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The sense of smell: multiple olfactory subsystems

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Abstract. The mammalian olfactory system is not uniformly organized but consists of several subsystems each of which probably serves distinct functions. Not only are the two major nasal chemosensory systems, the vomeronasal organ and the main olfactory epithelium, structurally and functionally separate entities, but the latter is further subcompartimentalized into overlapping expression zones and projection-related subzones. Moreover, the populations of 'OR37' neurons not only express a unique type of olfactory receptors but also are segregated in a cluster-like manner and generally project to only one receptor-specific glomerulus. The septal organ is an island of sensory epithelium on the nasal septum positioned at the nasoplatine duct; it is considered as a 'mini-nose' with dual function. A specific chemosensory function of the most recently discovered subsystem, the so-called Grueneberg ganglion, is based on the expression of olfactory marker protein and the axonal projections to defined glomeruli within the olfactory bulb. This complexity of distinct olfactory subsystems may be one of the features determining the enormous chemosensory capacity of the sense of smell.

Keywords. Main olfactory system; vomeronasal organ; septal organ, Grueneberg ganglion; receptors.

Introduction

Animals constantly survey their external environment for chemicals advising food sources and habitats but also for signals controlling social interaction and reproductive behavior. The chemical compounds are sensed by monitoring the respiratory airstream through highly specialized detectors, the chemosensory neurons, which are organized in structurally and functionally divergent subsystems in the nasal cavity. Generally, two systems are distinguished: the main olfactory epithelium (MOE), which is considered to be responsible for sensing and discriminating the myriads of volatile odorous compounds, and the vomeronasal organ (VNO), which is thought to mediate the detection of substances carrying specific information concerning species, gender and identity of an animal [1, 2, 3]. However, recent studies have challenged such a strict functional categorization of these two systems [4], and furthermore have disclosed that the chemosensory system is much more complex, composed of various unique entities, each of which is characterized by distinct structural features and appears to cope with a particular chemosensory task.

Subsystems of the main olfactory epithelium

In mammals, the MOE covers cartilaginous lamellae in the posterior nasal cavity, called turbinates, comprising a few million olfactory sensory neurons (OSNs). These bipolar sensory cells extend a single dendrite to the epithelial surface carrying numerous long cilia embedded in the nasal mucus, providing an extensive surface for interaction with odorants. The ciliary membrane contains receptor proteins [5] and elements of the olfactory transduction machinery [6] which render these cellular compartments chemosensoric. The receptors are distinct types of G-protein-coupled receptors (GPCRs), the odorant receptors (ORs) [7]. Out of a repertoire of about 1300 OR genes [8], an individual OSN appears to express only one type [9, 10]. Each OR interacts with a broad range of chemical compounds, albeit with different affinities; thus, one OR recognizes multiple odorants, and an odorant is recognized by various OR types. This combinatorial receptor strategy is used to encode odor qualities [10,

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11]. All OSNs which express the same OR send their axons to common glomeruli in the main olfactory bulb (MOB). Glomeruli are neuropil networks of synaptic interaction between the axon terminals of the OSNs and the dendritic trees of the olfactory bulb projection neurons; they are considered as modules for representing and processing sensory features; [12, 13]. The convergence of receptor-specific neuron populations onto mutually exclusive glomeruli generates a chemospecific map which is considered as the basis for a combinatorial processing of molecular entities, leading to the identification of odors [14–16]. The olfactory information processed by the complex neuronal network of the olfactory bulb is relayed by the mitral cells to higher brain regions for the perception of smell [17].

Topographical subsystems of the MOE

In rodents, OSNs expressing distinct receptor types are distributed in characteristic topographical zones of the epithelium. Although it was originally thought that the epithelium is subdivided into a few, well-circumscribed rostral/caudal zones [18–20], recent studies indicate that cells expressing ORs are rather organized in overlapping zonal positions which are continuously arrayed along the central to peripheral axis of the epithelium [21, 22]. Moreover, each of these zones can be subdivided: cells expressing the same OR appear to be randomly distributed within a designated area, but in fact, concerning their projection into the bulb they can be grouped into a medial and a lateral subpopulation, which converge separately onto medial and lateral glomeruli, respectively [23, 24]. The functional implication of such topographic expression patterns for defined groups of receptor types is still elusive; however, its importance is underscored by related patterns of expression for a variety of cell surface proteins, such as semaphorins [25] and ephrins [26]. The differential segregation of OSNs with distinct receptor types in spatially confined areas of the chemosensory epithelium has been related to complex airflow during inspiration [16]. Within the olfactory recesses of the rodent nose, turbinates form anatomically separate channels through which air and odorants flow during inspiration. The air entering the olfactory recesses first passes through a dorsal channel approaching the dorsal region, then courses more peripherally towards ventral and lateral regions [16]. It has been suggested that the nose of terrestrial vertebrates, which is lined with aqueous mucus, may act as a gas chromatograph; i.e. inhaled odorous molecules may be selectively adsorbed from the respiratory airstream [27]. Thus, according to their relative adsorptiveness, odorants migrate through the nose differently; hydrophilic molecules are retained to a greater extent than hydrophobic molecules. As the airflow will first approach the dorsal region, this area will preferentially encounter the most adsorptive hydrophilic odorous compounds. Interestingly, the ORs expressed in the dorsal zone include all of the phylogenetically old class I receptors that are most related to those found in aquatic vertebrates, such as fish and amphibians [28, 29], and which are expected to be particularly tuned to hydrophilic odorants [30]. The notion that the dorsal zone may be specialized in the detection of more hydrophilic odorants was supported by electrophysiological recordings and functional analyses indicating that the dorsal region is generally more responsive to highly adsorptive, more hydrophilic odorants, whereas the lateral-ventral areas are more responsive to less adsorptive, more hydrophobic odorants [31, 32]. Thus, the disproportional expression of distinct OR types along the inspiratory airstream may have important functional implications for efficiently matching the physicochemical features of odorous molecules and the relevant receptor types.

