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Zonal organization of the mammalian main and accessory olfactory systems

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Zonal organization is one of the characteristic features observed in both main and accessory olfactory systems. In the main olfactory system, most of the odorant receptors are classified into four groups according to their zonal expression patterns in the olfactory epithelium. Each group of odorant receptors is expressed by sensory neurons distributed within one of four circumscribed zones. Olfactory sensory neurons in a given zone of the epithelium project their axons to the glomeruli in a corresponding zone of the main olfactory bulb. Glomeruli in the same zone tend to represent similar odorant receptors having similar tuning specificity to odorants. Vomeronasal receptors (or pheromone receptors) are classified into two groups in the accessory olfactory system. Each group of receptors is expressed by vomeronasal sensory neurons in either the apical or basal zone of the vomeronasal epithelium. Sensory neurons in the apical zone project their axons to the rostral zone of the accessory olfactory bulb and form synaptic connections with mitral–tufted cells belonging to the rostral zone. Signals originated from basal zone sensory neurons are sent to mitral–tufted cells in the caudal zone of the accessory olfactory bulb. We discuss functional implications of the zonal organization in both main and accessory olfactory systems.

Keywords: olfactory bulb; zonal organization; olfactory epithelium; vomeronasal epithelium; odorant receptors; pheromone receptors

1. INTRODUCTION

Since the pioneering and classic works of Mountcastle (1957) and Hubel & Wiesel (1959), understanding the detailed functional organization of cortical regions of the brain has been an important and fascinating challenge for neuroscientists (Hubel & Wiesel 1998). Accumulating lines of evidence indicate that each cortical region is composed of large numbers of neurons that are organized in multiply replicated functional columns or modules. The functional modules are spatially arranged in characteristic and function-related ways in individual neocortical regions. The characteristic spatial arrangements can be seen, for example, as E-E bands (summation columns) and E-I bands (suppression columns) in the auditory cortex (Brugge & Merzenich 1973), as the barrel arrangement of the rodent somatosensory cortex (S-I) (Woolsey & Van der Loos 1970), and as the ocular dominance column arrangement and pinwheel-like structures of iso-orientation domains in the visual cortex (Hubel & Wiesel 1972; Hubel *et al.* 1978; Bonhoeffer & Grinvald 1991).

Functional modules similar to those seen in the above neocortical regions are present also in the simple cortical structure of the mammalian main olfactory bulb (MOB), the first station for the processing of odour molecule information in the central olfactory pathway. An emerging view is that the MOB is composed of thousands of signal-processing glomerular modules, each representing a single or a few type(s) of odorant receptors (ORs). Recent studies showed that these glomerular modules are spatially arranged in a zonal fashion and that each zone in the MOB represents a zonal subset of ORs (Buck 1996; Yoshihara & Mori 1997; Mori *et al.* 1999). In the first part of this review we focus on the zonal organization of sensory neurons in the olfactory epithelium (OE) and the zonal arrangement of glomeruli in the MOB. Because of the direct connection of sensory axons with bulbar neurons, the MOB provides a good model system with which to analyse the detailed functional organization of the cortical structure.

In addition to the main olfactory system, most mammals have an accessory olfactory system in which pheromonal information is processed (Keverne 1999). Sensory neurons in the accessory olfactory system lie in the vomeronasal epithelium (VNE) and project their axons to the accessory olfactory bulb (AOB). The AOB contains hundreds of glomeruli within which it receives axonal inputs from the vomeronasal sensory neurons. Each glomerulus represents one or a small number of vomeronasal receptors (VRs) (Rodriguez *et al.* 1999; Belluscio *et al.* 1999). In the latter part of § 3 we describe the zonal organization of the sensory neurons in the VNE and the zonal arrangement of glomeruli in the AOB. We compare the main and accessory olfactory systems in respect to the detailed modular organization and their zonal arrangement.

