is therefore probable that stimuli enter the lumen of the organ only by passive diffusion against the flow of mucus secreted by glands. Attempts to characterize neurone-like cells in the adult vomeronasal epithelium using histochemistry have been inconclusive (Johnson *et al.* 1985), although neurone-like cells have been described in one study (Takami *et al.* 1993). The density of these putative sensory neurones did not exceed approximately four immunoreactive cells per 200 µm of vomeronasal luminal surface. Some authors claim that the vomeronasal organ is functional in man on the basis of the existence of slow changes in the surface potential of the vomeronasal epithelium in response to chemical stimulation (Berliner *et al.* 1996; Monti Bloch *et al.* 1994).

A recent study has shown that the timing of menstrual cycles is influenced by chemical stimuli from the armpits of females at different phases of the menstrual cycle (Stern and McClintock, 1998), but it has not been demonstrated that the vomeronasal organ is involved in this process.

Other vertebrates

It seems probable that the vomeronasal organ first evolved in amphibians (Eisthen, 1992). It is conspicuous in relation to the olfactory organ in frog tadpoles (Burton, 1990), but the olfactory organ expands considerably after metamorphosis while the vomeronasal organ retains its size (Fig. 5) and is found medially beneath the vestibule. In mammals, the sensory cells are found on the medial wall of the lumen, and the ciliated cells are located on the lateral wall. In contrast, the sensory neurones of the vomeronasal organ in the frog are mixed with ciliated sustentacular cells. A series of grooves and cavities connect the anterior naris to the vomeronasal organ proper in the frog and also to the posterior naris and the mouth. Water enters these grooves and is transported to the vomeronasal organ even when the anterior naris is closed (Døving *et al.* 1993).

The vomeronasal organ is absent in fishes and birds. The organ is not found in crocodiles and chameleons, but it is present and may be even larger than the olfactory organ in some other reptiles. The vomeronasal organ is, for example, well developed in snakes (Figs 4, 6). The lumen of the organ opens into the mouth. The split tongue of snakes and lizards does not enter the vomeronasal organ. The similarity in design to the vomeronasal organ in mammals is evident.

Function

The first experiments indicating that the vomeronasal organ played a role in reproduction in mammals were performed by Planel (1953). He showed that when this organ in guinea pigs was impaired the males failed to mount. Females with impaired organs did not show lordosis, lost interest in their partner and seldom became pregnant. Peripheral deafferentation of the vomeronasal system produces severe sexual behaviour deficits in both male and female hamsters (Powers and Winans, 1975; Winans and Powers, 1977). These authors found that the effects of removal or prevention of normal function of the vomeronasal organ were acute when the animal was sexually naive, but that if the animal had experienced a partner in sexual interplay, the effect of removal of the vomeronasal organ was

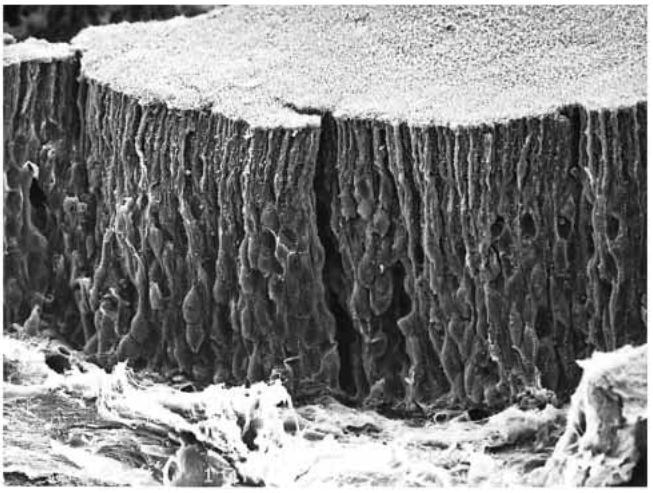


Fig. 3. Scanning electron micrograph of the sensory epithelium of the vomeronasal organ of rat. The specimen has been cracked to demonstrate the columnar structure of the epithelium. Epithelium depth about 120 µm. From Trotier *et al.* (1998).

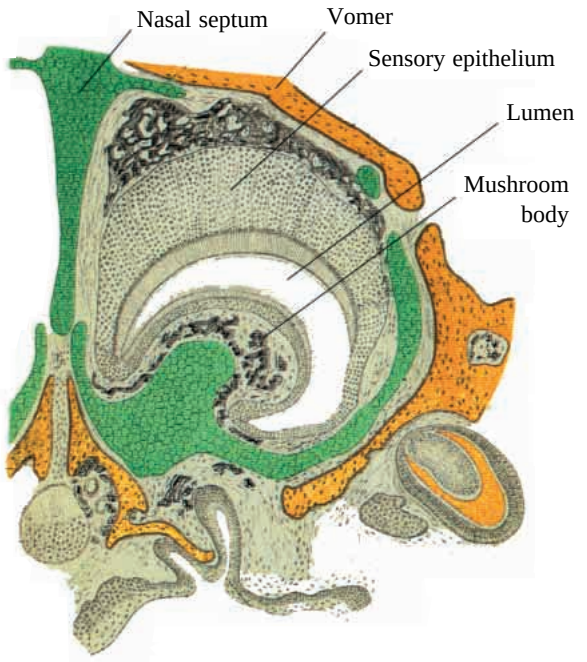


Fig. 4. Cross section of the head of a snake, *Coluber natrix*. The sensory epithelium of the vomeronasal organ is situated dorsally. The mushroom body is situated ventrally. Note the similarity to the arrangement of the mammalian vomeronasal organ. From von Mihalkovics (1899).

