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The Vomeronasal Organ of the Male Ferret

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Abstract

The vomeronasal organ (VNO) is known to play a major role in sexual behavior in many mammals. This study is the first report that the adult male ferret has a VNO, which is considerably smaller and morphologically different from the usually crescent-shaped epithelium in several mammalian species, particularly rodents. There were no differences in the size or structure of the ferret VNO between the mating season in spring and the sexually quiescent season in autumn, although plasma testosterone, testis size and brain size are dramatically increased in spring and behavior changes significantly. The histological data suggest that the VNO might be not as important a structure in male ferret sexual behavior as in rodents.

Introduction

The vomeronasal organ (VNO) plays a major role in the perception of stimuli related to social and/or reproductive behavior in many species of vertebrates (for review see Estes, 1972; Wysocki, 1979, 1989; Meredith, 1983; Halpern, 1987). The morphology of the VNO has been investigated in some detail in rodents (Gratiolet, 1845; Addison and Rademaker, 1927; Vaccarezza *et al.*, 1981), dogs (Klein, 1881; Read, 1908; Ramser, 1935; Adams and Wiekamp, 1984), cats (Harwey, 1882; Schwink, 1888; Herzfeld, 1889; Broom, 1900; Read, 1908; Seifert, 1971; Wöhrmann-Repenning, 1989; Wöhrmann-Repenning and Ciba, 1989; for reviews see Zuckerkandl, 1910; Dawley, 1998) and minks (Salazar *et al.*, 1994).

The VNO, also known as Jacobson's organ because it was first described by Jacobson in 1811, is a paired, non-connected, unbranched tube-shaped structure located in the anterior region of the nose within the ventral end of the nasal septum. It is generally enclosed in a bony or cartilaginous capsule. In carnivores it opens at its anterior end into the incisive or nasopalatine duct (Starck, 1978), thus connecting with the oral and nasal cavity. At its posterior extent it ends blindly (Adams and Wiekamp, 1984). The VNO consists of a sensory and a non-sensory epithelium lining the medial and lateral side of the tube respectively. The sensory epithelium in mammals is usually crescent-shaped (Addison and Rademaker, 1927; Adams and Wiekamp, 1984; for review see Farbman, 1992) and is an arrangement of a pseudostratified epithelium, containing the microvilli-bearing sensory neurons (for review see Menco, 1997), basal cells and supporting cells with nuclei in the apical compartment (Vaccarezza *et al.*, 1981; Garrosa and Coca, 1991). The axons of the sensory neurons collect in the lamina propria beneath the epithelium into a small number of nerve bundles which ascend in the nasal septum and pierce the cribriform plate, then project along the medial aspect of the main olfactory bulbs to their target, the accessory olfactory bulbs (McCotter, 1912).

The European polecat (*Mustela putorius* L.) is a mammalian carnivore which lives a solitary life except during the annual spring mating season (Marshall, 1905). In the polecat and its domesticated form, the European ferret (*Mustela putorius f. furo* L.), the reproductive cycle is reflected in the seasonal variations of many anatomical, behavioral and physiological parameters (Kästner and Apfelbach, 1987; Weiler, 1992). In spring the testes in males become significantly enlarged and produce spermatozoa (Neal *et al.*, 1977). Male ferrets have to find their mating partners and—as nocturnal animals—are known to depend predominantly on olfactory cues (Wheeler, 1978; Apfelbach, 1986). The main olfactory system is very well developed and has been intensively studied (Apfelbach and Weiler, 1985; Apfelbach, 1986; Rehn *et al.*, 1986; Weiler, 1986, 1993; Voigt, 1987; Apfelbach and Schmidt, 1989; Schmidt, 1989). In contrast, the accessory olfactory system has not been intensively investigated in this species. Although a very small accessory olfactory bulb was reported to be present (Lockard, 1982, 1985; Fox, 1988), a VNO has not, to our knowledge, been described in the adult male ferret.

The purpose of the present study therefore was to determine whether a VNO is present in adult male ferrets and, if

so, whether it undergoes morphological changes between sexually active and quiescent seasons. In addition, the serum testosterone was measured during the two seasons to verify that the males were primed for sexual activity. The histological studies revealed that a rudimentary VNO is present and does not undergo morphological changes seasonally although there is a dramatic increase in plasma testosterone in the mating season.

Materials and methods

Animals

All ferrets investigated in the present study were taken from the breeding colony of the University of Tübingen (Germany). Animals were kept under natural light and temperature conditions, and cared for by specially trained animal-care personnel.

'Adult' refers in this study to animals 1–3 years of age. Body weight changes seasonally (Weiler, 1992).

Testosterone quantification

Blood collected from the tail vein was analyzed to determine the plasma concentration of testosterone by radioimmunoassay. In the spring 54 ferrets were used, and in the autumn, 38. The antiserum reacted 100% with testosterone whereas the cross-reactivity with dihydrotestosterone (DHT) was 54%. In order to obtain sufficient sensitivity, it was necessary to remove the sex steroid-binding proteins from the samples. Ether was used for extraction of steroids. After addition of the tracer (^{3}H-labeled DHT) and reaction with the antibody until equilibrium was achieved, free and bound androgens were separated by adsorption of the free steroids by dextran-coated charcoal followed by centrifugation. Chemicals were obtained from Mallinckrodt Diagnostica, Dietzenbach, Germany.