The 'OR37' system, a subsystem of the main olfactory epithelium

Despite enormous sequence variability, OR genes encode proteins with a rather uniform size and membrane topology. However, there is one exception to this rule: a small group of ORs, the 'OR37' subfamily, is characterized by the insertion of six amino acids in the third extracellular loop (E3-loop) [33, 34]. The extended loop in the 'OR37' receptors has a high content of charged amino acids, which seem to be exposed mainly to one side of the helix [35]. The unique structural feature of the 'OR37' receptors suggests that they are specifically tuned to special odorous ligands. Comparative analyses have shown that potentially orthologous receptors exist in diverse mammalian species, such as mouse, dog, elephant and in primates [34a]. Even in humans, this group of receptors exists, with a surprisingly high fraction of potentially functional genes, a situation that is against the general trend of pseudogenization for OR genes in humans. When compared across all species, the 'OR37' receptors display an unexpected high degree of conservation (Fig. 1A); bioinformatic data reveal that these genes apparently are under a negative selective pressure, another feature that seems to be quite unusual for OR genes, which in general are rather under positive darwinian selection [36–39]. Positive selection is thought to favor diversification and thus variability of receptor types, whereas the observed negative selection for the 'OR37' receptors promotes their conservation, further supporting the idea that these are specially tuned receptors. The pronounced diversity in the surrounding noncoding sequences indicates a long co-existence of the genes. Indeed, phylogenetic analyses have demonstrated that 'OR37' genes already exist in 'ancient' mammals, like the opossum [34 a]. OR37' receptors are not found in non-mammals [35], suggesting that this subfamily of re-

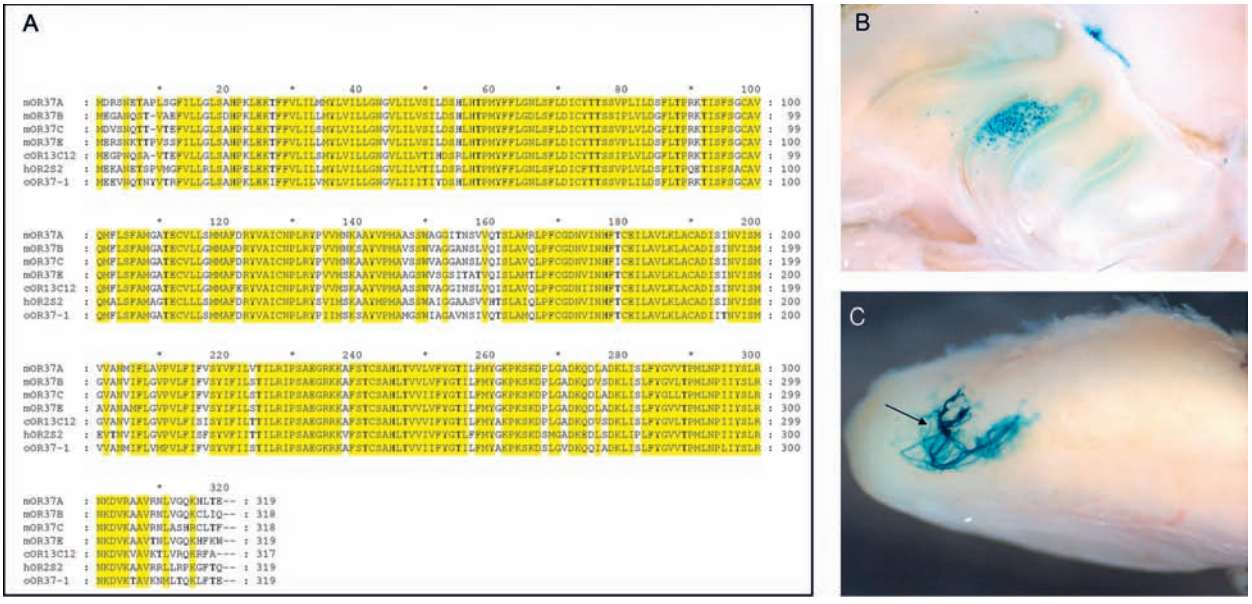

Figure 1. (*A*) Amino acid sequence alignment of representative 'OR37' receptors from mouse (m), dog (c), human (h) and opossum (o). Residues which are identical in all subfamily members are indicated in yellow. (*B*) Pattern of 'OR37'-expressing cells in the nasal cavity; whole-mount view of the right half-head from an mOR37-lacz mouse [42] stained with X-Gal. Cells are clustered in a central region of the turbinates. (*C*) Projection pattern of 'OR37'-expressing cells; whole-mount view onto the ventral surface of the main olfactory bulb reveals stained fibers converging onto a single glomerulus (arrow).

ceptors was specifically 'devised' by mammals and may serve a special function.