less dramatic. However, injection of LHRH (also called gonadotropin-releasing hormone, GnRH) into experimental animals lacking a vomeronasal organ re-established sexual behaviour. It seems that a reasonable chain of events is the following: stimulation of the vomeronasal organ is needed to release LHRH, which promotes an increase in the level of luteinizing hormone (LH), which in turn induces sexual behaviour (Clancy *et al.* 1988; Coquelin *et al.* 1984; Meredith and Fernandez-Fewell, 1994).

Bruce (1959) demonstrated in mice that fertilised eggs failed to implant if a strange male was exchanged for the mating male in the cage of a female within 4 days after copulation. This effect is mediated by chemical stimuli and is dependent upon a functional vomeronasal organ (Bellringer *et al.* 1980). The model to explain this phenomenon includes a memory of the odour of the mating male. It has been shown that memorization and recognition of the mating male takes place in the accessory olfactory bulb and that metabotropic glutamate receptors are involved in this process (Kaba *et al.* 1994). Subsequent stimulation with pheromones from a strange male enhances activity in the arcuate dopaminergic neurones, which release their transmitter into the portal plexus of the median eminence. Dopamine acts on the pituitary gland to suppress the release of prolactin. Prolactin is luteotropic, so the ovarian corpus luteum is no longer sustained, progesterone levels fall and oestrogen levels rise. It is the fall in the level of progesterone that allows the level of LHRH to increase, and this sustains follicular development and the rise in oestrogen levels (Brennan and Keverne, 1997; Keverne, 1982).

If a number of females rats are kept in small cages, they will stay in anoestrous. Exposing a crowded group of females to the smell of a male will induce oestrous. These effects are also dependent upon an intact vomeronasal organ (Johns, 1986; Johns *et al.* 1978).

All these results and those of many other experiments demonstrate that the vomeronasal organ is a sensory organ involved in reproduction by detecting pheromones. However, in some animals, such as snakes, it also mediates the trailing of prey and food detection (Halpern, 1987). The vomeronasal organ is not the sole organ used to detect pheromones, e.g. in pigs it is the olfactory organ that detects androstenone (Dorries *et al.* 1995). Since our inquiries about the function of the vomeronasal organ are still in their infancy, there are probably many more functional aspects to be uncovered. A number of review articles have dealt in great detail with the function of the vomeronasal organ in mammals and other vertebrates (Halpern, 1987; Meredith and Fernandez-Fewell, 1994; Schilling, 1987; Wysocki, 1979).

Behaviour related to stimulus access

Animals demonstrate different behaviour patterns that are adaptations to investigating odorant sources. Some of these behaviour patterns are certainly related to the investigation of the odorants, while others have to do with the entry of odorants into the vomeronasal organ. In frogs, water enters *via* the anterior naris and is transported by the ciliated lining of the epithelium to the vomeronasal organ without any obvious behavioural change (Døving *et al.* 1993). Salamanders display a nose-tapping behaviour that is presumably related to activation of the vomeronasal organ (Dawley and Bass, 1989), and lizards and snakes show a characteristic tongue-flicking behaviour (Halpern and Kubie, 1980) when actively searching for food and when aroused.

In many mammals, e.g. in the bull, the tongue plays a major part in directing stimuli to the vomeronasal organ (Jacobs *et al.* 1981). Dogs are frequently seen licking the urine deposits of congeners, especially those produced by bitches in heat. They also chatter their teeth in conjunction with this performance. In the grey short-tailed opossum, nuzzling behaviour is exhibited in response to markings of conspecific odours. Nuzzling consists of bouts of repeated forward rubbing motions with intermittent tapping with the ventral part of the snout over the odour source to moisten the source with naso-oral secretions (Poran *et al.* 1993). These authors also demonstrated that radioactive tracers were taken into the vomeronasal organ, but not into the olfactory organ, during this behaviour. Head-bobbing in guinea pigs, associated with the probing of congeners, has a characteristic frequency of 4 Hz (Beauchamp, 1973).

Flehmen behaviour

During sociosexual interactions, many mammals, especially felids and ungulates, display flehmen behaviour. Typically, the animals lift their head after contact with the odorant source,

