Chem. Senses 28: 581–593, 2003 DOI: 10.1093/chemse/bjg047

The Septal Organ of the Rat During Postnatal Development

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Abstract

The septal organ of Masera (SO) is a small, isolated patch of olfactory epithelium, located in the ventral part of the nasal septum. We investigated in this systematic study the postnatal development of the SO in histological sections of rats at various ages from the day of birth (P1) to P666. The SO-area increases to a maximum at P66–P105, just as the animals reach sexual maturity, and decreases thereafter, significantly however only in males, indicating a limited neurogenetic capacity for regeneration. In contrast, the main olfactory epithelium area continues to expand beyond P300. The modified respiratory epithelium ('zwischen epithelium') separating the SO and the main olfactory epithelium contains a few olfactory neurons up to age P66. Its length increases postnatally so that the SO becomes more ventral to the OE. Although the position of the SO relative to other anatomical landmarks changes with development it is consistently located just posterior to the opening of the nasopalatine duct (NPAL). Thus, a possible function of the SO is in sensing chemicals in fluids entering the mouth by licking and then delivered to the nasal cavity via the NPAL; therefore the SO may be involved in social/sexual behavior as is the vomeronasal organ (VNO). We suggest that the SO is a separate accessory olfactory organ with properties somewhat different from both OE and VNO and may exist only in species where the NPAL does not open into the VNO.

Key words: accessory olfactory organ, growth, nasopalatine duct, organ of Masera, olfactory epithelium, vomeronasal organ

Introduction

The septal organ (SO), also called the organ of Masera, was first observed in newborn mice (Broman, 1921) and named 'Riechepithelinsel' (island of olfactory epithelium). It is an isolated patch of olfactory sensory epithelium on the ventral region of the nasal septum and separated from the olfactory epithelium proper (OE) by a region of modified respiratory epithelium. The SO was subsequently described in several mammalian species by Rodolfo-Masera (1943) and others (Adams and McFarland, 1971; Bojsen-Møller, 1975; Katz and Merzel, 1977; Kratzing, 1978; Breipohl *et al.*, 1983, 1989; Mendoza *et al.*, 1989; Adams, 1992; Taniguchi *et al.*, 1986, 1993; Saito *et al.*, 1994; Takami *et al.*, 1994; for review see Farbman, 1992).

The SO is derived from the olfactory placode, as are the main OE and the vomeronasal organ (VNO) (Bojsen-Møller, 1975). Morphologically it consists of basal cells, supporting cells and ciliated olfactory sensory neurons. The axons of the sensory neurons project to the medial aspect of the posterior olfactory bulb (Bojsen-Møller, 1975; Benson *et al.*, 1985; Clancy *et al.*, 1985, 1994; Wysocki *et al.*, 1985; Pedersen and Benson, 1986; Astic and Saucier, 1986, 1988; Astic *et al.*, 1987; Ma *et al.*, 2001, 2003) where most of them terminate in a group of about 30 'septal glomeruli' (Giannetti *et al.*, 1992).

The results of physiological studies argue convincingly that the SO is involved in odor recognition. It responds to a broad range of odor stimuli, with sensitivities even higher than in OE (Marshall and Maruniak, 1986) using the cAMP signal transduction cascade (Ma *et al.*, 2003). One author suggested it functions as an early warning or monitoring system, sensing air entering while breathing at rest (Rodolfo-Masera, 1943). Others suggested it senses compounds of low volatility (Wysocki *et al.*, 1980). Efforts to observe behavioral changes to odorants after lesions of the SO were unsuccessful (Giannetti *et al.*, 1995b). Currently, the actual function remains unclear.

We made a systematic examination of the growth and development of the postnatal rat SO with the idea that an understanding of the anatomical details might be useful in disclosing its possible function(s). We report here that the area of the SO increases to a maximum around sexual maturity and decreases slightly thereafter. In contrast, the

area of the OE continues to expand into senescence. The position of the SO relative to other structures in the nasal cavity changes somewhat in development but maintains a close relationship with the opening of the nasopalatine duct (NPAL), which, in the rat, passes between the incisive papilla region of the oral cavity and the nasal cavity. We suggest the SO is an accessory olfactory organ, perhaps involved in sexual and social behavior (cf. Wysocki *et al.*, 1980) and may exist only in species where the NPAL does not open directly into the VNO.

Materials and methods

Animals

A total of 79 Sprague–Dawley rats of both sexes at different postnatal ages ranging from P1 (day of birth) to P666 were used in this study (Table 1). They were born in our own colony and housed under standard conditions in a temperature-controlled environment with a 12 h light/dark cycle and access to food and water *ad libitum.* Newborns were allowed to suckle until P40 (weaning). During that time, they were weighed every other day. After weaning, animals were separated by sex and kept in groups. Thereafter, they were weighed twice a week. Animals from different litters were used in each age group. All experiments were carried out according to regulations established by the National Institutes of Health and approved by the Animal Care and Use Committee at Northwestern University.

Perfusion and fixation

Rats were killed by i.p. injection of a lethal dose of sodium pentobarbital (250 mg/kg body wt). While under deep anesthesia, the animals were perfused intracardially through the left ventricle with 0.1 M PBS, pH 7.2, at room temperature to clear the vessels of blood. This was followed by a fixative of 4% paraformaldehyde (Paraformaldehyde Powder, EM Grade no. 30525-89-4; Polysciences Inc, Warrington, PA) in 0.1 M Sørensen's phosphate buffer, pH 7.0. Heads were removed, skinned and postfixed overnight in the same fixative at 4°C.

Histology (decalcification, embedding, sectioning, staining)

After washing several times in 0.1 M Sørensen's phosphate buffer, pH 7.0, noses were decalcified in 5% EDTA (ethylenediaminetetraacetic acid tetra sodium salt tetrahydrate, Sigma E-5391; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in the same buffer and processed for embedding in Paraplast®. Frontal (coronal) sections of 10 µm were cut serially through the total extent of the nasal cavity and each section was collected. Every 10th section was taken and placed on silane (3′-aminopropyltriethoxy-silane, Sigma A-3648) treated slides and stained with hematoxylin and eosin (H&E) (Burck , 1973). These sections (in combination with the OMP sections, see below) were used to locate the position of the septal organ, determine the antero-posterior extent, measure the dorso-ventral length as well as the epithelial height. The borders of the septal organ are very clearly defined as an island of pseudostratified epithelium (consisting of an upper row of supporting cell nuclei, and lower rows of neurons and basal cells) within the simple columnar epithelia dorsally and ventrally of the organ. The SO was consistently isolated along the ventral border by respiratory epithelium, which was measured from the