'OR37' receptors are expressed in OSNs, which are organized in a special topographic pattern; they are exclusively located in a small, central region of the olfactory epithelium (Fig. 1B). This so-called clustered expression pattern is clearly unique and different from the typical zonal expression pattern. Although the 'OR37'-expressing cells occupy only a small patch in the epithelium, the number of cells expressing a distinct OR type is not smaller; consequently, the density of cells expressing an 'OR37' receptor in the cluster is four to five times higher than for zonally segregated OSNs. The high concentration of cells expressing 'OR37' receptors could make this region of the epithelium particularly sensitive to the appropriate odorants. The basis for this topographic expression pattern of the 'OR37' genes is still elusive; however, analyzing the structure of 'OR37' genes within and across a variety of species has disclosed highly conserved DNA elements in the promotor region [40, 41]. The unraveled combination of DNA motifs is common to all 'OR37' genes, but is not present in promotors of zonally expressed genes; thus, they are considered as candidates for regulatory elements determining the unique clustered expression pattern of this gene subfamily.

Since zonally organized populations of OSNs converge their axons onto common glomeruli in the bulb, it has been suggested that the clustered 'OR37' cells might rather disperse their axons onto many different glomeruli. However, studies using gene-targeted mouse lines revealed that the clustered cells also converge their axons onto a common glomerulus [42]. However, again uniquely, axons for a distinct 'OR37' population target mainly onto a single glomerulus (Fig. 1C). This is in clear contrast to the cells that are zonally arranged and project to two distinct glomeruli which are distantly positioned at the medial and lateral side of each bulb. The distinct glomeruli for the various 'OR37' subtypes are gathered in a small subdomain of the bulb and positioned in close neighborhood [42]. The 'OR37' subdomain of the bulb is located in the ventral region, close to the axis of symmetry that divides the bulb into the two hemispheres. This ventral part of the bulb appears to play a particular role in processing odor information, since so far only very few odorants have been found that elicit any responses in this extreme ventral aspect of the bulb [43] (also see http://leonlab.bio.uci.edu/). The observation that a large fraction of glomeruli in the ventral regions of the bulb are activated upon stimulation with urine [44–46] has raised the intriguing possibility that neurons expressing 'OR37' receptors might be involved in detecting distinct compounds of urine, some of which are involved in social communication between individuals.

Subsystem of the GC-D-positive neurons

Although it is generally assumed that olfactory signal transduction in ciliated OSNs of the MOE is mediated by the canonial adenylate cyclase/cyclic AMP (cAMP) cascade, a small subpopulation of ciliated cells in the

MOE lacks the key elements of this olfactory signaling pathway, including the G-protein G_{olf}, type III adenylyl cyclase (ACIII), the phosphodiesterases PDE1C2 and PDE4A, and the olfactory cyclic nucleotide-gated channel [47, 48]. It was found that these sensory cells express a distinct subtype of the transmembrane receptor guanylyl cyclase, termed GC-D [47, 48]. The GC-D isoform is apparently specifically expressed in OSNs which are randomly distributed in a rather broad and central region of the MOE [49]. It is unknown whether the GC-D neurons comprise ORs, but it has been suggested that GC-D itself might interact with olfactory cues; in fact, it shares remarkable sequence similarities with other guanylyl cyclases which are transmembrane receptors for peptides. Due to the intrinsic guanylyl cyclase activity of GC-D [49], it has been speculated that in GC-D-neurons, cGMP instead of cAMP may mediate the transduction process. This notion was supported by the finding that GC-D neurons also express other signaling elements of a cGMP-mediated pathway, including the cGMP-stimulated phosphodiesterase PDE2 and a cGMP-specific cyclic nucleotide-gated channel. Moreover, these elements were found to be localized in the cilia, which are considered to be the chemosensory sites of these cells [47, 48]. Like other sensory cells of the MOE, the GC-D-expressing neurons extend axons which project to the MOB where they innervate about 12 glomeruli in the 'necklace glomerular complex' which encircles the caudal region of the bulb [47]. As the necklace glomeruli are supposed to be associated with pheromone-induced suckling behavior in pups, it has been speculated that GC-D neurons might be involved in the detection of pheromone-like compounds [47].

The vomeronasal organ and its subsystems

Most mammals possess a well-developed vomeronasal system, which is usually considered to be specialized for processing pheromonal information [50]. The VNO is a blind-ending, mucus-filled tube in the nasal septum with a crescent-shaped sensory epithelium located at the medial wall of the organ and a large blood vessel running laterally to the lumen [51, 52]. It is considered to be a 'specialized nose' tuned for detecting chemical signals which are emitted by other animals and convey specific information concerning species, gender and identity; they induce innate behaviors, such as aggression and mating [53–55]. Chemical signals from the environment reach the lumen of the tubelike VNO by a vascular pumping mechanism through the vomeronasal duct, which opens into the nasal cavity [56]. This mechanism also enables the VNO to receive relatively non-volatile compounds.