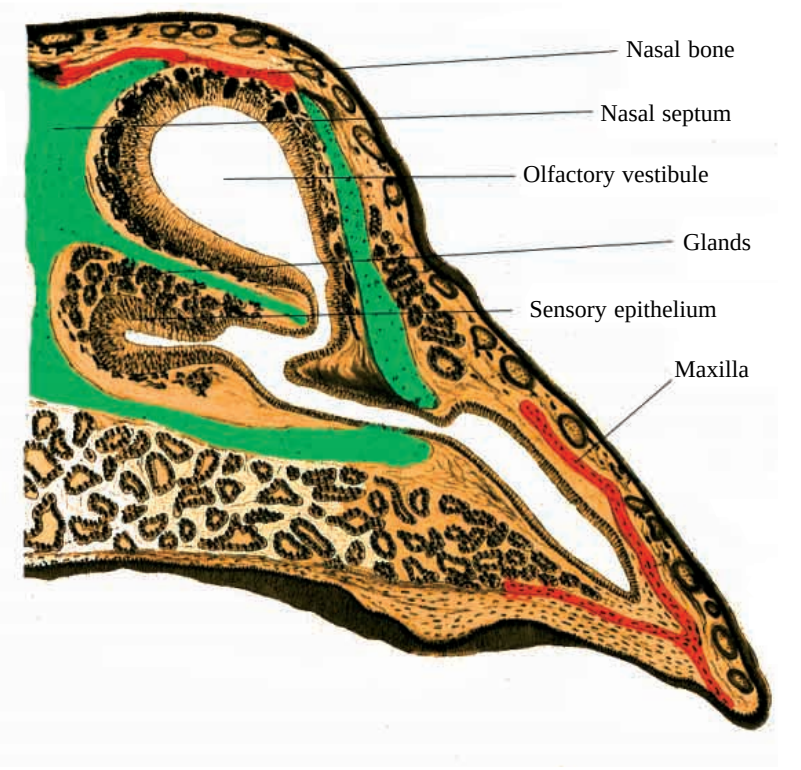


Fig. 5. Cross section of a head of the frog, *Hyla arborea*, made just posterior to the anterior naris. The vomeronasal organ is found beneath the olfactory vestibule, which lies anterior to the eminentia olfactoria of the main olfactory organ. A series of grooves runs from the anterior naris to the sensory epithelium of the vomeronasal organ. Water is transported along these grooves from the environment to the vomeronasal organ and out *via* the maxillary recess and the posterior naris. From von Mihalkovics (1899).

wrinkle their nose, lift their upper lip and stop breathing for a moment. In horses, flehmen behaviour is frequently associated with a neigh. In ungulates, this behaviour is evoked most readily by olfactory investigation of urine and vaginal secretions. In cats, flehmen behaviour is also preceded by naso-oral contact with the stimulus material. During heterosexual encounters in cats, flehmen behaviour is displayed by males only. However, females exploring a urine-marked room without another cat present also demonstrated flehmen behaviour. Thus, the sexual dimorphism is situation-specific (Hart and Leedy, 1987).

Detailed investigation has indicated that flehmen behaviour is associated with anatomical specialization. Thus, ruminants have an incisive papilla and incisive ducts located on the hard palate just behind the dental pad. Two species of alcelaphine antelopes, topi and Coke's hartebeest, lack the incisive papilla and incisive ducts constituting the oral connection to the vomeronasal organs. This distinctive anatomical feature is complemented in these species not only by a lack of flehmen behaviour but also by reduced chemosensory interest in female urine during sexual encounters. The common wildebeest, which is also an alcelaphine antelope, lacks the incisive papilla, but has small incisive ducts. Wildebeest males do perform flehmen behaviour when exposed to urine from females. However, during flehmen behaviour in the wildebeest, intermittent nostril licking apparently delivers the stimulus material to the vomeronasal organs *via* the nasal route, possibly compensating for reduced oral access. These observations on alcelaphine antelopes would appear to represent a unique feature among the world's ruminants (Hart *et al.* 1988).

Flehmen behaviour 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. The role of flehmen behaviour in facilitating the passage of non-volatile materials from the oral cavity to the vomeronasal organ has been studied using a tracer dye. The dye was applied to the oral cavity of six male goats in which flehmen behaviour was elicited by the presentation of female urine. Inspection of the vomeronasal organ after the animals had been killed revealed dye in the posterior part of the vomeronasal organ in most subjects that had performed flehmen behaviour but only in the anterior part of the vomeronasal organ of subjects that had not demonstrated flehmen behaviour (Ladewig and Hart, 1980).

Stimulus entry and egress

Blood supply

The vomeronasal organ contains cavernous tissue and is well vascularized. The sphenopalatine artery enters the nasal cavity in mammals and one of its branches, the septal artery, is the chief route of blood supply to the vomeronasal organ. One or two large veins run along the organ in the tissue forming the mushroom body. Between these are several smooth muscle cells radial to the lateral wall of the vomeronasal lumen (von Mihalkovics, 1899). Electron microscopic studies of the vomeronasal organs of rats, mice and rabbits show that several layers of smooth muscle encircle the wall of the venous sinus (Taniguchi and Mochizuki, 1983).

Innervation of the nasal mucosa

The autonomic innervation of the nasal mucosa and of the

vomeronasal organ is extensive and complex. Parasympathetic cholinergic nerve terminals from the sphenopalatine ganglion innervate blood vessels and exocrine glands. Sympathetic adrenergic nerve terminals from the superior cervical ganglion predominantly innervate blood vessels (Änggård *et al.* 1983). The erectile tissue of the nose has a dense adrenergic innervation (Änggård and Densert, 1974), and sympathetic stimulation causes a nasal vasoconstriction (Änggård and Edwall, 1974). The Vidian nerve (N. canalis pterygoidei) contains both postganglionic sympathetic nerves and preganglionic parasympathetic components subsequently relaying in the sphenopalatine ganglion. Electrical stimulation of the Vidian nerve induces vasodilatation and secretion in the nasal mucosa. Stimulation of the Vidian nerve after the parasympathetic pathways have been blocked induces vasoconstriction (Eccles and Wilson, 1974). The second branch of the trigeminal nerve also sends sensory fibres to the vomeronasal organ (Jacobson, 1813; Silverman and Kruger, 1989). These nerves contain substance P, and stimulation of the trigeminal branches causes vasodilatation in other types of tissue. The levels of biogenic amines and serotonin change in the vomeronasal organ of female mice upon stimulation with male urine (Zancanaro *et al.* 1997).