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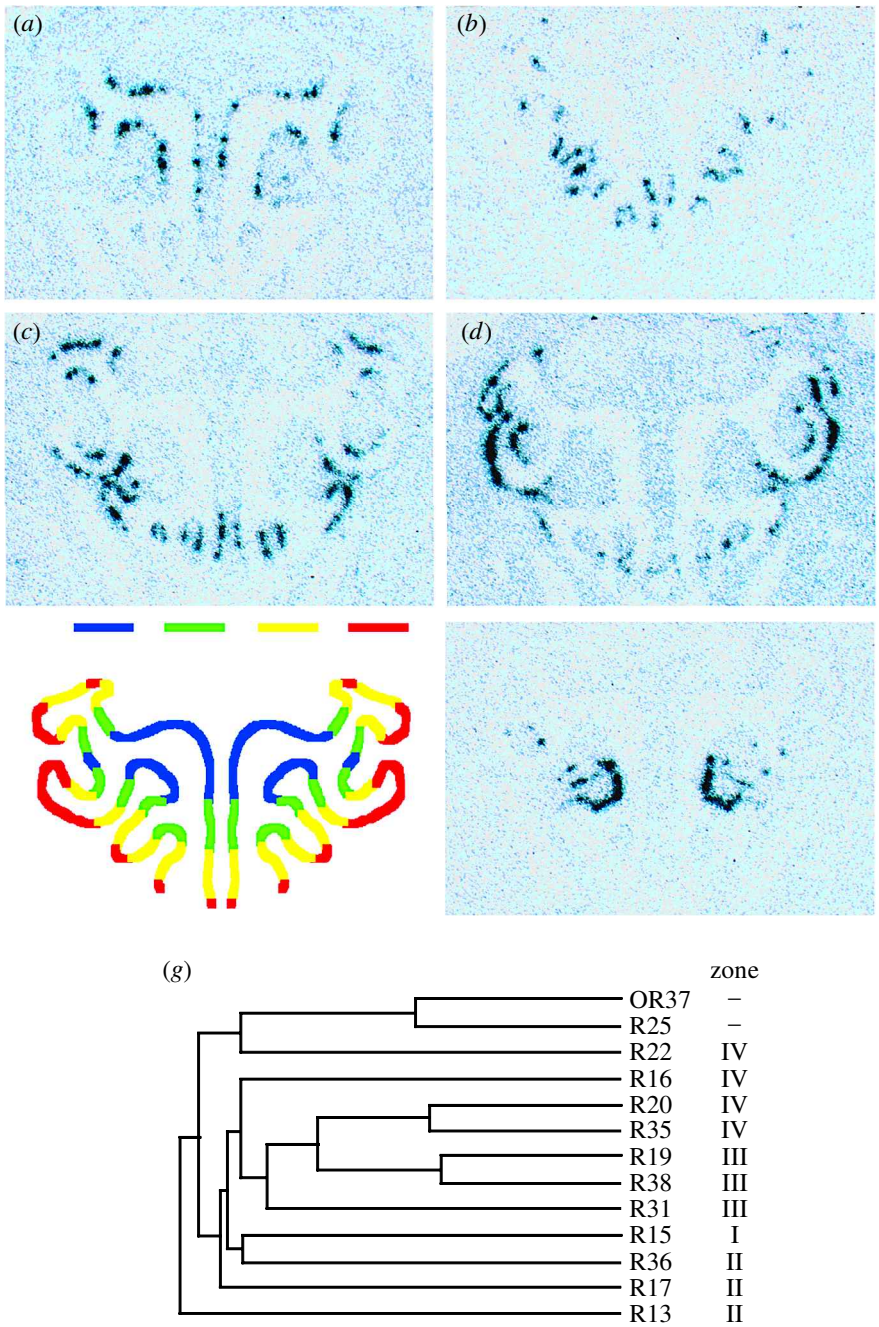


Figure 1. Zonal organization of mammalian olfactory epithelium (OE). (*a–d*) Odorant receptor (OR) expression zones in the mouse OE. Coronal sections were hybridized with OR cRNA probes: zone I OR (R15) in (*a*), zone II OR (R36) in (*b*), zone III OR (R38) in (*c*), and zone IV OR (R20) in (*d*). (*e*) A schematic diagram illustrating the spatial arrangement of four zones in a coronal section of the OE. (*f*) *In situ* hybridization showing an expression of a fifth type of OR (R25). (*g*) Structural relationship among several mouse ORs compared with rat OR37. Partial amino-acid sequences were compared using DNASIS phylogenetic tree program. Expression zones of ORs in the OE are indicated on the right. Rat OR37 and mouse R25 show strong homology and are unique in that they do not belong to any of the four zonal categories.

2. ZONAL ORGANIZATION IN THE MAIN OLFACTORY SYSTEM

(a) *Odorants and their receptors*

Odour molecules are detected at the cilial surface of olfactory sensory neurons in the OE. A wide variety of volatile odorants with different chemical structures are emitted from a given substance, inhaled into the nose, and reach the OE to bind OR on the cilia of sensory neurons. The binding of odorants to serpentine ORs initiates a metabotropic signal transduction cascade (for reviews, see Reed 1992; Buck 1996). An olfactory-specific GTP-binding protein (G_{olf}) mediates activation of adenylyl cyclase, leading to second messenger cAMP formation. The increased cAMP directly opens cyclic nucleotide-gated non-selective cation (Na^+ and Ca^{2+}) channels and then Ca^{2+}-dependent Cl^- channels (Kurahashi & Yau 1993), resulting in depolarization of the plasma membrane and generation of a train of action potentials that are transmitted to the MOB via olfactory