Histological preparation and analysis

For the histological studies adult male ferrets were used in spring (high testosterone level) and autumn (low testosterone level); in addition newborns were investigated for developmental aspects (see Table 1). Ferrets were injected i.p. with a lethal dose of Nembutal®. Under deep anesthesia, ferrets were perfused intracardially with a physiological saline solution at room temperature to clear the vessels of blood, followed by a fixative solution containing 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (Karnovsky, 1965). Heads were skinned and postfixed overnight. As much as possible of the skull bones was removed before decalcifying the noses in 5% EDTA in 0.1 M Sørensens phosphate buffer for several days. Snouts were embedded in Paraplast® and sectioned at 10 µm in a frontal plane from the tip of the snout to the posterior end of the olfactory epithelium. Each section was preserved; every 10th section was placed on silane-treated slides and stained with hematoxylin and eosin. For the newborns every section was stained. Sections were examined with a light-microscope at a magnification of ×400 and measurements performed with a calibrated ocular micrometer.

The sections were screened to note two landmarks: (i) the anterior end of the VNO where it opens into the nasopalatine duct; and (ii) the posterior end where the VNO ends in a one-cell-deep epithelium as the duct of glands. The sensory epithelium was defined by the clear distinction of a supporting cell nuclear layer from a neuronal nuclear layer and the number of sections containing sensory epithelium was used to measure its anterior–posterior extent.

The thickness of the sensory and non-sensory epithelia, from the basement membrane to the luminal surface, was measured in each section half way along their length. In addition, in sections with sensory epithelium, the thickness occupied by neuronal nuclei was measured and the percentage of the whole thickness was calculated.

The length of lateral (non-sensory) and medial (sensory) epithelium in its dorso-ventral extent was measured in each section. Because the epithelial thickness around the tube in a section was nearly constant from dorsal to ventral, the area was calculated by multiplying the epithelial thickness by the length. The volume of the VNO was then calculated by multiplying the extent by the area. The volumes of the sensory and non-sensory epithelium were calculated separately. In addition the volume of the lumen was determined by measuring the length (a) and width (b) of the oval-shaped space and calculating the area in each section using the formula $\pi/4ab$ (Segovia *et al.*, 1984).

For the newborns we used the *analySIS* morphometric system connected to a CCD camera on a Zeiss microscope at a magnification at ×400; this allowed us to measure area and thickness semiautomatically.

For statistical analysis to determine whether there was a significant difference between the values of spring and autumn we used the Mann–Whitney U test (Lienert, 1973).

OMP immunohistochemistry

Paraplast® sections were deparaffinized, rehydrated in phosphate-buffered saline (PBS) and incubated sequentially at 37°C in a blocking solution of normal rabbit serum and PBS 1:1 (30 min), the antibody against OMP (goat α-OMP, dilution 1:500, 60 min; a gift from Dr F. Margolis, University of Maryland, Baltimore, MD), the Vectastain Kit (Vector Labs, Burlingame, CA) containing biotinylated rabbit anti-goat antibodies (30 min) and the avidin–biotin complex (ABC) reagent (45 min). Each antibody treatment was followed by a 15 min wash with PBS at room temperature. Specimens were then incubated for 5 min at room temperature in a freshly made solution containing 0.01% H_2O_2 and 0.05% 3,3′-diaminobenzidine (DAB) in 0.1 M Tris–HCl buffer, pH 7.5, to visualize the immunoreactivity. PBS was substituted for the primary antibody in the negative control slides.

BrdU immunohistochemistry

For proliferation studies, some animals were injected i.p. with a single dose (50 mg/kg body wt) of 5-bromo-2′-deoxyuridine (BrdU; Sigma B 5002, Sigma-Aldrich Chemie GmbH, Deisendorf, Germany) 1 h before perfusion. The fixative was 4% paraformaldehyde in 0.1 M Sørensen's phosphate buffer. Following standard histological procedures, sections were placed under a UV light for 2 h (to enhance the BrdU signal; Weiler and Farbman, 1997), deparaffinized and rehydrated. Endogenous peroxidase was inactivated by incubation of the slides in 3% H_2O_2 in methanol for 30 min at room temperature. After several washes in PBS, sections were treated with trypsin (0.1% trypsin, Sigma T-8642, 0.1% $CaCl_2$ in 0.05 M Tris buffer for 5 min at room temperature) to increase the signal of the antibody reaction (Hayashi *et al.*, 1988), then washed several times in PBS. Sections were then incubated at 37°C in a 1:1 mixture of normal horse serum and 0.1 M PBS to block non-specific binding (60 min), then overnight at 4°C in an antibody against BrdU (Amersham anti-bromodeoxyuridine solution, containing mouse monoclonal antibody and a nuclease, Amersham RPN 202, Amersham Life Science, Arlington Heights, IL) diluted 1:10 in PBS, and sequentially at 37°C with the Vectastain Elite Kit for mouse antibodies (Vector Labs) containing biotinylated horse anti-mouse secondary antibody (30 min) and the ABC reagent (45 min). Each antibody treatment was followed by a 15 min wash with PBS at room temperature. Specimens were then incubated as above in a solution containing H_2O_2 and DAB in Tris–HCl buffer to reveal the BrdU immunoreactivity. To stop the reaction, specimens were placed in distilled water, washed several times and dehydrated in increasing concentrations of ethanol before being mounted with Permount® and coverslipped.