Table 1 Number and sex of animals at different ages. Extent of the septal organ during postnatal development. Mean values and standard deviations

Age	No. of animals total (n = 79)			Antero-posterior extent, average values (mm)		Dorso-ventral extent, mean values (µm)	
	M	F	n	M	F	M	F
P1	1	2	3	0.90	0.93 ± 0.05	153.9	171.4 ± 13.1
P3	2	1	3	0.90 ± 0.08	0.90	166.3 ± 17.5	172.2
P5	2	1	3	1.23 ± 0.21	0.90	259.5 ± 7.9	234.2
P11	2	1	3	1.50 ± 0.14	1.30	265.7 ± 3.1	219.5
P18	1	2	3	2.05 ± 0.07	1.60 ± 0.17	345.2 ± 12.8	330.3 ± 36.5
P21	4	2	6	1.69 ± 0.17	1.75 ± 0.24	373.7 ± 23.6	313.1 ± 24.8
P40–P41	9	6	15	2.33 ± 0.26	2.06 ± 0.23	491.4 ± 74.3	428.6 ± 48.9
P66–P68	9	6	15	2.89 ± 0.32	2.35 ± 0.27	489.4 ± 57.8	509.4 ± 90.7
P105–P108	6	5	11	2.92 ± 0.27	2.53 ± 0.30	585.2 ± 80.0	441.1 ± 57.6
P181	3	0	3	2.55 ± 0.41	—	451.4 ± 42.3	—
P324–P333[a]	4	0	4	2.73 ± 0.36	—	476.3 ± 86.0	—
P400–P456	4	4	8	2.91 ± 0.30	2.28 ± 0.32	432.2 ± 118.2	434.7 ± 64.2
P600–P666	1	1	2	2.80	3.15	454.1	514.0

[a]One animal did not have a septal organ and is not included in the calculations.

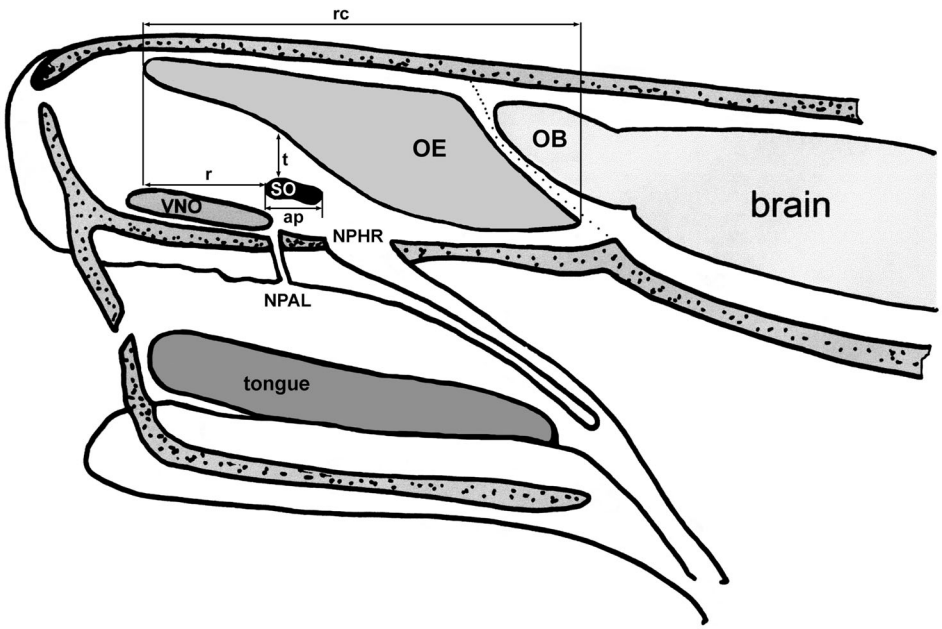

Figure 1 Schematic drawing of a parasagittal view through a rat's head showing the position of the SO. NPAL, nasopalatine duct; NPHR, nasopharyngeal duct; OB, olfactory bulb; OE, main olfactory epithelium; SO, septal organ; VNO, vomeronasal organ; ap, anterior–posterior extent of SO; t, length of ZE (epithelium between OE and SO); r, distance of rostral end of SO to rostral end of OE; rc, rostral–caudal extent of OE. [Adapted from Farbman, 1992.]

ventral end of the SO to the most indented point of the septal wall. For details of the histological characterization and categorization of respiratory epithelia, see Katz and Merzel (1977).

OMP immunohistochemistry

OMP (olfactory marker protein) is a protein expressed nearly exclusively in mature olfactory sensory cells (Farbman and Margolis, 1980; Margolis, 1982). To visualize mature olfactory sensory neurons in the septal organ, 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) (rabbit serum no. 16120-099, Gibco BRL, Life Technologies, Grand Island, NY), the antibody against OMP (goat no. 255 α-OMP) at a dilution of 1:1000 (60 min), the Vectastain® ABC Kit (Peroxidase Goat IgG, PK 4005, Vector Laboratories, Burlingame, CA) containing biotinylated rabbit anti-goat antibodies (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 for 5 min at room temperature in a freshly made solution containing 0.01% hydrogen peroxide and 0.05% 3,3′-diaminobenzidine in 0.1M Tris–HCl buffer, pH 7.5, to visualize the immunoreactivity. PBS was substituted for the primary antibody in the negative control slides.

Measurements and statistics

All measurements were done with a calibrated ocular micrometer on a Leitz microscope with a Zeiss objective at a magnification of 400×. Right and left sides of an animal were always investigated separately.

We refer to the rostral end as 'anterior' and to the caudal end as 'posterior' for the uniformity of the nomenclature in other developmental studies of the rat olfactory organs and studies on the projections from the sensory organs to the olfactory bulb (Pedersen and Benson, 1986; Pedersen *et al.*, 1986; Weiler and Farbman, 1997, 1998; Weiler *et al.*, 1999; Ma *et al.*, 2003).