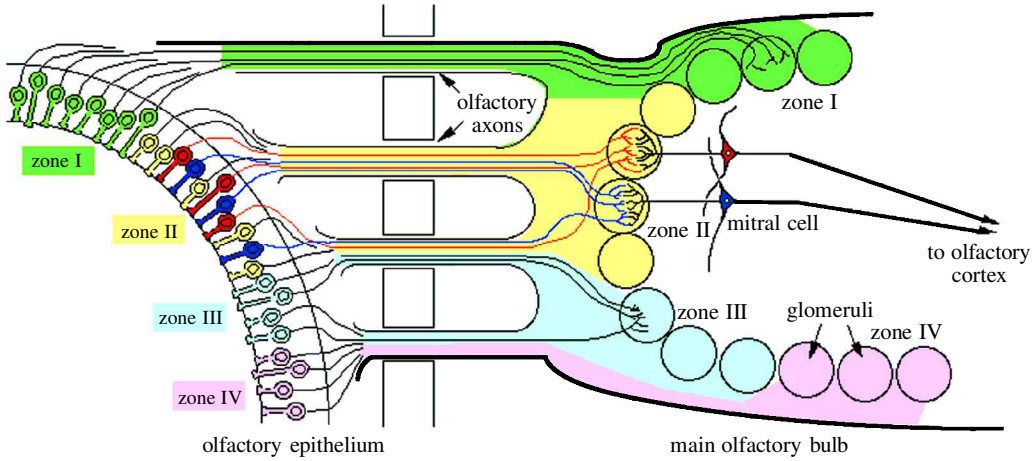


Figure 2. Zone-to-zone projection of olfactory axons. Four zones of the OE (zones I, II, III, and IV) connect with corresponding zones (*zones I, II, III* and *IV*) of the MOB. In a given zone, sensory neurons expressing the same OR converge their axons onto a few fixed glomeruli. Within the glomeruli, olfactory axons form excitatory synaptic connections on dendrites of mitral and tufted cells. Axons of mitral and tufted cells project to the olfactory cortex. Modified from Yoshihara *et al.* (1997).

axons. Thus, the signal transduction cascade in the olfactory sensory neurons initiated by odorant ligands is very similar to that in other types of cells that respond to a variety of neurotransmitters or hormones.

The mammalian main olfactory system exhibits a unique feature in that it furnishes *ca.* 1000 ORs, which constitute the largest multigene family ever identified (Buck & Axel 1991). This repertoire of a large number of ORs is required for animals to receive and to discriminate between a much more immense number of odorants. How many types of ORs are expressed in a single olfactory sensory neuron? Detailed *in situ* hybridization analysis revealed that each OR gene is detected in *ca.* 0.1–0.2% of olfactory sensory neurons on average, suggesting that individual neurons express only one out of *ca.* 1000 OR genes (Ressler *et al.* 1993; Vassar *et al.* 1993; Buck 1996). This notion was supported by the finding of allelic inactivation of OR genes, enabling olfactory sensory neurons to express only one allele of the selected OR gene (Chess *et al.* 1994). In addition, the 'one neuron–one receptor' hypothesis was recently confirmed by single-cell reverse transcriptase (RT)–PCR analysis of sensory neurons (Malnic *et al.* 1999). Thus, phenotypes of individual olfactory sensory neurons are primarily determined by the selection of OR to be expressed. In other words, the neurons are functionally distinct in their responses to odour molecules.

Genetic and molecular mechanisms underlying OR selection by olfactory sensory neurons have not been elucidated. In the human and mouse, OR genes are clustered within multiple loci that are broadly distributed on multiple chromosomes (Ben-Arie *et al.* 1994; Griff & Reed 1995; Sullivan *et al.* 1996). OR genes with very similar sequences tended to be localized in tandem in the same chromosomal locus. However, there is no precise correspondence between the chromosomal loci and the expression zones (see §2(b))—each cluster contains OR genes expressed in different zones and ORs in the same zone are scattered in different chromosomes (Sullivan *et al.* 1996).

The relationship of odour ligand–receptor interaction has been recently examined by three unique strategies. (i) An adenoviral vector-mediated *in vivo* expression of ORI7 followed by a physiological recording of electro-olfactogram from the OE revealed that *n*-octanal and structurally related aldehydes are specific ligands for ORI7 (Zhao *et al.* 1998). (ii) Screening of an expression library containing a large and diverse repertoire of OR sequences was performed to identify specific ligands for the ORs. This study indicated that even a single amino-acid substitution in the OR sequence leads to a change in ligand preference (Krautwurst *et al.* 1998). (iii) Specificity of ligand–receptor interaction has been analysed by using a combination of calcium imaging of individual sensory neurons and single-cell RT–PCR (Malnic *et al.* 1999; Touhara *et al.* 1999). The results indicated that one odorant can activate multiple ORs and that one OR can be activated by multiple odorants. In addition, different odorants activate different combinations of ORs, indicating that the olfactory system uses combinatorial OR codes for information processing in the OE.

(b) *Zonal classification of odorant receptors*

Rodent ORs are classified into four groups according to their zonal localization in the OE. A given OR is expressed by sensory neurons distributed within one of four circumscribed zones in the OE (Vassar *et al.* 1993; Ressler *et al.* 1993; Sullivan *et al.* 1995) (figure 1*a–e*). In other words, the OE is divided into four spatially segregated zones (from the dorsomedial zone I to the ventrolateral zone IV, figure 1*e*) that are defined by the expression of OR mRNA. Within a given zone, neurons expressing a given OR are randomly dispersed and intermingled with neurons expressing other types of ORs. This zonal organization of the OE seems to be applicable also to sustentacular cells—phenol sulphotransferase was shown to be expressed selectively by sustentacular cells within zone I of the OE (Miyawaki *et al.* 1996).