Basal and apical layer of VNO neurons

The vomeronasal sensory neurons can be categorized into two subpopulations organized in separate layers of the vomeronasal epithelium. Cells in the apical layer express V1R receptors and G_i proteins; they project to the anterior part of the accessory bulb. The cells in the basal layer express V2R receptors and G_o proteins; they project to the posterior part of the accessory bulb [50]. The V1R-expressing cells are responsive to small hydrophobic molecules, such as volatile urinary components [57]. The responsive cells are extremely sensitive, with thresholds in a range of 10^{-11} M e.g. for farnesene; in addition, they are highly selective for individual compounds [57]. Thus, sensory cells of the VNO differ significantly from sensory neurons of the main olfactory system, which are much less sensitive and typically respond to a broad range of odorants.

The V2R receptors expressed by sensory cells in the basal layer differ markedly from the V1R receptors; they comprise a large N-terminal domain which is supposed to form the ligand binding site. Furthermore, most of the about 100 V2R receptors seem to be co-expressed with a so-called V2R2 receptor which is expressed throughout the basal layer [58]. It has been suggested that this may reflect the formation of receptor dimers similar to the T1R taste receptors [59]. Although the experimental data are still limited, recent studies indicate that the V2R-expressing cells respond to proteinaceous components [60, 61].

Odorant-responsive neurons in the VNO

Several lines of evidence suggest that the vomeronasal system may also respond to general volatile odorants as readily as the main olfactory system. First, analyzing the responsiveness of sensory cells from the VNO, it was found that a small number of cells isolated from the VNO specifically respond to volatile compounds which also elicit distinctive smells [62]. In a more recent study analyzing mice with a non-functional main olfactory system due to the knockout out of ACIII [63], it was found that these mice still respond to particular odorants [64]. This observation led to the idea that ACIII-independent detection of odorants may be possible through the VNO; in fact, it was demonstrated that odorous compounds elicit electrical responses in the sensory epithelium of the VNO. From these data, it was concluded that – in addition to the main olfactory system – the accessory olfactory system may also have the capacity to detect and process odor information [64]. The molecular basis for the recognition of odorants by the sensory cells of the VNO remained elusive. It has been speculated that the odorous compounds may be able to activate certain VNO cells via their V1R or V2R receptors; however, this view would imply that odorant stimuli are processed like the pheromonal compounds

that are detected by the respective receptors. However, work by Trinh and Storm [64] indicated, that food odors are recognized as such and elicit the appropriate behavior. These observations suggest that there might be distinct subpopulations of sensory cells in the VNO which are specifically tuned for responding to odorants. In fact, recent *in situ* hybridizations and analyses of transgenic mouse lines have provided evidence for a third population of sensory neurons in the VNO which express OR subtypes that are concomitantly also expressed in the MOE (Fig. 2A, B) [64a]. This observation raises the question whether these are VNO cells expressing ORs, or typical MOE cells ectopically positioned in the VNO. Molecular phenotyping of these cells has indicated that the OR-expressing cells in the VNO do not express ACIII and also lack G_{olf}; instead, they are equipped with G_i and TRPC2 channels, suggesting that at least some OR subtypes appear to be promiscuous concerning G protein coupling. Since olfactory neurons in the MOE which express a distinct OR type project to glomeruli in the main olfactory bulb, the question arises whether cells expressing the same OR type, but 'misplaced' in the VNO, may nevertheless also project their axons to the receptor-specific glomerulus. Analysis of transgenic mice which enables visualization of OR-expressing cells and their axons reveals that labeled nerve fibers from the VNO do not project to the MOB, but terminate in the most anterior region of the accessory olfactory bulb (AOB) (Fig. 2C) [64a]. This characteristic wiring pattern of OR-expressing cells in the VNO and MOE suggests that certain odorants may be processed differently, both as odors and also as pheromones.

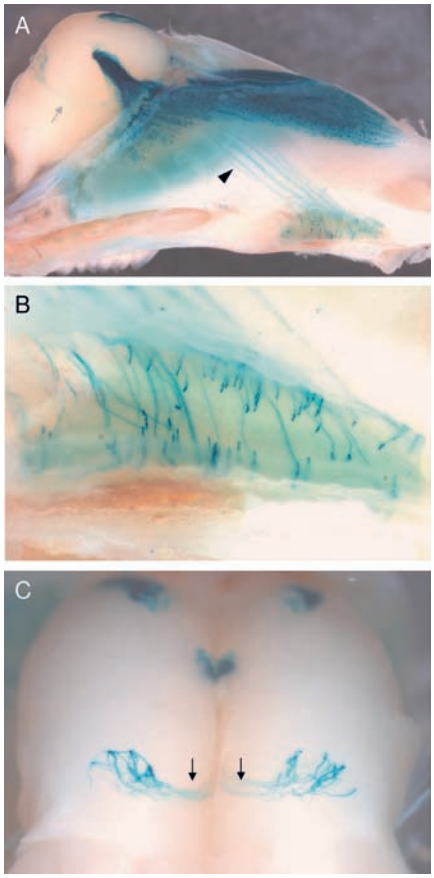

Figure 2. (*A*) X-Gal-stained whole mount preparation of the nasal septum and olfactory bulb from a transgenic mouse (MOL2.3-IGITL) [85] in which cells co-express the odorant receptor *mOR18-2* together with lacZ. The fibers of cells expressing *mOR18-2* in the vomeronasal organ (VNO) can be traced along the septum (arrowhead). In the bulb, fibers are visible cruising in posterior-dorsal direction (arrow). (*B*) A close view onto the VNO reveals homogeneously distributed *mOR18-2* expressing cells. (*C*) Fibers of *mOR18-2*-expressing cells from the VNO (arrows) reach the accessory olfactory bulb and terminate in a few, small glomerular-like spots. Figure based on unpublished data by T. Feistel.