Results

Hormonal data

Quantification of the testosterone level was performed in plasma of males between 1 and 3 years of age in spring and in autumn. There were no differences among the ages, therefore the values were taken together. However, there was a dramatic seasonal difference. In spring the testosterone plasma concentration was 2072 ± 215 pg/ml (mean value ± SE) while in autumn the concentration was only 397 ± 28.4 pg/ml. The difference between spring and autumn is statistically highly significant ($P < 0.001$).

Histological observations and data

Location, orientation, gross morphology and histological structure

A small VNO can be detected in adult male ferrets (Figure 1). As in other mammals, the VNO of the ferret is a paired, non-connected, elongated, unbranched tubular organ, located in the anterior part of the nose within the ventral septal wall (Figure 2A). Partially encapsulated by a layer of cartilage, it opens anteriorly via a narrow duct into the incisive canal (ductus nasopalatinus), as in other carnivores. Posteriorly it ends in a duct-like structure which branches to the ducts of caudally located glands.

In contrast to other mammals, the VNO of the male ferret does not appear crescent-shaped in a frontal section but is more tubular. The epithelium lining the medial wall is thicker than that lining the lateral wall (Figure 2B). The anterior end of the tube, where it opens into the nasopalatine duct, is between 11.5 and 13.5 mm from the tip of the nose and lined by a stratified squamous epithelium. The medial side (which more posteriorly will become the presumptive sensory epithelium) always shows a thicker epithelium than the lateral, non-sensory aspect (Figure 3). About 1 mm posterior to the nasopalatine opening, the medial epithelium has changed from a stratified squamous epithelium to a pseudostratified columnar epithelium, and an apical supporting cell layer can be distinguished from the neuronal nuclear layer beneath (Figure 2C). The surface of this sensory epithelium changes from a smooth to a 'hairy' appearance. The epithelium on the lateral side also becomes columnar (Figure 2D) and remains columnar throughout most of the length of the VNO. On the posterior end of the VNO the epithelium becomes a single layer of cuboidal cells which represents the common duct of posteriorly located glands. The sensory epithelium is located on the medial wall throughout its extent; it does not shift posteriorly to a ventral position, in contrast to other mammals, where a 90° rotation in the orientation is seen (Zuckerkandl, 1910; Barber and Raisman, 1978; Vaccarezza *et al.*, 1981). The

Table 1 Morphological parameters of the male ferret VNO, seasonal and developmental aspects.

Male ferrets	VNO (*n*)	Extent (mm)	Epithelium thickness (μm)			Volume ($\times 10^{-2}$ mm^3)		
			Sensory	Neuronal	Non-sensory	Sensory	Non-sensory	Lumen
Spring	6	2.83 ± 0.35	55.1 ± 3.8	32.0 ± 2.5	35.4 ± 4.1	13.1 ± 1.8	7.1 ± 1.7	17.8 ± 2.0
Autumn	4	2.58 ± 0.14	70.0 ± 1.6	41.8 ± 3.3	45.0 ± 1.6	15.9 ± 1.3	9.6 ± 0.8	15.1 ± 1.5
Newborn	3	0.69 ± 0.01	34.9 ± 7.5	–	9.7 ± 1.9	0.4 ± 0.04	0.1 ± 0.02	0.2 ± 0.03

Values are means and standard errors.