An exceptionally unique pattern of expression has been reported in the case of OR37 in rats (Strotmann *et al.* 1992). The OR37-expressing neurons are not restricted to one of the four zones but are much more clustered and located over multiple zones on endoturbinate II and ectoturbinate 3 (Strotmann *et al.* 1994). We also found another OR (R25) in mice that showed an unusual expression

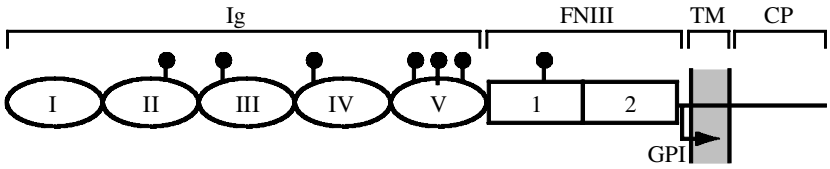


Figure 3. Structure of OCAM. (*a*) OCAM has five immunoglobulin (Ig)-like domains and two fibronectin type III (FNIII)-like domains in its extracellular region. OCAM has two isoforms: one has a transmembrane (TM) region and a cytoplasmic (CP) region, while the other is attached to the membrane via glycosylphosphatidylinositol (GPI) linkage.

Table 1. *Zonal expression pattern of OCAM in the main and accessory olfactory systems*

(In the main olfactory system, OCAM is expressed by olfactory axons originating from sensory neurons in zones II, III, and IV of the OE. Zone I sensory neurons do not express OCAM. In the accessory olfactory system, apical zone sensory neurons express OCAM, whereas OCAM is absent from basal zone sensory neurons. During development, mitral–tufted cells in MOB and AOB express OCAM in a zone-specific manner (Von Campenhausen et al. 1997; Trelor *et al.* 1998).)

	main olfactory system				accessory olfactory system	
zone	I	II	III	IV	apical	basal
sensory neurons	—	+	+	+	+	—
developing mitral or tufted cells	+	—	—	—	—	+

pattern similar to OR37 (figure 1*f*) and whose structure was closely related to that of OR37 (figure 1*g*). These results suggest that there exists a fifth group of ORs in addition to the four zonal groups.

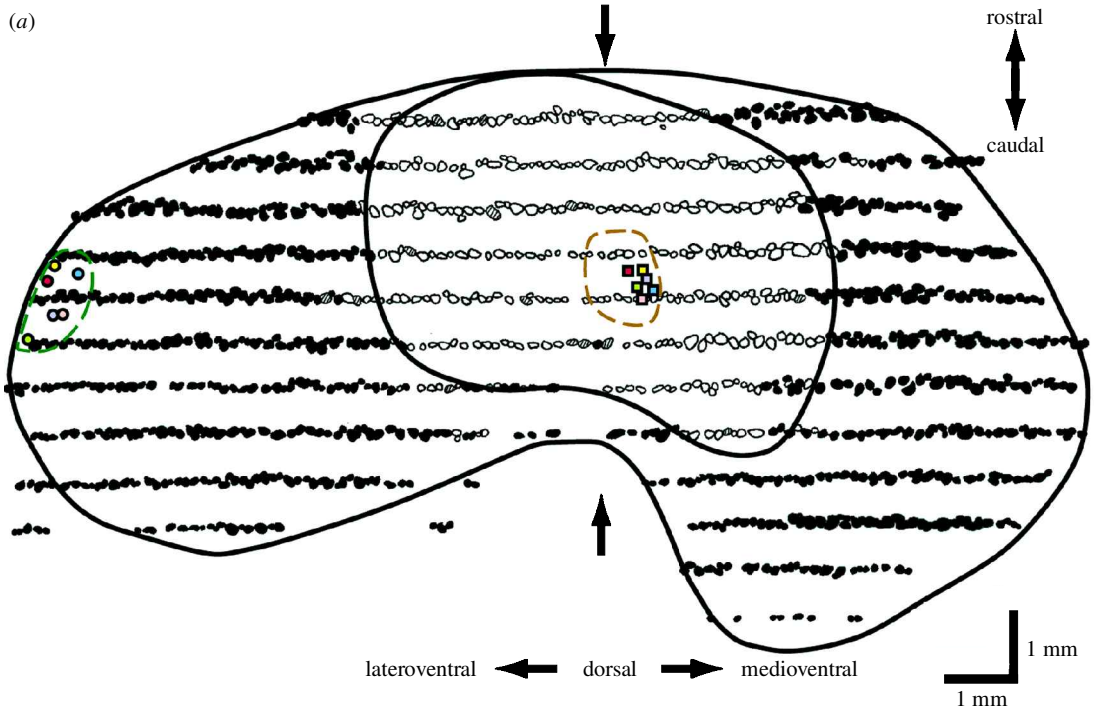
A spatial arrangement of ORs somewhat similar to the mammalian zones has been reported in the sheet of olfactory sensory neurons of zebrafish and *Drosophila*. In zebrafish, individual ORs are distributed in one of three overlapping concentric zones in the olfactory rosette (Weth *et al.* 1996). In *Drosophila*, individual ORs are expressed in one of the topographically restricted domains in either the antenna or the maxillary palp (Clyne *et al.* 1999; Vosshall *et al.* 1999; Guo & Chess 1999). These results suggest that olfactory information is first spatially classified into different zonal subsets at the level of the olfactory sensory neurons, and that this is conserved across a variety of animal species. Furthermore, a zone-like topographical sheet may be important for the pattern of axonal projections to different zones (regions) of the brain.