Pumping mechanisms

Broman (1920) was probably the first to assume that the vomeronasal organ was a *Wassergeruchsorgan* and that the fluids carrying the stimuli could be sucked into the lumen of the organ by a pumping mechanism. This process was

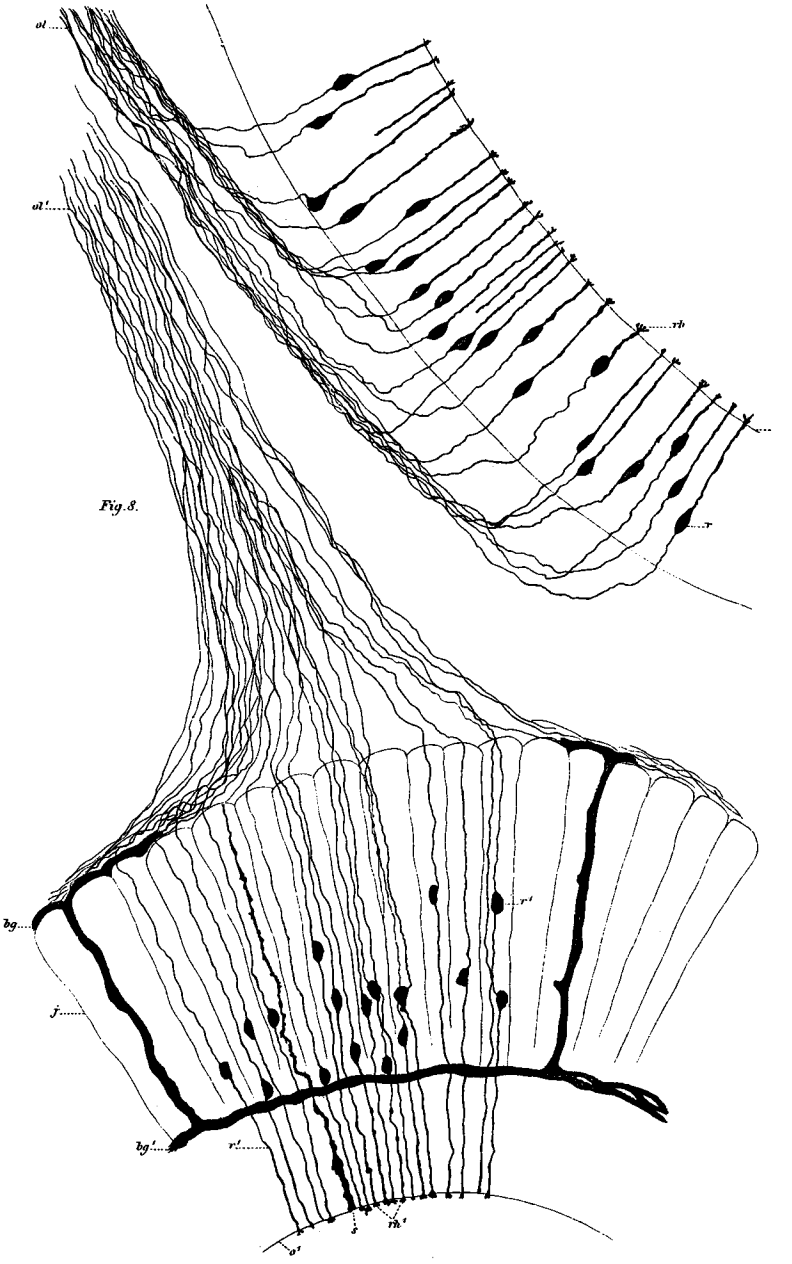
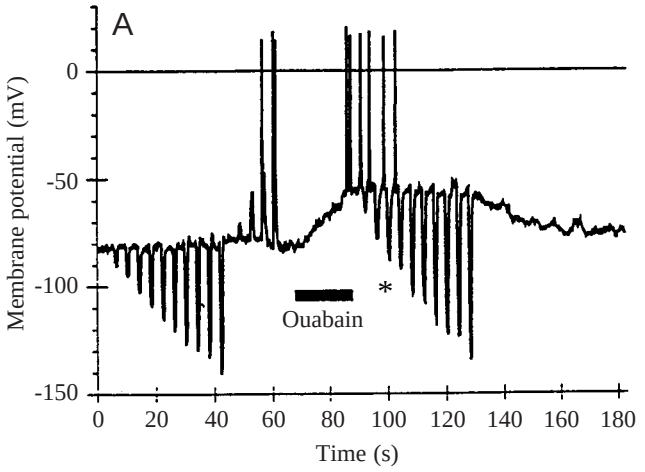


Fig. 6. Reproduction of Retzius' gravure of the olfactory placode from a snake embryo. The drawing demonstrates the similarity between the morphology of the sensory neurones of the olfactory epithelium (to the right) and those of the vomeronasal organ (lower part). From Retzius (1894).