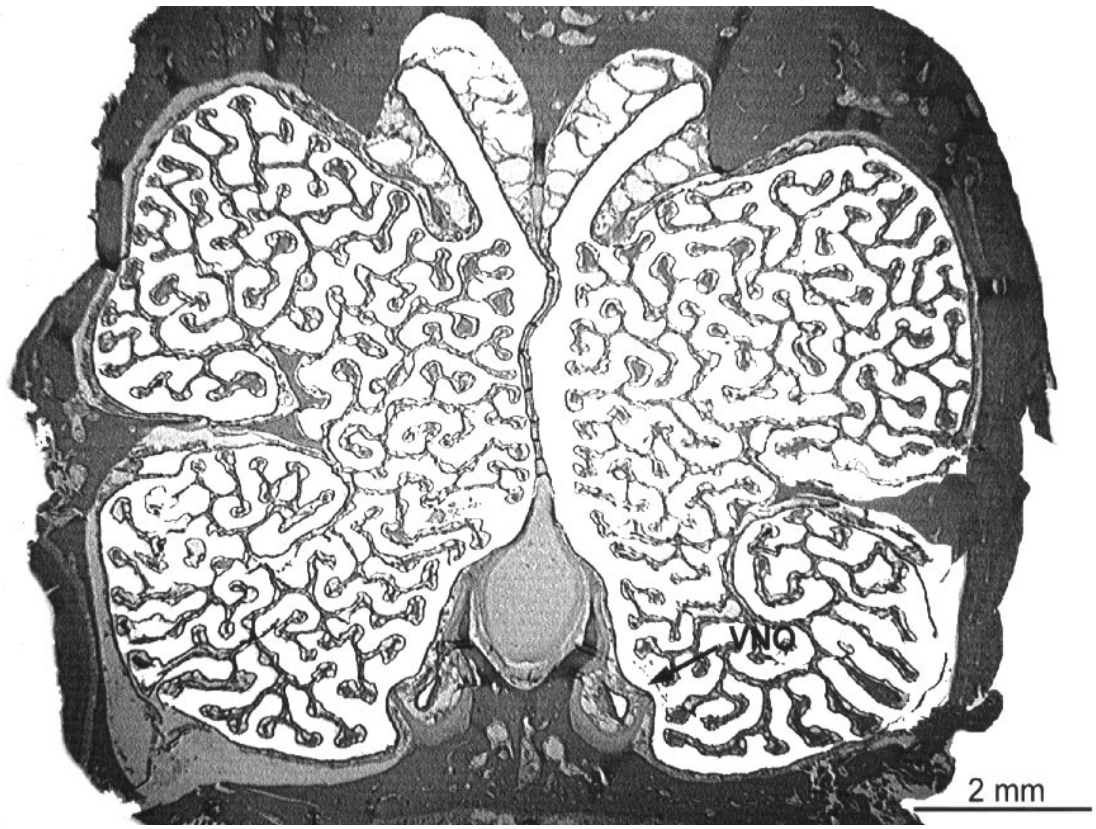

Figure 1 Frontal section through the snout of an adult male ferret in the anterior region, ~14 mm from the tip of the nose. The VNO is present at the ventral aspect of the septum as a rudimentary structure. The very elaborate, branched conchae in the nasal cavity are covered with respiratory epithelium.

extent of the VNO from anterior to posterior is on average 2.73 ± 0.32 mm.

Epithelium thickness, area and volume

The values for epithelial thicknesses, areas and volumes are listed in Table 1. There were no significant differences between the right and left sides (with one exception; see below, Unusual features) of the animals for any measured parameter. Therefore the individual adult VNOs were combined in the table. No significant differences between males at different hormonal status (spring, autumn) could be detected in the averages of most of the measured parameters (Table 1).

In the sensory epithelium only ~60% of the thickness is occupied basally by neuronal nuclei. The remainder consists of two zones, the elongated nuclei of supporting cells which span a very dense band and a small nuclear free zone in the apical region (Figure 2C, E). The non-sensory epithelium is organized differently: there are no rows of neuronal nuclei (Figure 2D, F).

From anterior to posterior the epithelium on both sides of the VNO-tube increases in thickness even before a sensory epithelium is distinguishable on the medial side (Figure 3), and further posteriorly the thickness remains relatively constant before it drastically decreases on its posterior end to become a one-cell layer epithelium of a glandular duct. The proportion of epithelium thickness occupied by neuronal nuclei to the whole sensory epithelial thickness is constant throughout the anterior–posterior extent (Figure 3). The non-sensory epithelium also increases in thickness from anterior to posterior but then decreases faster, commencing from the anterior third of the organ to the posterior end (Figure 3).

The area of sensory and non-sensory epithelium per section (Figure 4) increases from anterior to posterior rapidly but then decreases because of the decrease in lumen size.

Histological observations

In the sensory epithelium mitotic figures were observed infrequently and were randomly distributed, close to the basement membrane. The distribution of BrdU-immunoreactive cells was similar (Figure 5). Condensed nuclei (probably apoptotic cells) were also seen, but higher in the epithelium (Figure 2E). We did not observe intruding capillaries into the epithelium nor piercing glands through the epithelium as has been reported in the rodent VNO (Weiler *et al.*, 1999).

In the non-sensory epithelium cells with round nuclei were interspersed with cells having an elongated spindle-shaped nucleus; polymorphonuclear leucocytes were frequently observed (Figure 2F).