What kind of genetic and molecular mechanism governs the zonally restricted expression of OR genes? Qasba & Reed (1998) generated transgenic mouse lines that express *LacZ* transgene (β-galactosidase) under the control of 5′-flanking region of the *M4* OR gene. They demonstrated that a 6.7 kb region upstream of the *M4* coding region was sufficient to direct expression of the transgene in a zone-specific manner. This result suggested that crucial *cis*-regulatory elements directing zone-specific expression were located in close proximity to transcriptional initiation sites of the OR genes. However, responsible transcription factors that may restrict the zone-specific expression of ORs have not been identified.

ORs with highly homologous amino-acid sequences tend to be expressed in the same zone of the OE (Sullivan *et al.* 1995; Malnic *et al.* 1999; Tsuboi *et al.* 1999). Because homologous ORs may bind to distinct but overlapping ranges of odour molecules, sensory neurons in the same zone may have a tendency to show similar tuning specificity to odorants. In accordance with this hypothesis, recordings of electro-olfactogram (odour-evoked summed potentials of sensory neurons; Ezeh *et al.* 1995) and Ca^{2+} imaging of individual sensory neurons (Sato *et al.* 1994; Malnic *et al.* 1999) have shown that sensory neurons with similar tuning specificity to odour molecules tend to be localized in a particular zone of the OE.

Figure 4. Unrolled map of OCAM-positive and OCAM-negative glomeruli in the rabbit MOB (*a*) and comparison of response specificity of mitral–tufted cells in one region in zone I (*b*) and in another region in zone III or IV (*c*). Response specificity to odorants differs systematically between neurons in different domains or zones of the rabbit MOB. (*a*) Positions of mitral–tufted cells in two different regions of the MOB were shown on the unrolled OCAM map. The flattened glomerular layers with OCAM-positive glomeruli (shown by filled spots) and OCAM-negative glomeruli (open spots) were aligned from dorsal to caudal using the dorsal edge (arrows). A cluster of fatty-acid-responding neurons were localized within the region surrounded by the brown broken line. For comparison, response specificity of mitral–tufted cells in the region (a green broken line) in the most ventral part of the OCAM-positive zone was examined. (*b*) Response specificity of six representative mitral–tufted cells (shown by squares with different colours) in the fatty-acid-responding region (brown line in *a*) within the OCAM-negative zone. Each neuron typically responded to a range of aliphatic acids and/or aldehydes (surrounded by coloured lines) having similar molecular structures. Stimulus odorants are shown by their molecular formula. (*c*) Response specificity of neurons (circles with different colours) in the most ventral region (green line in *a*) within the OCAM-positive zone. Each neuron in this region responded to a range of aliphatic aldehydes, alcohols and/or alkanes, but did not respond to fatty acids.

(*a*)



(*b*)

no. of carbon atoms	aliphatic acids	aliphatic aldehydes	aliphatic alcohols	alkanes
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

(*c*)

no. of carbon atoms	aliphatic acids	aliphatic aldehydes	aliphatic alcohols	alkanes

(c) *Zone-to-zone projection of olfactory axons*

Each olfactory sensory neuron in the OE projects a single axon to a single glomerulus and forms synaptic connections with the dendrites of MOB neurons. How do the olfactory sensory neurons in individual OE zones project their axons to the MOB topographically? Several lines of evidence suggest that zonal organization in the OE is preserved basically at the level of the glomerular sheet of the MOB, i.e. zone-to-zone projection of olfactory axons (figure 2).

Studies of anatomical tracing between the OE and the MOB suggested topographical organization of the olfactory axon projection (Le Gros Clark 1951; Saucier & Astic 1986; Schoenfeld *et al*. 1994). This notion was clearly demonstrated by detailed immunohistochemical analyses with specific monoclonal antibodies (MAb). MAb R4B12, which was raised against a homogenate of rabbit olfactory bulb, recognizes an antigen molecule (designated as olfactory cell adhesion molecule (OCAM)) expressed by a subpopulation of the olfactory sensory axons in a zone-specific manner (Fujita *et al*. 1985; Imamura *et al*. 1985; Mori *et al*. 1985, 1987; Yoshihara *et al*. 1997). OCAM is expressed by the olfactory axons originating from zones II, III, and IV of the OE, but not by the axons from the most dorsomedial zone I. OCAM-positive and OCAM-negative axons show clearly segregated termination into glomeruli in the caudoventral and rostrodorsal zones of the MOB, respectively. RNCAM, a rat homologue of OCAM, was independently discovered with MAb RB-8 and proved to be expressed in a zone-specific pattern similar to that of rabbit OCAM (Schwob & Gottlieb 1986, 1988; Alenius & Bohm 1997).

A staining pattern complementary to OCAM was reported with another MAb CC2 that recognizes a unique carbohydrate epitope on a subset of the rat olfactory axons (Schwarting & Crandall 1991). Expression of the CC2 antigen is restricted to the olfactory axons originating from epithelial zone I and projecting into glomeruli in the most rostrodorsal zone of the MOB. Thus, several molecular markers have been used as convenient tools for distinguishing axonal projections from different zones of the OE.