Location, length and area of the septal organ

The location and extent of the SO (Figure 1) was determined in the H&E-stained series and confirmed by OMP-immunostained sections. Each H&E section from the most anterior to the most posterior end of the nasal cavity was screened and the start and end of the SO as well as of the main OE was noted; the antero-posterior extent of the SO was then given by the number of sections. The position and extension of the SO was defined in relation to the OE. In each section the dorso-ventral length of the SO was measured. Because the SO is not always one continuous stretch in the dorso-ventral extent but sometimes appears as pieces separated by non-sensory epithelium, all stretches of sensory epithelium in a section were summed to give one value. For each animal the dorso-ventral extent was graphed for the whole antero-posterior length; the measurements were spaced 100 μm in the antero-posterior axis. All dorso-ventral measurements per SO were averaged to the mean dorso-ventral extent value. The area was calculated by multiplying the mean dorso-ventral extent with the antero-posterior length per SO. No correction factor (Abercrombie, 1946; Coggeshall

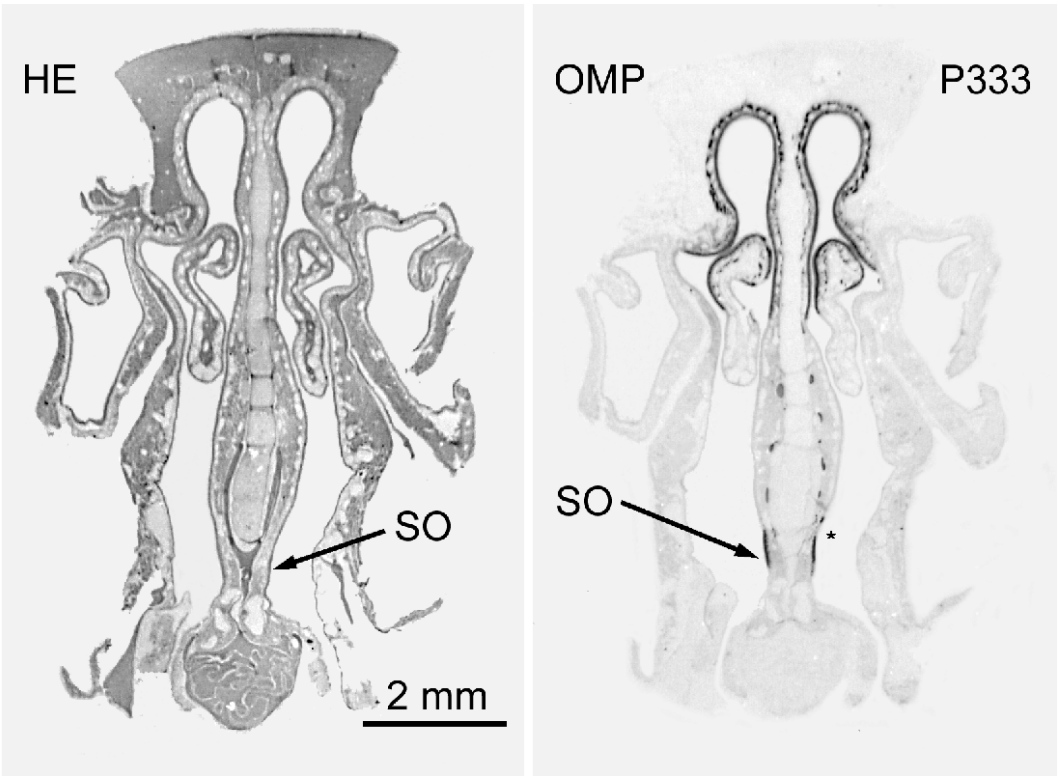

Figure 2 Frontal section through the snout of a male rat at P333. The septal organ (SO) is located on the ventral aspect of the septal wall. Left: hematoxylin and eosin (HE) staining; right: OMP immunostaining. Note the unstained patch within the SO on the right side (*).

and Lekan, 1996) was used for any shrinkage or change which the histological procedure could have caused because all tissues were processed the same way. Mean values and standard deviations were calculated for each age group, separately for males and females.

In all animals the length of the 'zwischen epithelium' (ZE, the epithelium between the OE on the septal wall and the SO) was measured for the whole antero-posterior extent of the SO and graphed to show the shape and position of the organ.

Height and volume of the epithelium

The height (thickness) of the epithelium was measured from the basement membrane to the apical surface for the whole antero-posterior extent at the middle of the dorso-ventral length in each section. Average values were calculated for each side of each animal. The volume was defined for each animal by multiplying the area by the mean epithelium height. Mean values and standard deviations were calculated for the age groups separately for males and females.

Analysis of data and statistics

All parameters were noted for individual animals and mean values and standard deviations were calculated within an age group. Males and females were compared within one age group and differences were statistically analyzed with the non-parametric Mann–Whitney *U*-test (Lienert, 1973). Differences among age groups were statistically verified the same way. Differences between the right and left sides were statistically analyzed with the sign test (MacKinnon, 1964).

Results

Location and extent

In coronal sections of 78 of the 79 rats at various ages, we found isolated, elongated SOs bilaterally on the ventral region of the nasal septum just dorsal to the area where the septal wall is indented (Figure 2). A zone of modified respiratory epithelium separates the SO from the more dorsally situated OE (Figure 3). For the purposes of this paper we refer to this zone as the ZE. In the younger age groups, scattered olfactory sensory neurons are found within the ZE, up to age P66. Nevertheless, the borders of the septal organ are very clearly defined by the onset of the island of the pseudostratified epithelium of the SO (consisting of an upper row of supporting cell nuclei, and lower rows of neurons and basal cells) within the simple columnar epithelia dorsally and ventrally of the organ. The SO is also consistently isolated along its ventral border by respiratory epithelium. The bilateral organs never merge into one, not even in the region where the septal wall retracts from the hard palate and the nasopharyngeal duct allows contact of the two sides of the nose.