The septal organ

The septal organ (SO), first observed by Broman [65] in newborn mice and named as 'Riechepithelinsel', is a patch of sensory epithelium completely separated from the MOE by respiratory tissue. It was later investigated in more detail by Rodolfo-Masera [66] and has since been known also as the 'Organ of Masera'. Despite its discovery decades ago it remained relatively unexplored for a long time, and its relevance is still enigmatic. Towards an understanding of whether the SO operates as a completely independent chemosensory pathway, sensing distinct odors and projecting to distinct brain regions, or whether it contributes to the sense of smell by supplementing the activities of the MOE and/or the VNO, great efforts have been made in recent years to explore the molecular phenotype of sensory cells populating the SO and to visualize the projection patterns of their axons.

The SO is an island of sensory tissue on each side of the nasal septum positioned posterior to the nasopalatine and anterior to the nasopharyngeal duct (Fig. 3C). The area occupied by the SO is highly variable among individuals and sometimes even varies between contralateral surfaces of the same animal [67]. The sensory epithelium of the SO appears to be quite similar to the MOE: it is composed of ciliated OSNs as well as basal and sustentacular cells, but it comprises only one to three layers of sensory neurons (Fig. 4B), compared with six to eight in most regions of the MOE [24, 68]. The notion that the SO is indeed a chemosensory system has been confirmed by physiological studies, demonstrating that it responds to a broad range of odor stimuli [69]. In order to unravel the molecular basis for the chemosensitivity of the SO, attempts have been made to identify receptors which are expressed in sensory cells of the SO. Tissue was isolated from OMP-GFP transgenic mice, where the SO can be visualized due to intense fluorescence (Fig. 3A, B), thus

allowing precise isolation of the complete organ [70]. Different approaches, including reverse transcription-polymerase chain reaction (RT-PCR) experiments and microarray assays led to the identification of 50–80 OR types [70, 71]. Categorizing the ORs expressed in the SO reveals that these are exclusively class II ORs which belong to various families. However, no class I ORs were found. From certain small families, more than a third of the members are expressed in the SO, whereas from a particularly large family only a single member is present [70]. These observations suggest that the choice of an OR type from the large repertoire by a given SO neuron does not occur at random. All OR genes expressed in the SO are also expressed in the MOE, mainly in the medial or lateral zone of the nasal neuroepithelium [70, 71]. The number of cells expressing a particular OR type varies considerably. Most of the ORs are found only in a small number of cells; a few receptors are found in several hundred cells; however, receptor type *mOR244-3* is expressed in ~20% of all cells [70] (Fig. 4C) and *mOR256-3* in approximately half of all SO neurons (Fig. 4D) [71]. Despite such a high proportion of cells expressing a particular OR, there is no evidence for expression of more than one receptor type per cell.

Concerning the axonal connections of SO neurons to the olfactory bulb, earlier studies [72] have demonstrated that axons from the SO are organized in only two discrete bundles, which remain separated from axon fascicles originating from the VNO or from the MOE. Subsequent tracing studies provided evidence that all the SO projec-