A zone-to-zone projection pattern of olfactory axons was implied also by *in situ* hybridization studies using several OR probes (Vassar *et al*. 1994; Ressler *et al*. 1994). A very sensitive *in situ* hybridization analysis with ^{33}P-labelled cRNA probes for OR genes yielded an unexpected but interesting result that OR mRNAs are detectable not only in the OE but also in the glomeruli in the MOB. This finding suggested that OR mRNAs transcribed in the cell bodies of sensory neurons are anterogradely transported through axons to their terminals in the glomeruli. Hybridizing signals in the MOB were detected within a few topographically fixed glomeruli onto which the olfactory sensory neurons expressing a given OR mRNA converge their axons (glomerular convergence, see §2(d)). Furthermore, these studies indicated that olfactory axons from one topographic zone in the OE tend to converge on glomeruli that are localized in a discrete zone of the MOB. Thus, the zonal organization in the OE is preserved, to some extent, in the projection of sensory axons to the MOB (figure 2).

OCAM is a candidate molecule involved in formation and maintenance of the zone-to-zone projection of olfactory axons (Yoshihara *et al*. 1997; Yoshihara & Mori 1997). OCAM is a member of the immunoglobulin superfamily, the structure of which is most closely related to that of NCAM (neural cell adhesion molecule) (figure 3). Several lines of evidence suggest that OCAM may be involved in selective fasciculation of zonal subsets of the olfactory axons. First, OCAM is expressed on a subset of olfactory axons in a zone-specific manner in both the developing and mature olfactory systems (Fujita *et al*. 1985; Imamura *et al*. 1985; Mori *et al*. 1985, 1987; Schwob & Gottlieb 1986, 1988; Yoshihara *et al*. 1997). Second, an *in vitro* adhesion assay demonstrated that OCAM is a homophilic adhesion molecule (Yoshihara *et al*. 1997). Third, an immuno-electron microscopic analysis revealed that OCAM is localized to closely apposed surface membranes of neighbouring olfactory axons in nerve bundles (Yoshihara *et al*. 1993).

(d) *The glomerulus as a functional unit*

Olfactory glomeruli are spherical neuropils that contain olfactory axon terminals forming excitatory synaptic connections with dendritic tufts of mitral and tufted cells—the principal output neurons of the MOB. The diameter of glomeruli ranges between 75 and 250 µm in different mammalian species. In mice, the surface of the MOB is covered by about 1800 glomeruli (Royet *et al*. 1988), each receiving converging axonal inputs from several thousand sensory neurons. Recent studies show that olfactory sensory neurons expressing a given OR converge their axons onto a few defined glomeruli (Vassar *et al*. 1994; Ressler *et al*. 1994; Mombaerts *et al*. 1996; Wang *et al*. 1998). Furthermore, studies using P2 OR-*IRES*-*tauLacZ* knock-in mice (Mombaerts *et al*. 1996; Wang *et al*. 1998) indicated that all the axons converging onto the P2 glomeruli originate from P2-expressing olfactory sensory neurons (Bellusico *et al*. 1999). Thus, each of the P2 glomeruli presumably represents exclusively P2 OR. As a simple working model, we hypothesize in this review that the 'one glomerulus–one receptor' correspondence occurs in all glomeruli of the MOB. We speculate that an individual glomerulus is an olfactory axon convergence centre, receiving signals originating from one type of OR. However, it is not known whether individual glomeruli other than P2 represent a single type or multiple types of OR(s). It is possible that some glomeruli receive convergence of olfactory axon inputs from a few different ORs.

Physiological examination of response specificity of individual mitral–tufted cells is in favour of the 'one glomerulus–one receptor' hypothesis (for a review, see Mori & Yoshihara 1995; Mori *et al*. 1999). Because individual mitral–tufted cells in the mammalian MOB project a single primary dendrite to a single glomerulus, the response specificity of a given mitral–tufted cell strongly reflects that of the glomerulus it innervates. When examined with a battery of odour molecules with systematic variations of molecular conformation, individual mitral–tufted cells in the rabbit MOB responded with increased spike discharges to a range of odour molecules having similar molecular conformation. In other words, individual MOB neurons are tuned to detect a range of odour molecules that have specific molecular features. If we assume

that a single type of OR binds to a range of odour molecules with similar molecular features, the above observation can be explained easily according to the 'one glomerulus–one receptor' hypothesis. A recent study using optical imaging of intrinsic signals of rat MOB demonstrated that individual glomeruli respond to a range of odour molecules with similar molecular features (Rubin & Katz 1999).

If we refer a glomerulus together with its associated neurons as a 'glomerular module' (Mori *et al.* 1999), neuronal architecture of the mouse MOB can be viewed as a repetition of 1800 such modules. An individual glomerular module may be tuned to detect specific molecular features and different glomerular modules are tuned to detect different molecular features. A single MOB may thus be equipped with 1000 different types of molecular-feature-detecting modules. Olfactory glomeruli are commonly seen in the MOB of virtually all vertebrate species. They are also seen in invertebrates, including insects and molluscs (Hildebrand & Shepherd 1997). Glomeruli are functional modules commonly used among different species and essential for the processing of odour molecule information at the initial stage of the central olfactory system (Shepherd & Greer 1998).