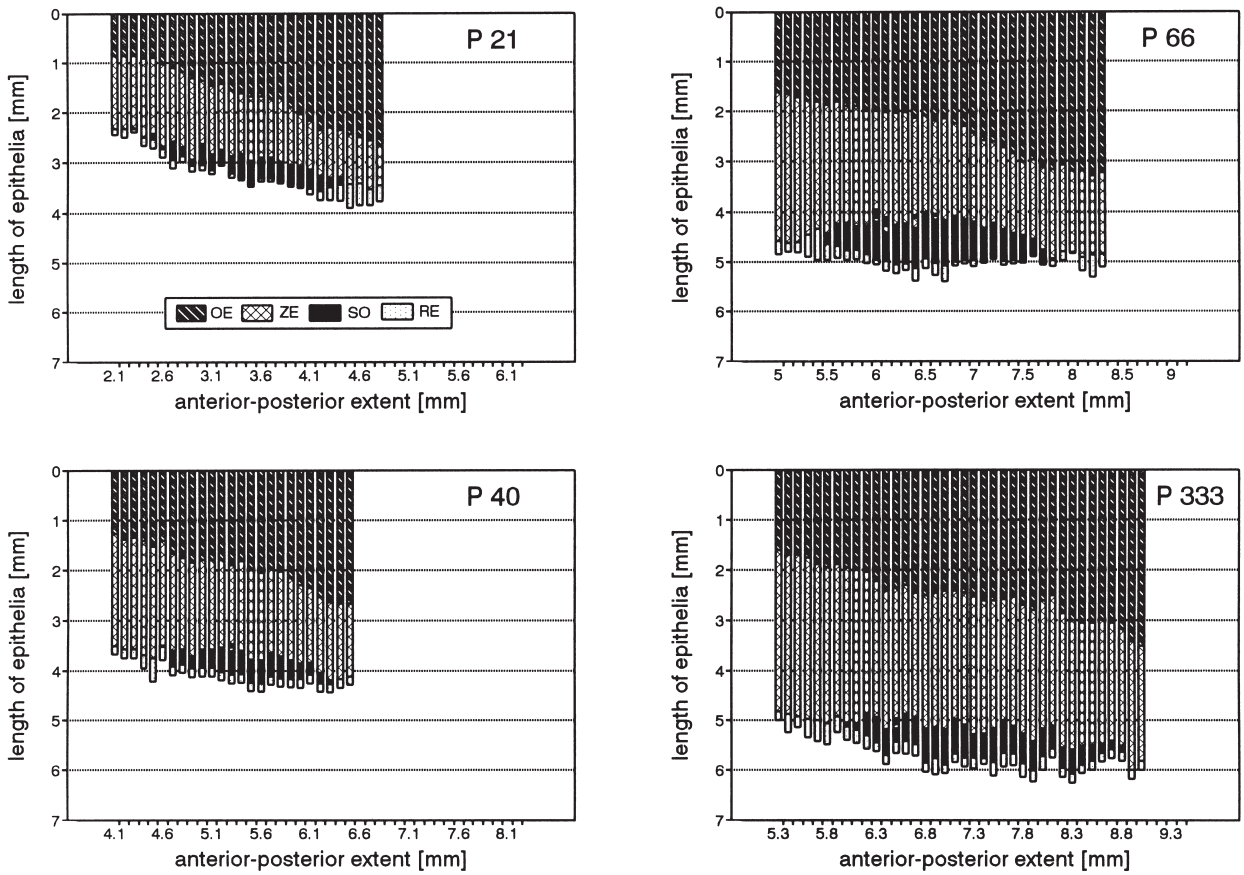

Figure 3 Position, location and shape of the SO relative to OE and ZE at different ages during postnatal development. On the *y*-axis are represented the dorso-ventral length of the main olfactory epithelium, OE, the ZE, the SO and the respiratory epithelium (RE) ventral to the SO on the nasal septum. On the *x*-axis are represented (at 100 µm intervals) the distances in the anterior–posterior extent; the zero would be the most anterior end of the OE. Connection of the SO (black) segments gives an approximate outline of the SO area as it would be viewed on the septal wall from a lateral position. A few small patches of non-sensory epithelium within the dorsal-ventral extent of SO are indicated by tiny patches of white on the black background within the bars. Dorsal is up, ventral is down. Representative animals.

In all age groups, the rostral end of the SO is located just posterior to the opening of the NPAL. The length of the SO in the *antero-posterior direction* (distance a-p in Figure 1) increases with growth of the animal from P1 (0.9 mm) until P66 and then reaches a plateau (2.8 mm in males, 2.4 mm in females; Table 1). There are no statistically significant differences in a-p length between the two sides of an individual animal. However, the differences between the age groups are significant (Mann–Whitney *U*-test; P1–P5/P11–P21, P40/P66, $P < 0.001$). Although the a-p extent of the SO in females is less than that in males at the same age, when the values of females and males are correlated to their body weight and not to their age, there is no sex-dependent difference in the extent of the SO.

The *dorso-ventral extent* of the SO is narrow anteriorly, usually bulges in the middle and narrows posteriorly (Figure 4). The *mean* value of the dorso-ventral extent (160 µm in neonates) reaches a maximum at P105 (585 µm) and declines thereafter in males (450 µm; Table 1) (Mann–Whitney *U*-test; P105/P181–P666; $P < 0.005$), suggesting the size of the SO actually shrinks during adulthood. This is verified by (i) the fact that the *maximal* values decline in parallel with the mean values, and (ii) the fact that the SO area declines (see below, Figure 4). In contrast, the dorsal-ventral extent of the main OE in the SO-region continues to expand until well after one year of age (0.9 mm at P1, 2.5 mm at P105, 2.9 mm at P450) as does the total OE area (Weiler and Farbman, 1997). In adults, small patches of non-sensory epithelium are sometimes intercalated within the SO (Figures 2, 3 and 7). These patches are occasionally visible as early as P40 but become more prominent with increasing age.

Area

The area of the SO was calculated by multiplying the mean dorso-ventral length with the antero-posterior extent per side per animal. The area increases continuously from P1 (0.15 mm^2) to P105 (1.75 mm^2) in males when a maximum is reached and decreases significantly ($P < 0.001$) afterwards to a plateau of 1.25 mm^2 (P181–P666). In females, the decrease is less obvious and not statistically significant. In contrast,