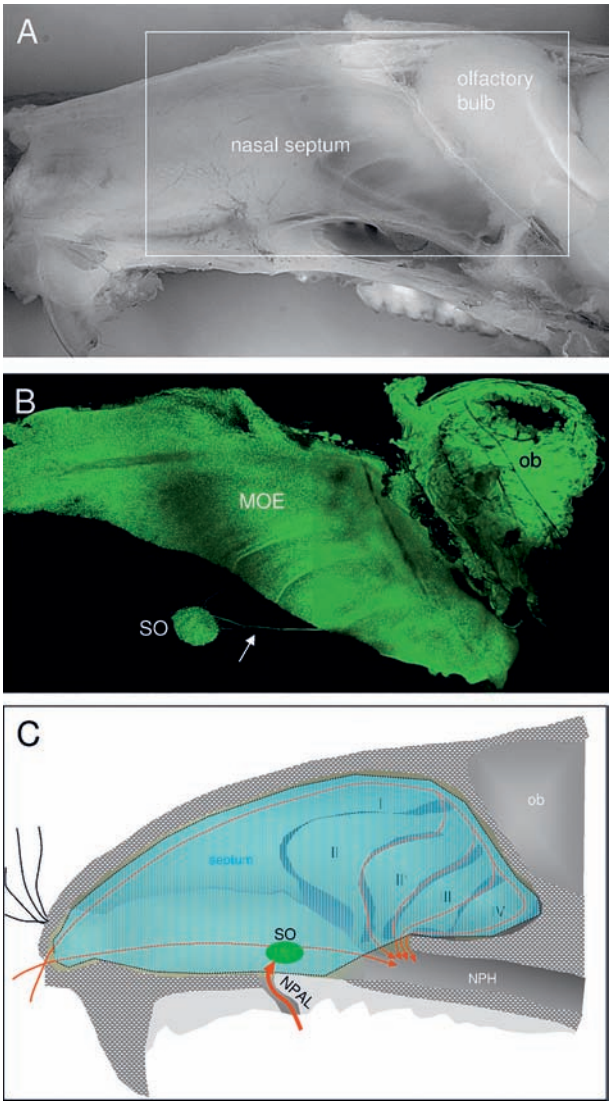

Figure 3. (*A*) View onto the nasal septum and olfactory bulb of an OMP-GFP mouse [86]; (*B*) under GFP illumination, the septal organ (SO) becomes visible as a fluorescent patch within the respiratory epithelium of the septum. Axons from the SO neurons extend in posterior direction (arrow) and can be followed until they enter the main olfactory epithelium (MOE) sheet. (*C*) Schematic drawing of the rodent nasal cavity displaying a cutaway view of the right nose exposing the medial surfaces of the endoturbinates and the olfactory bulb; the nasal septum is drawn as a transparent blue structure. The position of the SO on the septum is indicated by the green area. Nasal airflow patterns as determined for rats are indicated by thin red lines. Air flow from the oral cavity through the nasopalatine duct is indicated by thick red line. I–IV, endoturbinates; NPAL, nasopalatine duct; NPH, nasopharyngeal duct. Panels *A* and *B* from [23] with permission from Springer; panel *C* redrawn after [87].

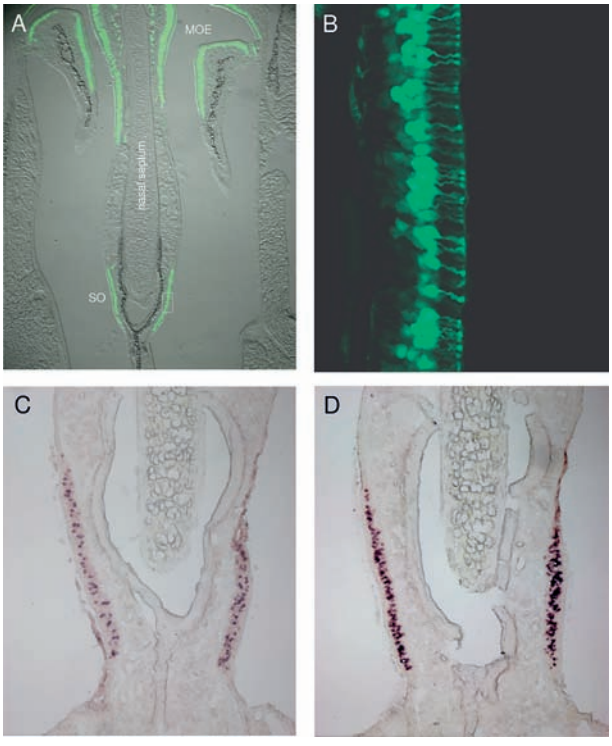

Figure 4. (*A*) Coronal section through the anterior region from the nose of an OMP-GFP mouse. The SO is located in a ventral position on both sides of the nasal septum, separated from the MOE by respiratory epithelium. (*B*) At higher magnification, the knob, dendrite and soma of individual SO neurons are visible. The cell bodies are organized in only one to three layers. Distribution pattern of the OR gene *mOR244-3* (*C*) and *mOR256-3* (*D*), respectively, in the SO.

tions are confined to the MOB; however, the reports were inconsistent regarding dorsomedial [73] or ventromedial [74] projection. Using transgenic olfactory marker protein-green fluorescent protein (OMP-GFP) mice for precise deposition of anterograde tracers and complete coverage of the sensory epithelium, it became obvious that the SO fibers navigate towards the bulb in a variable number of bundles [23]. They all reach the cribriform plate at a well-circumscribed site, and all bundles cross the plate at a very narrow window located almost exactly in the center of the dorso-ventral extension of the cribriform plate. The SO fiber bundles enter the MOB on the medial side; from here, two principles of projection can be distinguished. One branch turns towards the ventral surface of the MOB, where the thick bundle defasciculates into smaller bundles which terminate in one of a few sites (glomeruli), which are about 70–100 µm in diameter and located in a small domain of the bulb (Fig. 5A, C). The number of glomeruli varies between four and six. The second branch remains at the medial side of the MOB, where the bundles defasciculate, forming a dense mesh of very thin fibers (Fig. 5B, D). These fibers terminate in multiple glomeruli; each of them targeted by a different number of SO fibers. About 30 glomeruli receive a noticeably number of SO fibers; they are clustered at the ventro-medial surface of the bulb (Fig. 5F). About 150 glomeruli receive only a single or at most a few SO fibers, which show extensive branching. Thus, the vast majority of glomeruli receiving input from the SO appear to be of a 'mixed phenotype'; i.e. they receive their main input from the MOE and to varying degrees additional input from the SO. A few glomeruli appear to be distinct in that they receive input exclusively from the SO, thus representing 'septal' glomeruli (Fig. 5E).

The molecular phenotype and projection patterns of sensory cells in the SO support the notion that this 'island' of sensory epithelium on the nasal septum is not just an ectopic area of the MOE or the VNO, but rather a unique chemosensory entity. Due to its strategic location directly at the opening of the nasopalatine duct, which connects the mouth with the nasal cavity (Fig. 3C), the SO is perfectly suited to sample odorous compounds from the oral cavity. These could be airborne substances but also non-volatile compounds transferred by licking activities. Consequently, it is conceivable that the SO may have a dual functional role: being involved in surveying food odors, as well as in detecting social-sexual signal molecules. This view is in fact in line with the dual principle for both the expression pattern of receptor types (a few receptor types in most of the sensory cells; many receptor types each in just a few cells) as well as the projection pattern of the SO nerve fibers (either a dense projection into a few 'septal' glomeruli or minor projections of a few axons to many glomeruli) [75]. Thus, the SO may be viewed as a 'mini-nose' with a dual function, contributing to the olfactory system receiving and processing myriads of odors.