(e) *Glomerular zones and odour information processing in the main olfactory bulb*

As described in § 2(c), olfactory sensory neurons in a given zone of the OE project their axons to a corresponding zone of the MOB. Thus, glomeruli are parcelled into four zones in the MOB. Zone I glomeruli are localized in the rostrodorsal portion of the MOB and receive inputs from zone I ORs in OE. Zones II, III, and IV glomeruli are distributed progressively more ventrally and caudally in a concentric circle-like fashion and receive inputs from sensory neurons in the corresponding zone of the OE. An integration into a coherent map of the results of spatial arrangement of glomeruli obtained from *in situ* hybridization studies (Vassar *et al.* 1994; Ressler *et al.* 1994) and OR-*tauLacZ* studies (Mombaerts *et al.* 1996; Wang *et al.* 1998) suggested that each MOB represents two symmetrical sensory maps of ORs, one in the lateral hemisphere and the other in the medial hemisphere of the MOB. Further comparison of the sensory maps and OCAM expression maps suggested that the four zones exist in both medial and lateral maps (Nagao *et al.* 2000).

It should be noted that spatial segregation of glomerular zones is somewhat distorted in the MOB. For example, zone I of the MOB contains some glomeruli filled with OCAM-positive olfactory axons that originate in zones II, III or IV (Mori *et al.* 1985; Schwob & Gottlieb 1986). Moreover, a small number of glomeruli contain both OCAM-positive and OCAM-negative olfactory axons. It is possible that these glomeruli might receive olfactory axon inputs from multiple zones. Further analysis is necessary to determine the detailed spatial arrangement of glomeruli.

Structural comparison of various ORs, in relation to their expression zones in OE, revealed that the ORs with highly homologous amino-acid sequences tended to be localized in the neighbourhood within the same zone (Sullivan *et al.* 1996; Malnic *et al.* 1999; Tsuboi *et al.* 1999). To examine whether glomeruli representing ORs with similar tuning specificity are assembled within specific zones, we recorded spike responses of mitral–tufted cells to odour stimulation in the rabbit MOB (Mori *et al.* 1992; Imamura *et al.* 1992; Katoh *et al.* 1993) and superimposed on the unrolled OCAM map the positions of mitral–tufted cells showing similar response characteristics (figure 4*a*). A panel of homologous series of aliphatic compounds was used as stimulus odour molecules and single unit recordings were made from the dorsomedial part of the MOB. We noticed within the OCAM-negative zone (zone I) a focal domain where mitral–tufted cells responsive to fatty acids are concentrated (shown by a brown broken line in figure 4*a*) (Mori *et al.* 1992). Among 105 fatty-acid-sensitive mitral–tufted cells recorded in the dorsomedial part of the rabbit MOB, 98% were localized within this domain. As shown in figure 4*b*, mitral–tufted cells in this domain characteristically responded to similar ranges of odour molecules covering aliphatic acids and/or aliphatic aldehydes. In contrast, they rarely responded to aliphatic alcohols and never to alkanes. Aliphatic acids and aldehydes smell 'fatty and rancid' to humans. This suggests that the dorsomedial part in zone I might handle signals related to fatty and rancid olfactory quality.

For comparison, we examined the response characteristics of mitral–tufted cells in a region located in the most ventral part of the OCAM-positive zone (shown by the green broken line in figure 4*a*). Neurons in this region responded to a range of odour molecules covering aliphatic aldehydes, alcohols and/or alkanes (figure 4*c*). However, no neuron in this region was activated by fatty acids. These results may indicate that a local region in a given zone contains clusters of neurons with similar response characteristics, and that response specificity to odorants differs from zone to zone or domain, further suggesting that a local region in a domain preferentially receives inputs from similar ORs. Such spatial arrangement of glomeruli in zones or domains seems to be important for the processing of the odour molecule information in the local neuronal circuit. The dendrodendritic synaptic pathways via granule cells can mediate interactions between mitral–tufted cells that are associated with different glomeruli and thus with different ORs (Mori 1987; Mori *et al.* 1999). The zonal organization of the glomerular sheet in the MOB suggests that strong synaptic interactions occur among signals derived from ORs belonging to the same zone. The synaptic interaction might result in the enhancement of the tuning of individual mitral–tufted cells to detect specific molecular features through the mechanism of the lateral inhibition (Yokoi *et al.* 1995). In addition, the synaptic interactions via the local circuit cause the synchronized oscillatory discharges of mitral–tufted cells associated with different glomeruli (Kashiwadani *et al.* 1999), which may contribute to the integration of signals from distinct ORs in the olfactory cortex (Mori *et al.* 1999).

3. ZONAL ORGANIZATION OF THE ACCESSORY OLFACTORY SYSTEM

(a) *Zonal organization of the vomeronasal sensory epithelium*

The accessory olfactory system, or vomeronasal system, is vital for conspecific communication and for sexual