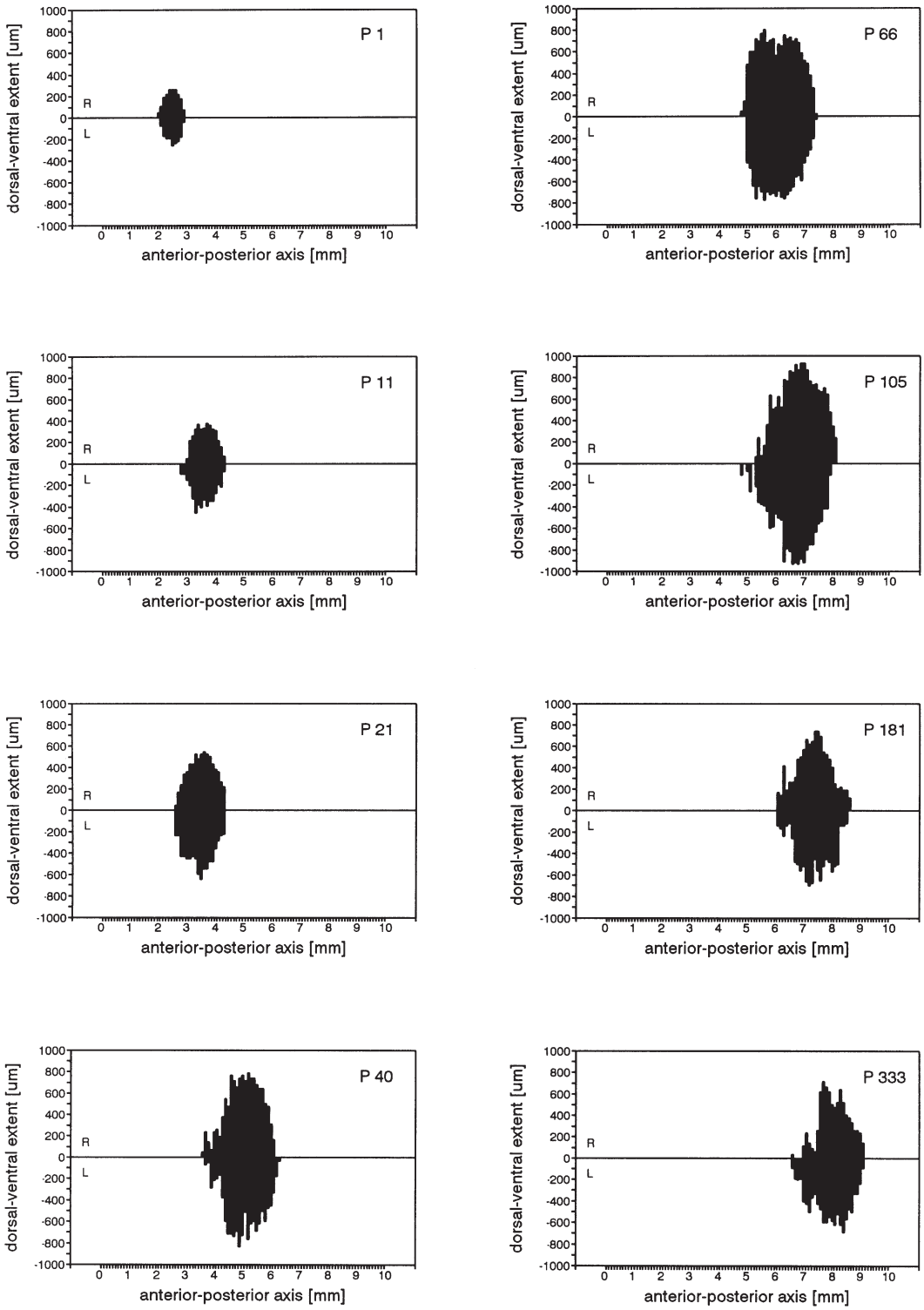

Figure 4 Area of SO at different ages. The *x*-axis represents the antero-posterior axis, the *y*-axis the dorso-ventral extent; the right side of each animal is above the horizontal line across the middle of each graph, the left side below. In the older animals the areas of non-sensory epithelial patches within the dorso-ventral extent were subtracted from the total before plotting. Each graph is of one representative animal in the age group. The anterior end of OE is arbitrarily set to zero on the *x*-axis. Note the shift of the SO in the posterior direction and the decrease in area from the peak at P105 to lower values at P181 and P333.

the OE area expands significantly with age (Weiler and Farbman, 1997).

Epithelial height

The height of the SO epithelium is relatively constant during postnatal development and is significantly less than the average height of the OE (cf. Weiler and Farbman, 1997). The mean value in newborns is 44.9 μm and stays around this value with little variation. The slightly higher value in the older animals might be related to an increase in supporting cell size (Weiler and Farbman, 1998).

At the anterior end, the epithelium reaches a height of ~35–40 μm immediately, increases to ~55 μm near the middle and rapidly decreases at the posterior end. Small changes in the epithelial height within a section occur because of the uneven basement membrane, which shows indentations caused by gland acini intruding into the basal region of the epithelium. There is no difference between males and females in epithelial height at any age. However, we did find a significant difference between the two sides of an animal, with the right side being higher in 80% of the cases ($P < 0.001$) with differences up to 15%.

Comparison of SO with OE

To determine whether the rate of SO growth is comparable to that of OE, we calculated the ratio between the average antero-posterior length of SO (distance ap in Figure 1) and the average length of OE (distance rc in Figure 1). The ratio (ap/rc) decreases significantly with age, from 20.8% at P1 to 17.5% at P66 and 14.6% in adults (P181–P450). This indicates that during postnatal development the rate of increase of SO size in the *antero-posterior axis* is retarded compared to OE growth. In the *dorso-ventral direction* the growth of SO is proportional to that of the septal wall up to the age of P66 (an increase of 3.1-fold between P1 and P66; occupying constantly 9.4% of the septal wall), but then is slower and the SO even shrinks in size (so that the SO occupies only 6.5% of the dorso-ventral septal wall at P450). The septal wall continues to grow in adults, as does the OE. At no age does the dorso-ventral growth of the SO appear to be related to the dorso-ventral growth of the OE.

Position of the septal organ

During postnatal growth the position of the SO shifts noticeably in relation to other structures in the nasal cavity, both in its antero-posterior and dorso-ventral locations.

With respect to the OE

The anterior end of the SO is always posterior to the rostral extent of the OE (distance r in Figure 1). The absolute distance between the two increases continuously during postnatal development from 2.1 mm at P1, 3.3 mm at P21, 4.3 mm at P40, 5.3 mm at P66, 5.6 mm at P105 to 6.2 mm at P181 and further to 6.6 mm at P400 in males, suggesting that the relative SO position shifts posteriorly. However, because the length of the nasal cavity increases relatively more than the distance between anterior SO and anterior OE, the relative position of the SO within the nasal cavity is a bit more anterior in adults. In P1, the SO starts approximately in the middle of the OE. In adults, the SO is located in the anterior third of the OE. In females, the absolute values are smaller; however, females have a smaller body size, and therefore there is no sex-dependent difference in the extent of the SO but rather a size-dependent one.

The differences in this *antero-posterior distance* in males are significant between P21 and P40 (Mann–Whitney *U*-test, $P < 0.001$), P40/P66 ($P < 0.001$), P105/P181 ($P < 0.01$), P181/P400 ($P < 0.05$), P66/P400 ($P < 0.001$).

The *dorso-ventral distance* between OE and SO, i.e. the length of the ZE, is not constant but declines from anterior to posterior. This is evident upon examination of Figure 3, in which the values derived from measurements of epithelial length (OE, ZE, SO, RE) in a dorso-ventral direction are plotted for four individual animals. The mean values of the length of the ZE increases dramatically (P1: 0.37 mm, P600: 3.2 mm), nearly 10-fold, over the lifespan. The proportion of the ZE length occupies an increasing region, from 20% of the septal wall length in neonates to 45% in adults. Non-sensory patches within the SO are indicated in Figure 3 by tiny white stripes on the black bars of the SO.

In all age groups, turbinates covered by olfactory epithelium are seen in coronal sections at the level of the SO. In the oldest age group investigated, at least the second endoturbinate appears (Figure 2). In newborns, the SO is even seen together with the olfactory bulb (Figure 5; see also Weiler *et al.*, 1999).