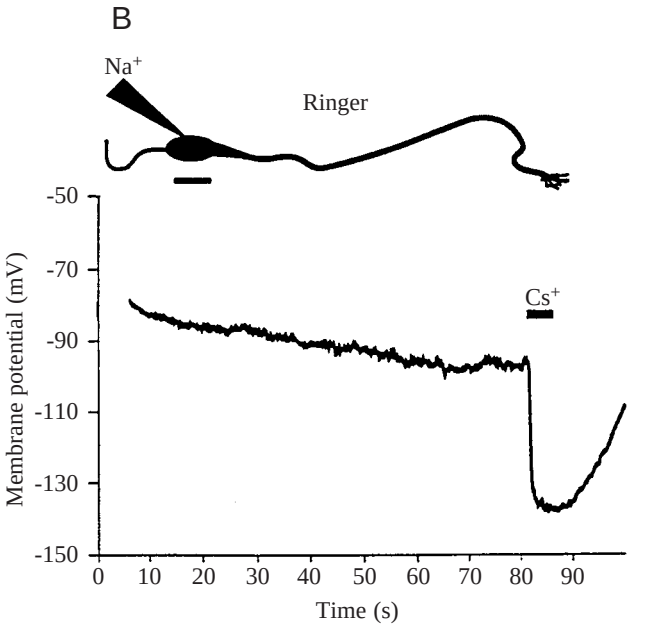


Fig. 7. The resting potential of frog vomeronasal receptor cells is not generated by passive diffusion of K^+ but by the hyperpolarizing current created by the Na^+/K^+-ATPase (Na^+ pump). (A) The resting potential recorded in standard ionic conditions was −88+20 mV (mean ± S.D., *N*=56). The addition of 50 µmol l^{-1} ouabain, a specific inhibitor of the Na^+/K^+-ATPase, depolarized the membrane by 25 mV, with no change in the membrane conductance, and triggered action potentials. The asterisk indicates that the membrane potential reached the original resting value in response to injection of a pulse of −3 pA, illustrating the high input resistance of these cells. (B) The polarization of the membrane was not abolished when internal K^+ was replaced by Na^+, illustrating that passive diffusion of K^+ did not contribute to the membrane potential. The addition of 5 mmol l^{-1} Cs^+ to the bath blocked the current I_h, which is steadily activated at the resting potential. Consequently, the Na^+ pump current polarized the membrane to approximately −140 mV. Other recordings indicated that the membrane was depolarized by decreasing the K^+ concentration in the bath, because the activity of the Na^+ pump current was decreased. From Trotier and Døving (1996*b*).

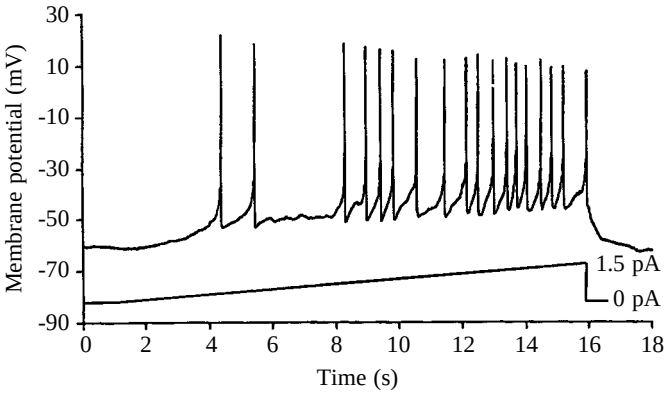


Fig. 8. Whole-cell recording from a vomeronasal receptor neurone from a hamster demonstrating the persistent discharge of action potentials in response to a slowly increasing depolarizing current (lower trace; 0.1 pA s^{-1}).

confirmed by a series of experiments by Meredith and co-workers (Meredith *et al.* 1980; Meredith and O'Connell, 1979) and Eccles (1982). These authors showed that stimulation of the cervical sympathetic nerve caused fluid to be sucked into the organ, and intravenous administration of adrenaline caused fluid to be sucked into the vomeronasal organ in the cat (Eccles, 1982).

Stimulation of the Vidian nerve or ganglion sphenopalatinum caused an increased secretion of fluid from the organ. Secretion from the vomeronasal organ started approximately 45 s after stimulation of the Vidian nerve and continued for 1–2 min after the stimulus had been switched off. Atropine blocked secretion induced by stimulation of the Vidian nerve (Eccles, 1982).

It is evident that filling of the cavernous tissue of the vomeronasal organ with blood will cause an efflux of fluid from the organ, especially if this action is concomitant with increased glandular secretion. How the suction of fluid into the lumen of the vomeronasal organ is accomplished is less obvious, although a vasoconstriction of the walls of the large vein of the organ will increase the volume of the lumen and create suction. The veins of the mushroom body exhibit thicker walls and a wider lumen than the medial veins and are, therefore, better suited for the pumping mechanism. Connective tissue surrounding the organ aids the pumping mechanism of the veins (Hedgewig, 1980).

Interruption of the efferent nerves controlling the pump results in behavioural deficits similar to those produced by interruption of the afferent nerves carrying information from the vomeronasal organ to the brain. Pump activation is thus a prerequisite for normal vomeronasal stimulation in animals (Meredith *et al.* 1980). Both the suction of fluid into the organ and the egress of fluid from the organ are active processes.