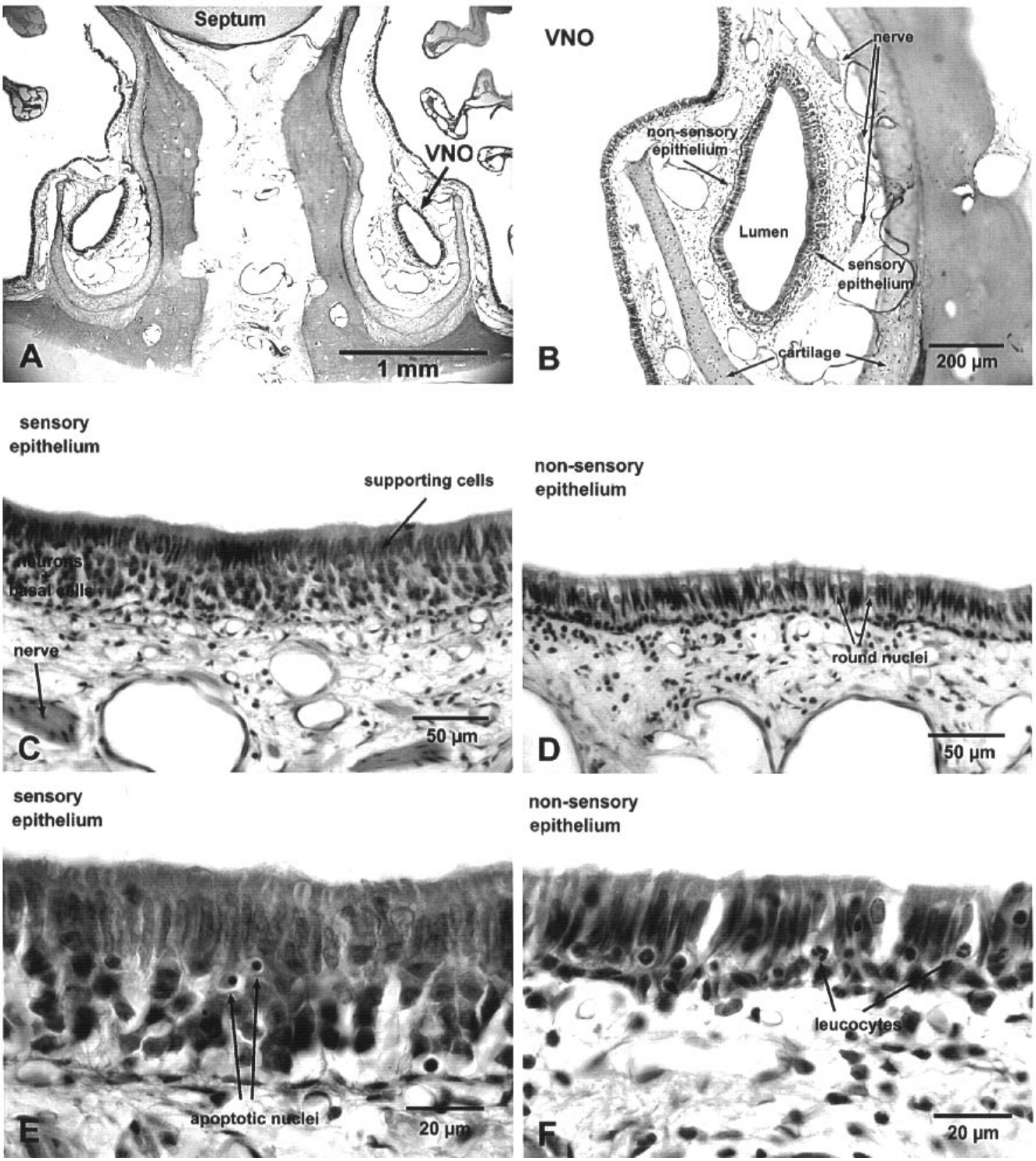

Figure 2 **(A)** The VNO appears as a paired, non-connected, unbranched tube-like structure. **(B)** Small in size, it is a thickening of the wall of the duct, with a medially thicker sensory epithelium and a laterally non-sensory epithelium. **(C, E)** In the sensory epithelium the apically located row of spindle-shaped nuclei can be distinguished from the underlying neuronal population including basal cells. Apoptotic nuclei are infrequently observed. **(D, F)** Non-sensory epithelium showing the interspersed cells with round nuclei between cells with elongated nuclei. There are some polymorphonuclear leucocytes within the epithelium.

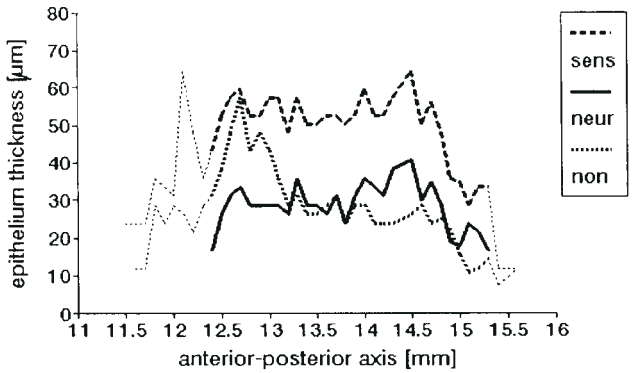

Figure 3 Thickness of the sensory and non-sensory epithelium in its anterior-posterior extent. Zero of the *x*-axis is the tip of the nose. At ~11.5 mm the VNO tube starts (light dotted lines). At 12.5 mm from the tip of the nose a dense band of apically located spindle-shaped nuclei of supporting cells can be distinguished from the round nuclei beneath, defining the epithelium on the medial side as sensory (sens); the epithelium on the lateral side is then called non-sensory epithelium (non). The neuronal thickness (neur) is a relatively constant portion of the sensory epithelium throughout its anterior–posterior extent.