With respect to the VNO

In newborns, the SO is seen together with the VNO in a coronal section (Figure 5). The SO starts approximately in the middle of the antero-posterior length of the VNO and extends posteriorly slightly beyond its caudal end. During postnatal development, the relative positions of the two organs change resulting in a relatively more anterior position of the VNO (Weiler *et al.*, 1999). The rostral end of the SO is then located dorsal to the posterior end of the VNO. In animals older than P181, the SO is completely posterior to the VNO (Figure 6) so that from that age onwards the two organs are not seen together in coronal sections.

With respect to the nasopharyngeal duct (NPHR)

The nasopharyngeal duct emerges where the two sides of the nasal cavity become confluent because the septal wall retracts from the hard palate (Katz and Merzel, 1977; Weiler *et al.*, 1999). At P1 the SO is completely anterior to the NPHR, whereas at P18 the SO ends just where the NPHR begins. With increasing age, the posterior end of the SO is located relatively more caudally and extends into the region of the NPHR (Figure 6). In the 6–15 month age groups 25%

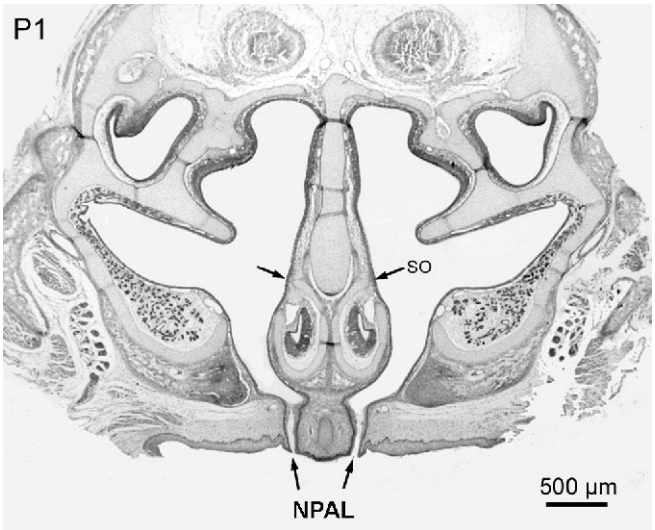

Figure 5 The paired nasopalatine ducts (NPAL) connect the nasal cavity with the mouth, just at the anterior end of the SO. Hematoxylin and eosin stained section of a P1 animal.

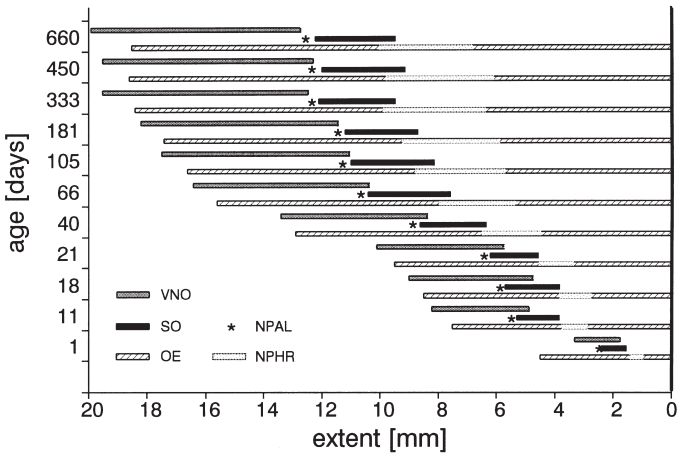

Figure 6 Shift of the relative position of SO relative to the OE, VNO, NPHR and the NPAL (asterisk) in the antero-posterior direction. The values in the graph are from male rats. Zero is set to the *posterior* end of OE (the *anterior* end of the animal's nasal cavity would be to the left of the *x*-axis as in Figure 1). The opening into the VNO would be near the anterior end of the VNO, to the left in this graph.

of the SO is on the septum above the NPHR. This percentage increases to 32% at P600.

With respect to the nasopalatine duct (NPAL)

The NPAL is paired and connects each side of the nasal cavity with the mouth. At P1 the NPAL extends in a vertical direction between the nasal cavity and the oral cavity at the mid-level of the VNO, just at the anterior end of the SO (Figure 5). In adult rats the NPAL connects the nasal and oral cavities at an oblique angle, rostro-dorsal to caudo-ventral, just below the caudal end of the VNO (Wöhrmann-Repenning, 1981). Again the nasal opening of the duct is just anterior to the SO (see also Clancy *et al.*, 1994; Schoenfeld *et al.*, 1994). In all age groups, therefore, the SO starts close to the NPAL (between 0 and 400 µm posterior to NPAL opening) and stretches posterior to this point.

In summary: during postnatal development, there is a shift of SO relative to OE, VNO and NPHR, but SO consistently remains close to the NPAL opening.

Sensory cells in the septal organ are OMP-positive

Olfactory marker protein is a protein expressed nearly exclusively in mature olfactory sensory cells (Farbman and Margolis, 1980; Margolis, 1982). In all age groups we saw OMP-immunoreactive sensory neurons in the SO (Figure 2). The number of OMP-positive cells increases postnatally in the SO. In contrast to OE, there are usually only one to three rows of OMP-positive neurons in the SO; this is related to the thickness of the epithelium. In general, the SO neurons appear to stain much more intensely than neurons in the OE. At least in the rat, neurons in thin areas of OE are often much more intensely immunoreactive with OMP antibody than those in thick epithelia. The nerve bundles in the lamina propria are also more intensely reactive and can easily be distinguished from the much lighter staining VNO nerve bundles which pass through the submucosa beneath the SO (Figure 7). The OMP immunoreactivity reveals that especially in older animals the SO contains patches of non-sensory epithelium interspersed within the sensory epithelium (Figure 7). In neonates, P5 and P11 animals, the ZE between OE and SO contains many OMP-positive cells (Figure 7) but their number declined with increasing age. The oldest age containing single OMP-positive neurons in ZE was P66. Scattered OMP-positive neurons are also seen anterior to the SO. The scattered single OMP-positive cells within the ZE lay basally, in contrast to the SO or OE where they lay in rows higher up in the epithelium, above the basement membrane (Figure 7). This allowed us to establish a dorsal boundary of the SO with assurance.

Discussion

Our results show that the rat SO grows until the animals are mature, then stops growing and even decreases in size in males, whereas the OE continues to expand. The SO position shifts relative to other nasal cavity structures, but remains close to the NPAL. The SO contains fewer neurons/unit area than the OE and its capacity for neurogenesis is limited.