Stimulus composition

The precise identification of the ligands that are released in biological fluids and are detected by vomeronasal receptor

neurones is still incomplete. Aphrodisin, a protein secreted in vaginal discharge (Henzel *et al.* 1988; Kruhoffer *et al.* 1997; Singer *et al.* 1986), elicits copulatory behaviour by male hamsters (Singer *et al.* 1986), and its effect depends on the integrity of the vomeronasal system. In mice, the relevant information is introduced by a mixture of small ligands [2-(sec-butyl)thiazoline and 2,3-dehydro-exo-brevicomin] in association with major urinary proteins (MUPs) (Guo *et al.* 1997). The small ligands may activate vomeronasal receptor neurones (Moss *et al.* 1997; Zhou and Moss, 1997).

Other experiments indicate that, after treatment that probably releases their naturally bound small molecules, MUPs (or even a small sequence from these proteins) may trigger the pheromonal effect. Vandenbergh (1969) found that substances emanating from the male accelerated puberty in mice. It was later demonstrated that male urine contains the active molecules (Vandenbergh, 1975; Vandenbergh *et al.* 1976). Mucignat Caretta *et al.* (1995) suggested that MUPs without bound ligands induce the pheromonal effect, the increase in the mass of the uterus in juvenile mice (Table 1). The urine from a juvenile male, alone or together with natural ligands, including 2-(sec-butyl)thiazoline and 2,3-dehydro-exo-brevicomin, had no pheromonal effect on the uterus. A hexapeptide (N-Glu-Glu-Ala-Arg-Ser-Met) similar to the N-terminal sequence of MUP (N-Glu-Glu-Ala-Ser-Ser-Thr) induced the acceleration of puberty. The authors suggest that vomeronasal receptor neurones recognize the N-terminal sequence of MUP.

The role of the volatile components is not understood. It seems that, in the rodent environment, urine deposits can be signalled by volatile substances. Thus, the presence of pheromones is flagged by the entrapped odorants, attracting congeners *via* the olfactory system and inducing a particular behaviour pattern that introduces the pheromones into the vomeronasal organ.

Morphology of the receptor neurones

Differentiation and renewal process

Vomeronasal receptor neurones are bipolar with a dendrite extending to the surface of the epithelium (Figs 3, 6). At the tip of the dendrite is a terminal knob or vesicle covered, in most species, by a tuft of numerous microvilli. A long axon emerges from the basal pole of the cell body and terminates in the vomeronasal bulb.

The differentiation and renewal of the primary sensory neurones have been studied in detail (Wang and Halpern, 1980, 1982*a*,*b*, 1988). In snakes, new chemosensory cells grow out from the basal cells (stem cells) at the basal lamina. Some of them have cell bodies close to the basal lamina and are consequently equipped with a long dendrite. Others migrate further towards the surface and have a short dendrite (Wang and Halpern, 1980). In adult mice, a population of dividing cells has been described at the boundary between the neurosensory epithelium and the ciliated respiratory epithelium (Barber and Raisman, 1978*a*), and replacement of receptor neurones has been observed after section of the vomeronasal nerves (Barber and Raisman, 1978*b*). It has been estimated that the survival time of the neurosensory cells of the vomeronasal epithelium is 2–3 months (Wilson and Raisman, 1980; for a review, see Ichikawa, 1996).

Table 1. *Acceleration of puberty*

Treatment	Number of animals	Uterine mass	Statistical values
Water	22	25.5±6.3	$F(1,42)=7.639$
Adult male urine	22	48.9±12.7	$P=0.0084$
Juvenile male urine	12	24.2±3.1	$F(1,22)=5.044$
Juvenile male urine + MUP	12	42.0±8.7	$P=0.0351$
Juvenile male urine + pyrazine	15	26.4±4.4	$F(1,29)=5.597$
Juvenile male urine + MUP + pyrazine	16	52.6±10.2	$P=0.0249$
Juvenile male urine	15	19.7±1.9	$F(1,28)=10.122$
Juvenile male urine + empty MUP	15	41.7±7.6	$P=0.0036$
Juvenile male urine + CH_2Cl_2	16	22.7±2.5	$F(1,29)=0.526$
Juvenile male urine + ligands + CH_2Cl_2	15	25.7±3.1	$P=0.474$
Juvenile male urine + CH_2Cl_2	16	20.5±2.6	$F(1,29)=11.459$
Adult male urine + CH_2Cl_2	15	46.9±8.3	$P=0.0021$
Juvenile male urine	18	24.5±4.3	$F(1,34)=4.194$
Juvenile male urine + hexapeptide	18	44.4±6.9	$P<0.05$

Values are means ± S.E.M.

The experiments were performed on mice exposed on days 30, 31 and 32 to the solutions shown to the left in the table. The uterine mass was determined on day 33 (from Mucignat-Caretta *et al.* 1995).

MUP, major urinary protein.

The hexapeptide (N-Glu-Glu-Ala-Arg-Ser-Met) is similar to the N-terminal sequence of MUP.