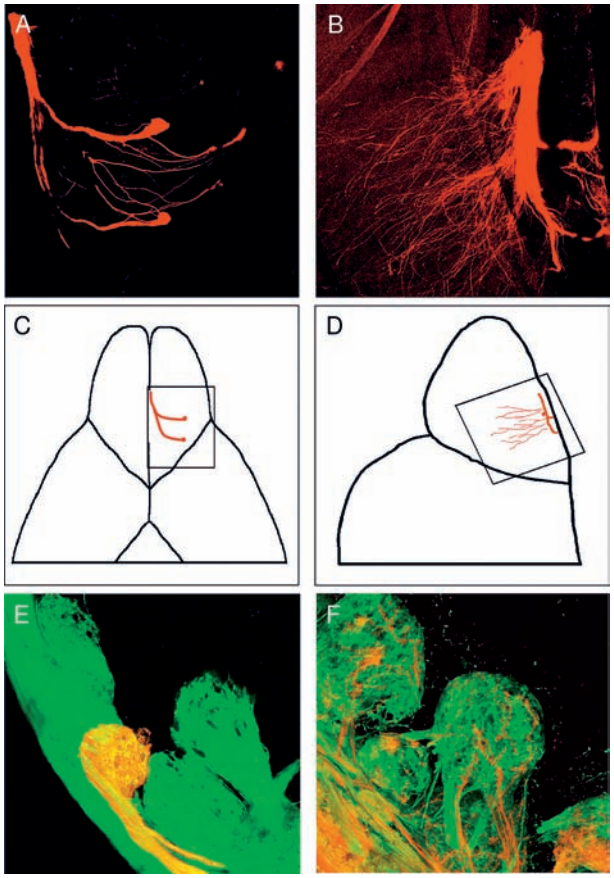

Figure 5. Projection pattern of SO neurons into the main olfactory bulb visualized by DiI tracing. Labeled SO fibers enter the bulb on the medial side and then curve towards a posterior and ventral domain where they form a few glomerular structures in the ventral (*A*, *C*), or a dense network on the ventro-medial (*B*, *D*) region, respectively *E, F*) Small sector from the ventral region of a cross section through the olfactory bulb showing a glomerulus which receives input exclusively from DiI-labeled fibers originating from the SO (septal glomerulus) (*E*) and glomeruli located in the ventro-medial aspect of the MOB receiving input from a small number of DiI-labeled fibers (*F*). Data from [23] with permission from Springer.

The Grueneberg ganglion: a putative olfactory organ

In a study examining nasal glands, Grueneberg [76] discovered a 'ganglion of unknown function', situated bilaterally in the rostro-dorsal area of the nasal cavity in the corners formed by the septum and the nasal roof. The neuronal cells of the ganglion form small groups or larger clusters and are accompanied by satellite cells [76]. The ganglion was originally considered as part of the *Nervus terminalis*; however, the absence of luteinizing hormone-releasing hormone (LHRH) [77], a characteristic marker of the terminal nerve [78, 79], argues against an affiliation of the Grueneberg ganglion (GG) to the terminal

nerve, and several recent studies suggest that this 'ganglion' may in fact be a chemosensory organ. This notion is based both on the expression of OMP (Fig. 6), indicative of OSNs, and the projection of axonal processes to glomeruli in defined areas of the olfactory bulb [80, 81]. On the other hand, GG cells lack some typical features of other chemosensory cells, such as cilia or microvilli. Ultrastuctural analyses have indicated the presence of several modified cilia [82], a feature also described for distinct chemosensory cells in the olfactory epithelium of fish, designated as 'crypt cells' [83, 84]. Although chemosensory stimuli for the GG cells are still elusive, it has been speculated that the ganglion may sense gaseous stimuli, such as oxygen, or may receive stimuli by direct contact with the source [81]. Based on their axons projecting to the region of the 'necklace glomeruli' in the olfactory bulb, which are active during suckling behavior of pups, it has been suggested that the GG may be involved in detecting important cues for newborns [80, 81]. Due to the very anterior position of the GG, it seems more likely that the cells may serve in detecting chemical cues with a limited volatility or such that require rapid detection [81].

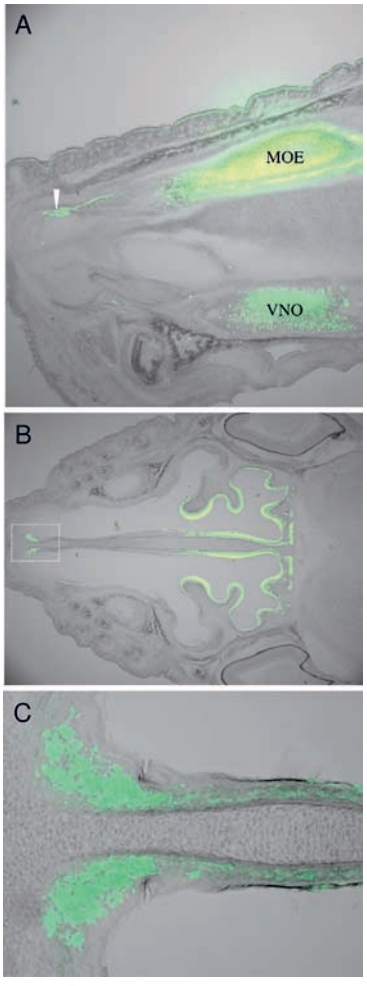

Figure 6. Expression of olfactory marker protein in the Grueneberg ganglion. Sagittal (*A*) and horizontal (*B*) section through the head of a newborn OMP-GFP mouse. In the anterior nasal area, a cluster of green fluorescent cells is visible (arrowhead in *A*, boxed area in *B*). (*C*) Higher magnification of the boxed area in *B*. Data from [77] with permission from Springer.