Morphometric characteristics of SO compared to OE and VNO

SO area increases until the rat reaches sexual maturity (P66–P105), thereafter it decreases in males, whereas OE area continues to expand well beyond P300 (Weiler and Farbman, 1997). Consequently the relative proportion of the SO area to the total olfactory surface sheet declines from 1% at birth

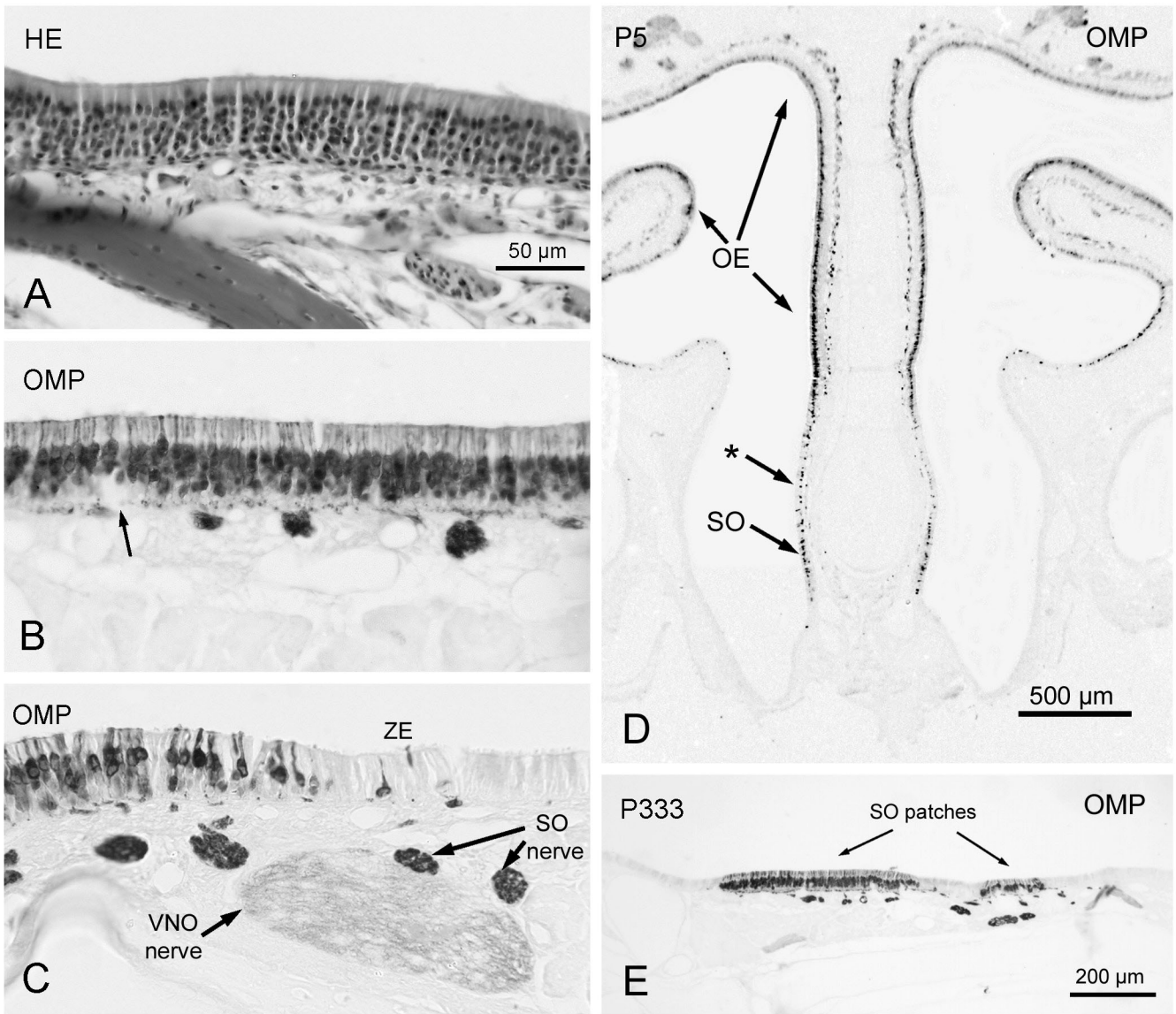

Figure 7 **(A)** Septal organ in an hematoxylin and eosin (HE)-stained section revealing the pseudostratified columnar organization of its epithelium. Scale bar applies to A–C. **(B)** In the OMP-antibody-treated section, two or three rows of positive cells can be seen. The arrow indicates an intruding gland. **(C)** At the boundary of the SO and ZE, OMP immunoreactivity in SO is present in 1–3 rows of neurons, all located at the mid-level of the epithelium, whereas in the ZE the immunoreactivity is in a few cells located more basally. The SO nerve is much more intensely immunoreactive than the accompanying VNO nerve. **(D)** OMP-positive cells within the SO and in the ZE (asterisk) in young animals. **(E)** OMP-immunoreactivity reveals that the SO in old animals is interspersed with patches of non-sensory epithelium. The SO frays.

to 0.3% in adults. A decrease in *area per histological section* was also reported in the VNO after maturity (Weiler *et al.*, 1999); however, the VNO continues to expand in the antero-posterior direction as does the OE. Thus, the growth pattern of the SO is different from both OE and the VNO.

The thickness of SO epithelium is less than that of either OE or VNO and remains constant throughout development. Epithelial thickness in OE and VNO is correlated with the number of neurons (Weiler and Farbman, 1997; Weiler *et al.*, 1999). The SO contains only 1–3 rows of OMP-positive neurons, compared with 6–8 in most regions of OE and more in VNO. Low epithelial thickness was also reported in the hamster SO (Schoenfeld *et al.*, 1994), indicating that SO contains fewer neurons/unit area than the OE.

Projections to the olfactory bulb

In the adult male rat, the SO shrinks when SO neurons are lost. SO neurons project via two nerve bundles to ~1% of the glomerular population, predominantly to ~30 'septal' glomeruli (e.g. Wysocki *et al.*, 1985; Ma *et al.*, 2003), although some fibers innervate glomeruli shared by OE axons (Giannetti *et al.*, 1992). If individual SO neurons, like

OE neurons, express a single odorant receptor and all neurons expressing a particular receptor project to the same glomeruli (Ressler *et al.*, 1994; Vassar *et al.*, 1994; Mombaerts, 1996), the projection pattern suggests the SO has its own cohort of odorant receptors, unique to the SO. However, using a mixture of four odorant receptor probes, neurons in the OE and the SO were labeled, suggesting SO and OE neurons express common odorant receptors (Kishimoto *et al.*, 1994), consistent with the observation of common glomeruli to SO and OE axons (Giannetti *et al.*, 1992). When the SO area decreases in adults, the loss might affect the entire palette of receptors, or neurons bearing certain receptors are selectively lost, diminishing the number of septal glomeruli.