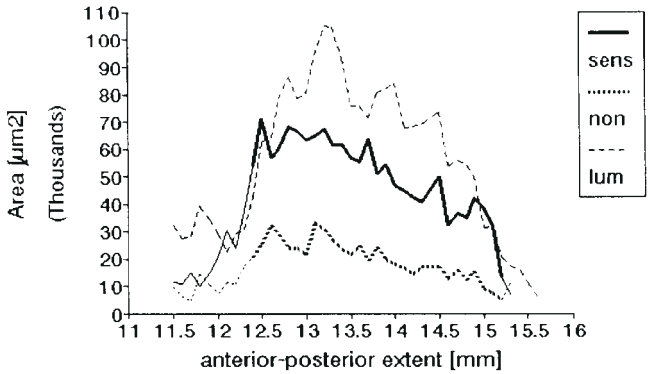

Figure 4 Area of the sensory (sens) and non-sensory (non) epithelium in sections from anterior to posterior changes closely related to the change in lumen area (lum). The VNO tube starts at ~11.5 mm from the tip of the nose, but becomes sensory at ~12.5 mm (thicker lines). Posteriorly it ends as a duct of glands.

Developmental aspects

The morphometric parameters in the newborns are listed in Table 1. Notably the thickness ratio of non-sensory to sensory epithelium in newborns was ~28%. During postnatal development, the thickness of non-sensory epithelium increases more than that of the sensory epithelium so that the non-sensory epithelium reaches ~60% that of the sensory epithelium in adults. In the newborn VNOs it is very difficult to distinguish a borderline between neuronal and supporting cell rows and therefore no data are included in the table for neuronal layer thickness.

OMP staining

Mature sensory cells of the VNO in mammals usually express olfactory marker protein (OMP) (Farbman and Margolis, 1980; Johnson *et al.*, 1993; Tarozzo *et al.*, 1998). The OMP immunohistochemical reaction was positive in some cells of the sensory epithelium although it appeared weak (Figure 6). Non-sensory epithelium and the negative control were not stained.

BrdU labeling

The sensory and non-sensory epithelium of the VNO showed BrdU-positive nuclei (Figure 5). Basal cells and supporting cells were stained. The staining appeared to be randomly distributed in contrast to the adult rodent VNO, where most proliferating cells appear in the edges of the sensory epithelium adjacent to the non-sensory epithelium. The non-sensory epithelium seemed to contain even more BrdU-positive nuclei than the sensory epithelium and seemed to be more basally located. In the sensory epithelium, sometimes clusters of labeled cells are found; this was never observed in the non-sensory epithelium.

Unusual features

In one animal in spring we observed on one side that the epithelium lining the vomeronasal tube appeared to be sensory epithelium all around (Figure 7A). No non-sensory epithelium faced the sensory epithelium opposite. OMP staining confirmed this observation (Figure 7B). However, this pattern was seen only in the middle part of the organ. Anteriorly and posteriorly there was non-sensory epithelium in its typical location and extension. The VNO of the other side of that particular animal was typical, with sensory epithelium on the medial side and non-sensory epithelium on the lateral side.

Discussion

This study is the first to describe the existence of a VNO in the adult male ferret. Our results show that it is a small, rudimentary structure, compared with that of rodents. Moreover, there are no apparent morphological differences between the mating and the sexually quiescent seasons, although there are significant differences in physiological parameters [the testosterone level and the metabolic rate (Kästner and Apfelbach, 1987)], as well as in morphological parameters, e.g. brain size and weight and testis size and weight (Weiler, 1992), and in behavior (Mauz, 1985).

Is the VNO in the ferret functional?

An adult male ferret has a body wt of >1 kg, but the length of its VNO is only 2.7 mm, comparable to the length of the VNO in a 10 day old 10 g hamster (Taniguchi *et al.*, 1982) and far less than the 7 mm VNO of the albino rat (Addison and Rademaker, 1927; Weiler *et al.*, 1999) or the 6 mm VNO of the chinchilla (Oikawa *et al.*, 1994). Correlated with the smaller length, the volume of the sensory epithelium in the male ferret VNO is 0.14 mm^3, much less than that of a rat and even less than the VNO in a mouse of 25–30 g body wt