Endocytosis

Endocytotic structures are present between the microvilli of mouse vomeronasal receptor cells (coated invaginations and vesicles) and within the dendrites (small uncoated vesicles and tubules, multivesicular endosomes) (Adams, 1992; Bannister and Dodson, 1992). The base of the dendrite is generally enlarged and contains much agranular endoplasmic reticulum and many dense lysosomes. These observations indicate the presence of an endocytotic system able to take into the receptor neurones exogenous materials from the overlying mucus. The contents then move in vesicles and tubules to the lysosomal apparatus of the perikaryon (Bannister and Dodson, 1992). The nature of the materials is not known. The endocytotic process is reasonably fast and, in the olfactory epithelium, tracer experiments have shown that after 10 min the tracers begin to enter vesicles in receptor endings at the cell surface (Bannister and Dodson, 1992).

The acquisition of stimuli into the lumen of the vomeronasal organ is seemingly an efficient and fast mechanism. In contrast, the removal (egress) of the ligands from the lumen must be a slow process. It could be that, after binding to the receptors, the stimuli are internalized by endocytosis. Thus, the endocytotic processes could aid in reducing the background levels of ligands.

Subclasses of vomeronasal receptor neurones

Several studies have demonstrated using different methods that the chemosensory neurones of the vomeronasal organ consist of two populations of cells. A hint to this distinction into two populations is that the vomeronasal bulb in the grey short-tailed opossum can be distinguished into a rostral and a caudal portion. The first distinction of subclasses of neuroreceptors in the vomeronasal organ was observed by Mori *et al.* (1987) using monoclonal antibodies generated against a homogenate of the axons (vomeronasal nerve fibres). A monoclonal antibody (R4B12), which has been shown to bind selectively to, and thus to identify, a subclass of olfactory nerve fibres also labels a subclass of vomeronasal nerve fibres in the rabbit. The R4B12-positive subclass of vomeronasal nerve fibres projects to the glomeruli in the rostrolateral part of the vomeronasal bulb. Another monoclonal antibody (R5A10) recognises a complementary subclass of vomeronasal nerve fibre projecting to the glomeruli in the caudomedial part of the accessory bulb. The olfactory cell adhesion molecule OCAM (R4B12 antigen) is expressed in vomeronasal sensory neurones with short dendrites, and these cells project to the rostral part of the vomeronasal bulb in mice (von Campenhausen *et al.* 1997). Monoclonal antibodies CC1 and CC6, which react to glycolipids, label receptor neurones connected to the rostral and caudal vomeronasal bulb, respectively (Schwarting and Crandall, 1991; Schwarting *et al.* 1994).

The lectin *Vicia villosa* agglutin (VVA), the NADPH-diaphorase reaction and antibodies to olfactory marker protein (OMP) all stain the rostral part of the vomeronasal bulb more intensely than the caudal part (Shapiro *et al.* 1995; Shnayder *et al.* 1993, 1994).

G-proteins

The distribution of G-proteins has been studied in the vomeronasal epithelium and in the vomeronasal bulb of the opossum (Halpern *et al.* 1995) and mouse (Berghard and Buck, 1996) using immunoreactivity. In the vomeronasal epithelium, $G_{\alpha o}$ antibodies stained neurones with long dendrites and with cell bodies found near the basal part of the epithelium. These neurones project to the caudal part of the vomeronasal bulb. $G_{\alpha i2}$ antibodies stained neurones with short dendrites located in the middle part of the epithelium. These neurones project to the rostral part of the vomeronasal bulb. Both types of G-protein are enriched in the microvilli of the receptor neurones, suggesting that they may be involved in the sensory transduction mechanisms.

Putative receptor proteins

Dulac and Axel (1995) isolated a family of approximately 30 coding sequences for putative receptor proteins (V1Rs) expressed in vomeronasal receptor cells. Sequence analysis of these genes indicates that they make up a separate family of proteins with seven transmembrane domains that are unrelated to the putative receptors expressed in the olfactory epithelium (Buck and Axel, 1991). The olfactory and vomeronasal organs utilise different molecular entities to translate the chemical signal into electrical activity, despite their common origin in the olfactory placode (Retzius, 1894).

In 1997, three independent groups discovered that there was a novel family of approximately 100 genes that coded for a class of receptor molecule with seven transmembrane domains (V2Rs) (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; for a review, see Bargmann, 1997). These molecules have a sequence similarity to Ca^{2+}-sensing and metabotropic glutamate receptors, having large hydrophilic sequences on both sides of the seven membrane-spanning domains (Masu *et al.* 1991).

To recapitulate, the V1Rs are expressed in receptor cells with short dendrites. These cells also express $G_{\alpha i2}$, OCAM and CC1 and project to the rostral part of the vomeronasal bulb. The V2Rs are expressed in sensory neurones with long dendrites that also express $G_{\alpha o}$ and CC6 and project to the caudal part of the vomeronasal bulb. There must be several functional implications of the existence of two segregated pathways for detecting stimuli. Small ligands and large binding proteins could be detected by different types of receptor neurone, expressing receptor proteins of different classes.

The probable differences in the projections of the mitral cells from the rostral and caudal vomeronasal bulb to brain centres have not yet been studied in detail. Information about these pathways could promote our understanding of the functional role of the dichotomy of receptor neurones.