OMP in the SO and the ZE

Sensory neurons of the SO express OMP (e.g. Breipohl *et al.*, 1989; Chen *et al.*, 1992). The OMP immunoreactivity of SO (and ZE) neurons was more intense than of most OE neurons. We found OMP-positive cells within the ZE up to P66, extending data of Giannetti *et al.* (1995a) who report ZE neurons up to the age of P7. The OMP-positive cells in the ZE lie more basally than those in the SO and their number decreases with age. This suggests that in adults the ZE lacks progenitors for neuronal replacement.

Limited regeneration

Our results show that the SO in unperturbed old animals (i) shrinks and (ii) is interspersed by patches of respiratory epithelium. This was also observed in hamster, where the SO was described as variable in size and shape and often difficult to identify as a continuous epithelium because sensory neurons appeared scattered (Clancy *et al.*, 1994). BrdU studies showed the total number of proliferative cells in the adult SO after P105 is lower than at the age P66–105 (Weiler and Farbman, 2001). These observations strongly imply that the SO has a limited neurogenetic ability, unlike the OE and VNO, which have the capacity for continuous replacement of neurons.

Shifting positions of SO and the NPAL

Differential growth rates of nasal structures during development result in a relocation of the relative position of the SO within the nasal cavity. The most significant point to emerge is that the SO remains immediately posterior to the opening of the NPAL. In adult rodents the NPAL traverses the palate in a dorso-rostral to ventro-caudal direction (Wöhrmann-Repenning, 1984a,b,c) opening into the nasal cavity posterior to the VNO but close to the SO (Clancy *et al.*, 1994; Schoenfeld *et al.*, 1994).

In many other mammalian species the NPAL opens directly into the vomeronasal duct (e.g. Cooper and Bhatnagar, 1976; Hedewig, 1980a,b; Schilling *et al.*, 1990), thus providing the VNO with direct access to the oral cavity. Those species, for example carnivores (cat: Breipohl *et al.*, 1983; ferret: our unpublished observations) do not possess a SO. It is possible, then, that the SO is present only in animals in which a patent NPAL does not open into the vomeronasal duct.

Possible function

The close anatomical proximity of NPAL and SO strongly suggests that a special function of the SO might be to sample odorants brought into the mouth by licking and passing through the NPAL to the nasal cavity. This is supported by experiments in which guinea pigs were allowed to lick conspecific urine containing rhodamine, a non-volatile fluorescent dye. The dye appeared in the SO and the VNO as well as in the NPAL, but was not present in OE (Wysocki *et al.*, 1980). The ability of the SO to bind non-volatile substances in urine implies an involvement of the SO in *social/sexual behavior* supported by the overshoot in SO size at sexual maturity. Overshoot phenomena are related to imprinting of behavioral cues (Nottebohm, 1981; Apfelbach *et al.*, 1985). Thus SO might act as an accessory olfactory organ.

The SO also reacts to airborne substances, brought in via the nares or with the retronasal airflow via the NPHR, and responds to several odorants (Ma *et al.*, 2003) with an even higher sensitivity than the OE (Marshall and Maruniak, 1986). This suggests another possible function of the SO, *testing food odors*, which might be related to the taste buds described within the NPAL (Kolmer, 1927; Wöhrmann-Repenning, 1978, 1980, 1982, 1993; Settembrini, 1987; Liem *et al.*, 1990a,b).

Is the SO just an ectopic piece of OE ?

Whereas the general cellular composition of the SO is similar to that of OE, and both are derived from the olfactory placode, as is the VNO (Bojsen-Møller, 1975), some of our data support the argument that it is a distinct accessory organ.

1. The SO area reaches maximum size at maturity, whereas the OE continues to grow into senescence.
2. The capacity for neurogenesis is significantly poorer than of VNO or OE (Weiler and Farbman, 2001). Foci of non-sensory epithelium appear in the SO (also in hamster: Clancy *et al.*, 1994).
3. The SO epithelial height is less than that of OE; it contains fewer neurons (cf. Schoenfeld *et al.*, 1994); its height does not change postnatally in contrast to OE and VNO (Weiler and Farbman, 1997; Weiler *et al.*, 1999).

In addition, data from other laboratories lend support to this argument:

1. The SO axons form their own nerve consisting of two fascicles which differ in histology, course and termina-

tion from OE (Bojsen-Møller, 1975). The projection of SO is predominantly to 'septal glomeruli' within the olfactory bulb (e.g. Giannetti *et al.*, 1992; Ma *et al.*, 2003), suggesting SO has its own odorant receptors.

2. Following 3-methylindole treatment, axons of OE sensory neurons entering the main olfactory bulb are severely damaged (Setzer and Slotnick, 1998). The region of the SO terminations and the VNO terminations in the AOB are preserved (Zaiens and Slotnick, 2002).
3. Freeze–fracture studies showed a higher density of intramembranous particles in SO ciliary membranes than in OE, but similar to VNO (Breipohl *et al.*, 1983; Miragall *et al.*, 1984).
4. The maturation of the SO with respect to PGP9.5 positive sensory cells is complete after birth, whereas this occurs in the OE before birth (Oikawa *et al.*, 2001).
5. Bowmans glands intrude into the SO frequently, a characteristic not true for OE, where only the ducts pass. Respiratory glands are located beneath the lamina propria of the SO, whereas they never extend into the OE sheet.
6. Calbindin, a calcium-binding protein essential for signal transduction, is more heavily expressed in SO and VNO than in the OE (Kishimoto *et al.*, 1993). Immunhistochemical studies showed that SO neurons exhibit calcitonin-gene-related peptide (CGRP) immunoreactivity; OE and VNO neurons do not (Silverman and Kruger, 1989).

Conclusion

The present study shows that the SO differs in several aspects from OE and VNO, and that it has a limited neurogenetic capacity. The relation between the NPAL and the SO remains constant throughout development; this suggests the SO functions as a sensor of odorants that reach it via the NPAL from the mouth, with possible involvement in sexual behavior. We speculate that only species where the NPAL is not connected to the VNO possess a SO, and that the SO should be considered as a separate accessory olfactory organ.

Acknowledgements

The research described in this paper was supported by NIH grants nos. P01 DC 00347, R01 DC02126. The antibody against OMP (Olfactory marker protein) was generously provided by Dr Frank Margolis (University of Maryland).

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Accepted June 27, 2003