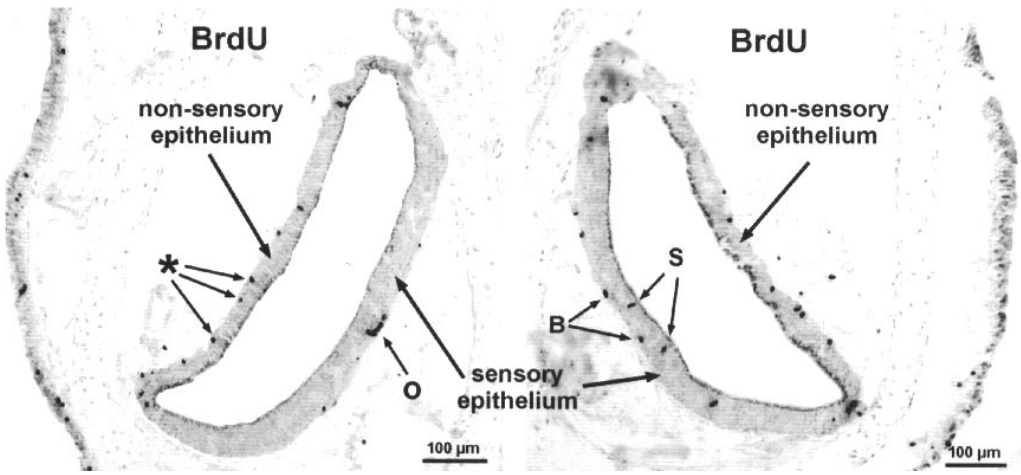

Figure 5 BrdU-immunohistochemically labeled nuclei showing proliferating cells in the sensory and non-sensory epithelium. In the sensory epithelium BrdU-labeled nuclei sometimes appear in patches (o) which are located basally, thus representing basal cells (B). Supporting cells (S) also proliferate. In the non-sensory epithelium proliferating cells are located predominantly basally and do not appear in patches but as single cells (*).

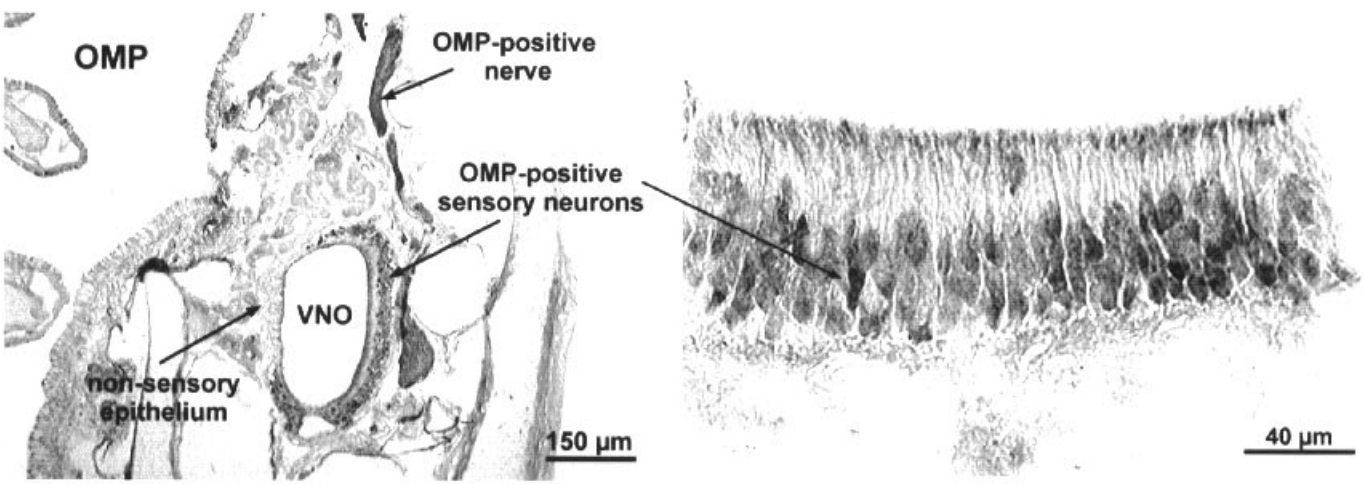

Figure 6 Immunostaining against olfactory marker protein revealed OMP-positive neurons in the sensory epithelium of the vomeronasal organ. Non-sensory epithelium and respiratory epithelium are OMP-negative.

(Wilson and Raisman, 1980). It is also smaller compared with other carnivores (for review see Dawley, 1998).

In addition to its small size, several other histological facts suggest that the VNO in ferrets is rudimentary compared with that of other animals.

1. The epithelium is very thin, approximately the thickness of the VNO of a 13 day rat embryo (Yoshida *et al.*, 1993).
2. Only about three rows of neurons are seen.
3. The ratio of the volumes of sensory to non-sensory epithelium is ~1.7:1, comparable to that described for the immature VNO in rat embryos (Garrosa *et al.*, 1986, 1992; Yoshida *et al.*, 1993). The ratio is considerably higher in the adult rat.
4. Proliferating cells in the developing VNO in the mouse and rat are found throughout the epithelium (Cuschieri and Bannister, 1975; Weiler *et al.*, 1999) but are concentrated on the edges of the sensory epithelium adjacent to the non-sensory epithelium in the mature VNO (Barber and Raisman, 1978; Weiler *et al.*, 1999). In the ferret, mitotic figures as well as BrdU-labeled cells were found infrequently, but were distributed near the basement membrane, again similar to an immature stage.
5. We did not observe intruding capillaries in the sensory