In mice, the first evidence for the development of the GG appears at embryonic day 14 (E14). This initial finding by Grueneberg [76] recently received substantial support from staining approaches using an RNA antisense probe specific for the neuronal marker βIII tubulin, which enabled visualization of the presence of neurons in the very anterior part of the nasal epithelium [77]. Later in development, βIII tubulin-positive cells are no longer positioned in the epithelium but in a ganglionic cell cluster beneath the epithelial layer, indicating that the GG is derived from the local epithelium. Expression of the OMP is first detectable at E14 [77, 80, 81]. The number of OMP-positive cells in the GG reaches a peak in the perinatal phase (about 500 cells per GG) and slightly decreases postnatally [77, 81]. In adults, the GG no longer has a clusterlike shape, but is organized as a thin and interrupted layer of cells located beneath the nasal epithelium [76, 77], indicating that full maturation of the GG might not be accomplished at birth. In spite of a slight reduction of OMP cells, the GG persists throughout life and even exists in 2-years-old mice [76, 77, 81]. In any case, unlike in the MOE, the VNO and the SO, in the GG, OMP-positive cells are not harbored in a pseudostratified epithelium.

The GG is apparently associated with two distinct types of nerve fibers: an afferent fiber and an efferent one, the latter clearly growing out from the central nerve system during embryogenesis. It is interesting to note that during embryonic development, formation of the GG starts when the efferent nerve reaches the area of the presumptive GG, leading to the hypothesis that efferent fibers may induce generation of the GG [76]. During their course, efferent and afferent fibers are intermingled and project caudally along the dorsal aspect of the septum. When they reach the level of the MOE, they join up with olfactory axons originating from the MOE, making it difficult to follow them further [76]. From each neuron of the GG, an axonal process emerges at a random position [82] projecting in the caudal direction. By application of the fluorescent tracer DiI (a substance that spreads in the cell membrane), it is possible to visualize the entire trajectory along the roof of the nasal cavity. The axon bundles penetrate the cribriform plate, reach the MOB and terminate in its caudal region. They innervate a group of 10 glomeruli in the region of the so-called necklace glomeruli [77, 81, 80].

Conclusion

The sense of smell is a highly versatile chemodetector that receives myriads of odorous compounds and

is involved in governing characteristic behaviors. This multifunctional task is accomplished by an orchestrated interplay between several olfactory subsystems each of which probably serves distinct functions. For the two major nasal chemosensory systems, the MOE and the VNO, which are supposed to be involved in detecting common odorants and pheromones, respectively, this is reflected in different cell types (cilia vs. microvilli), different receptors and transduction cascades as well as projection sites into different brain regions. The MOE is further subcompartmentalized in various spatially separated, albeit overlapping expression zones for distinct receptors and projection-related subzones. This zonal organization is of particular interest in view of the recent consideration concerning respiratory airflow within the rodent nose; this notion is underscored especially for the dorsal zone, where most of the fishlike class I receptors are expressed and which will encounter preferentially the most adsorptive hydrophilic odorous compounds. Interspersed in the caudal recess of the nasal cavity are the so-called GC-D neurons, which express the receptor type guanylate cyclase-D and project axons to the necklace glomeruli; this subsystem seems to be involved in receiving distinct maternal chemical cues during the perinatal phase. The populations of 'OR37' neurons not only express a unique class of highly conserved olfactory receptors but are also located in clustered manner as a distinct island at a particularly exposed site within the MOE; moreover, they generally project to only one receptor-specific glomerulus in the rostroventral aspect of the bulb. Although the adequate odorous signals for this unique subsystem are still elusive, it is quite obvious that the mammalian-specific 'OR37' system plays a very special role in odor reception. The septal organ is a spatially well separated patch of sensory epithelium on the nasal septum and is a sole chemosensory entity. Based on its strategic location at the nasopalatine duct, as well as the dual patterns of OR expression and axon projection by the sensory neurons, the septal organ is considered as a 'mini-nose' with a dual function: detecting airborne chemicals as well as non-volatile compounds transfered by licking, thus receiving food odors as well as social-sexual signals. The most recently discovered subsystem, the so-called Grueneberg ganglion, is located in the rostral nasal vestibule far anterior of any of the other subsystems. Although the identity of the receptors is still elusive, its chemosensory function is based on the expression of olfactory marker protein and the axonal projections to defined glomeruli within the olfactory bulb. It has been suggested that this subsystem may participate in receiving social signals most relevant during the early postnatal phase. Thus, the emerging picture indicates that the olfactory system comprises a variety of morphological, molecular and functional subsystems with defined projection patterns. Each of them appears to contribute in a unique manner to accomplish the daunting tasks of the sense of smell.

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