Physiological properties of receptor neurones

Transduction current

Since molecular biological studies indicate that putative receptor proteins are linked to G-proteins, an intracellular

second messenger should be involved in the activation the transduction current. Vomeronasal receptor cells do not express cyclic-AMP-gated channels, which are found in the ciliary membrane of olfactory receptor cells: no depolarizing inward current could be observed during injection of cyclic AMP (Liman and Corey, 1996; Trotier *et al.* 1994). Inositol 1,4,5-trisphosphate (InsP_3) is apparently involved in the transduction process because stimulation with aphrodisin leads to an increase in the level of InsP_3 in hamster vomeronasal organs (Kroner *et al.* 1996). Incubation of microvillous membranes from the vomeronasal organ from prepubertal female pigs with boar seminal fluid or urine results in an increase in the production of InsP_3. The dose–response curve for InsP_3 production was found to be biphasic, with a GTP-dependent component at low stimulus concentrations and a nonspecific increase in InsP_3 level at higher stimulus concentrations. The GTP-dependent stimulation was mimicked by GTPγS and blocked by GDPβS (Wekesa and Anholt, 1997).

Urine-evoked responses of rat vomeronasal receptor neurones are blocked by specific antagonists of the InsP_3 cascade (Inamura *et al.* 1997*a*). Inamura *et al.* (1997*b*) indicated that the injection of 100 μmol l^{-1} InsP_3 into rat vomeronasal receptor neurones increased the membrane conductance in approximately half the cells. The exact effect of InsP_3 is not known. It could release Ca^{2+} from intracellular stores or have a direct effect on membrane channels.

Membrane potential, Na^+/K^+-ATPase and the current I_h

In the frog, the resting potential of most vomeronasal receptor cells was found to be more negative than the equilibrium potential for K^+ (Trotier and Døving, 1996*a*). Substitution of intracellular K^+ by Na^+ had little effect on membrane polarization, and changing the external K^+ concentration had effects opposite to those expected assuming a resting K^+ permeability of the membrane. The input resistance of these cells is very high, and the polarization of the membrane is due to the outward current generated by the Na^+/K^+-ATPase (Trotier and Døving, 1996*a*). The application of ouabain, a specific Na^+/K^+-ATPase inhibitor, depolarizes the cells (Fig. 7A). The level of polarization is limited by the activation of the current I_h, a cationic non-specific membrane current steadily activated at membrane potentials more negative than approximately –80 mV (Trotier and Døving, 1996*b*). When this current is blocked by bath application of Cs^+, the cells hyperpolarize as the current caused by the Na^+/K^+-ATPase prevails (Fig. 7B).

Firing properties

Various Na^+, K^+ and Ca^{2+} conductances, activated by membrane depolarization, have been characterized in vomeronasal receptor cells from many species (Inamura *et al.* 1997*b*; Liman and Corey, 1996; Trotier *et al.* 1998, 1993) and contribute to the voltage responses to depolarizing currents.

The membrane resistance of approximately –70 mV is high and, consequently, action potentials are easily elicited in these neurones in response to depolarizing current pulses in the picoampere range (Inamura *et al.* 1997*b*; Liman and Corey, 1996; Trotier *et al.* 1993, 1998). We recently observed in hamsters that this high sensitivity is maintained even if the membrane is depolarized using a very slow ramp of depolarizing current (e.g. 0.1 pA s^{-1} as illustrated in Fig. 8) instead of a current pulse. This observation indicates that vomeronasal receptor cells are able to convert small and slowly increasing depolarizing currents into long-lasting repetitive firing of action potentials. This property could be important if long-lasting receptor cell spiking were involved in the activation of the vomeronasal pathway. For example, it has been shown that the long-lasting activation of the vomeronasal organ of the female mouse by male pheromones, in conjunction with mating, leads to a modification of the synaptic circuitry in the vomeronasal bulb (Brennan and Keverne, 1997). It is not known whether the different types of vomeronasal receptor cells, bearing a long or a short dendrite and connected to different areas in the vomeronasal bulb, code the sensory information in the same way.

Conclusions and perspectives

The discovery made by Jacobson nearly 200 years ago was a remarkable achievement. The organ he described was named the vomeronasal organ, but the true nature and function of this organ remained a secret for 150 years. It appears that the vomeronasal organ is pivotal for mediating pheromonal information in mammals and is a major sensory organ in amphibians and some reptiles. Stimulation of the organ has an astonishing and profound influence on the endocrine system and adjusts the reproductive function and behaviour in a most amazing way. When the vomeronasal organ fails to function in naive mammals, mating behaviour is perturbed, indicating the important role of this organ in some stages of reproduction.

We are still far from understanding all the functions of the vomeronasal organ. For example, the mechanism of stimulus intake is still unclear and many of the cellular mechanisms in the sensory neurones remain to be discovered. The surprising and unusual way of generating the membrane potential is certainly related to the function of the organ, but we are still unable to explain why these neurones function in this way. The transduction mechanisms are not fully understood. Many specific studies remain to be conducted to identify the molecules or mixture of molecules that are relevant stimuli for the vomeronasal receptor cells. Finally, we await an explanation of the functional and anatomical implications of the dichotomy of the sensory neurones of the vomeronasal organ.

The vomeronasal organ is encapsulated like Pandora's box, and attempts to reveal its secrets may be frustrating, but also give hope and pleasure.

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