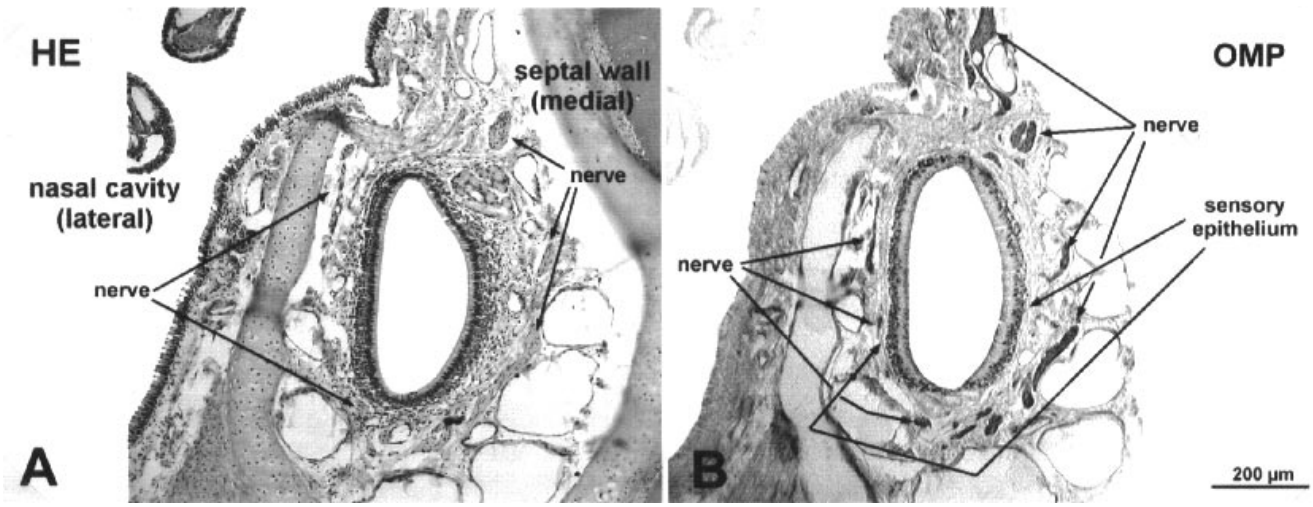

Figure 7 Unusual feature. Sensory epithelium lining both sides of the tube, medial as well as lateral sides. Nearly adjacent sections are shown. **(A)** HE staining, **(B)** OMP-immunolabeling, revealing cells as sensory neurons; supporting cells are OMP-negative. Notice the OMP-positive nerve is present all around the VNO.

epithelium of the ferret VNO as described for other mammals (Cuschieri, 1974; Barber and Raisman, 1978; Wilson and Raisman, 1980; Breipohl *et al.*, 1981; Vaccarezza *et al.*, 1981; Taniguchi and Mochizuki, 1983; Garrosa *et al.*, 1986, 1992; Mendoza and Szabo, 1988; Szabo and Mendoza, 1988; Yoshida *et al.,* 1993; Berghard and Buck, 1996; Weiler and Farbman, 1998). Intruding capillaries usually reflect a high metabolic activity (Cuschieri, 1974; Vaccarezza *et al.*, 1981). Their absence suggests a low metabolism and is described in species with a thin epithelium in which the VNO seems not to play an important role in behavior (Schilling, 1970; Jordan, 1972; Loo and Kanagusuntheram, 1972; Bhatnagar, 1980; Mendoza *et al.*, 1994). In species with a functionally important VNO, capillaries usually intrude prenatally when the VNO has reached a certain developmental stage (Breipohl *et al.*, 1981).

6. In the newborn ferret a rudimentary VNO can be detected (Kabioll, 1989). During postnatal development the volume of non-sensory epithelium increases more than the sensory volume. However, compared with the body size increase of ~200 times between newborn and adult, the VNO volume increases only about 60 times.
7. An accessory olfactory bulb, the target of the vomeronasal nerve, has been reported to be very small in the ferret (Lockard, 1982, 1985; Fox, 1988).
8. In contrast, the main olfactory system in the ferret is very well developed, the olfactory bulbs comprising about 5% of the whole brain (Weiler and Apfelbach, 1995). In male ferrets, seasonal, hormone-dependent changes occur in the total brain weight (Weiler, 1992) whereas the VNO shows no major differences between spring and autumn. The epithelium in spring seems to be even thinner and the volume of the lumen slightly bigger. The number of neurons and of OMP-positive neurons are relatively small compared with those seen in rodents.

Thus, the overall relative size and rudimentary appearance of the male ferret VNO compared with that of animals in which the VNO plays a role in sexual behavior suggest it has a more limited function.

Male ferrets show signs of agitation as well as an increase in exploratory behavior and vocalization when placed in an open field in which a female ferret in estrous had been previously introduced and then removed (Cowley, 1978). But male ferrets have to learn the odor of estrous females; only sexually experienced males prefer the odor of estrous versus non-estrous or male odor (Mauz, 1985). Therefore there might be no preprogrammed chemical signal for pheromones detected by the VNO as proposed in the hamster (Keverne *et al.*, 1986), where the VNO is very well developed (Winans and Powers, 1977) and plays a critical role in mediating sexual behavior (Powers and Winans, 1975). In the present study on the ferret VNO and in an earlier study on its main olfactory organ we discerned no distinguishable morphological changes between spring and autumn (Weiler and Bensemann-Ryvkin, 1995). If the VNO plays a role in male ferret sexual behavior it is not reflected in discernible morphological changes in the mating season, comparable to the dramatic increases in brain and testis sizes associated with mating in this species.

Acknowledgements

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