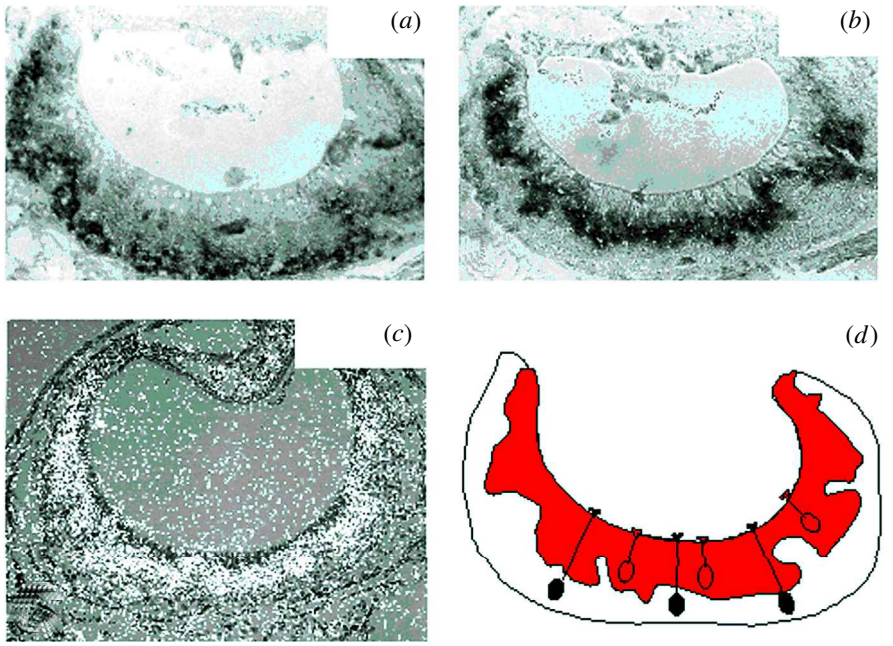


Figure 5. Zonal organization in the VNE. (*a*) Basal zone sensory neurons expressing $G_{o\alpha}$. (*b*) Apical zone sensory neurons expressing $G_{i2\alpha}$. (*c*) Hybridization signal with the OCAM probe is localized in the apical zone of the VNE. (*d*) A schematic diagram of the spatial arrangement of apical zone (red) and basal zone of the VNE. (*a* and *b* are modified from Saito *et al.* (1998).)

behaviour. Upon stimulation with pheromones, the vomeronasal system can trigger stereotyped innate behaviour. Pheromones are likely to be detected by vomeronasal receptors (VRs) expressed in microvilli of the vomeronasal sensory neurons (Dulac & Axel 1995; Herrada & Dulac 1997; Matsunami & Buck 1997; Ryba & Tirindelli 1997; Saito *et al.* 1998). These neurons are located inside the vomeronasal organ, a tubular structure situated at the base of the nasal septum.

In contrast to the overall sequence homology among ORs of the main olfactory system, the accessory olfactory system furnishes two structurally different families of VR genes. The first family of VRs (V1Rs) consists of 30–100 genes and are seven transmembrane-type receptors with a relatively short *N*-terminal extracellular region (Dulac & Axel 1995). The V1Rs are expressed by sensory neurons in the apical zone of the VNE (figure 5*d* and figure 6). The second family of VRs (V2Rs) comprises about 140 genes encoding seven transmembrane-type receptors with a long *N*-terminal extracellular region resembling the metabotropic glutamate receptors and Ca^{2+}-sensing receptor (Herrada & Dulac 1997; Matsunami & Buck 1997; Ryba & Tirindelli 1997). The V2Rs are expressed by vomeronasal sensory neurons in the basal zone of the VNE (figures 5*d* and 6).

Thus, sensory neurons are classified into two functional subsets by their selective expression of either V1Rs or V2Rs. Apical zone sensory neurons express G-protein $G_{i2\alpha}$, while basal zone sensory neurons express $G_{o\alpha}$ (figure 5*a*,*b*). OCAM is expressed by apical zone sensory neurons and not by basal zone sensory neurons (figure 5*c*). Taken together these findings suggest that there are two different zones of the vomeronasal sensory neurons (figure 5*d*). Sensory neurons in the two zones can detect structurally different types of pheromone molecules.

The repertoire of VRs is about one logarithmic unit smaller than the repertoire of ORs. One reason for such size differences might be that the set of structurally different odorants to be distinguished by the main olfactory system is much larger than the set of pheromone molecules to be detected and discriminated by the accessory olfactory system. The zonal organization of the OE and the VNE differs in two respects: the rodent OE consists of four zones, while the VNE is divided into two zones. More strikingly, the zones of the OE are segregated regionally whereas the VNE zones are separated in a laminar fashion into apical and basal zones.

(b) *Zonal organization of the vomeronasal axon projection*

The connectivity pattern of the vomeronasal axons with AOB appears to be a simpler version of that of olfactory axons with the MOB in terms of zonal organization. Apical and basal zones of the VNE are mapped onto rostral and caudal zones, respectively, of the AOB, preserving the strict spatial segregation of sensory neurons in the projection domains along the rostrocaudal axis (a zone-to-zone projection, figure 6) (for a review, see Halpern *et al.* 1998). Using OCAM immunohistochemistry we showed that vomeronasal sensory neurons located in the apical zone (OCAM positive) project their axons to glomeruli in the rostral zone of the AOB, whereas axons of the basal zone sensory neurons (OCAM negative) terminate in the caudal zone of the AOB.

Recently, by using genetic strategy, Rodriguez *et al.* (1999) and Belluscio *et al.* (1999) analysed the patterns of axonal projections of vomeronasal sensory neurons. *TauLacZ* transgene was introduced into a given VR genomic locus and axons of VNE neurons expressing the VR gene were selectively visualized. They showed that

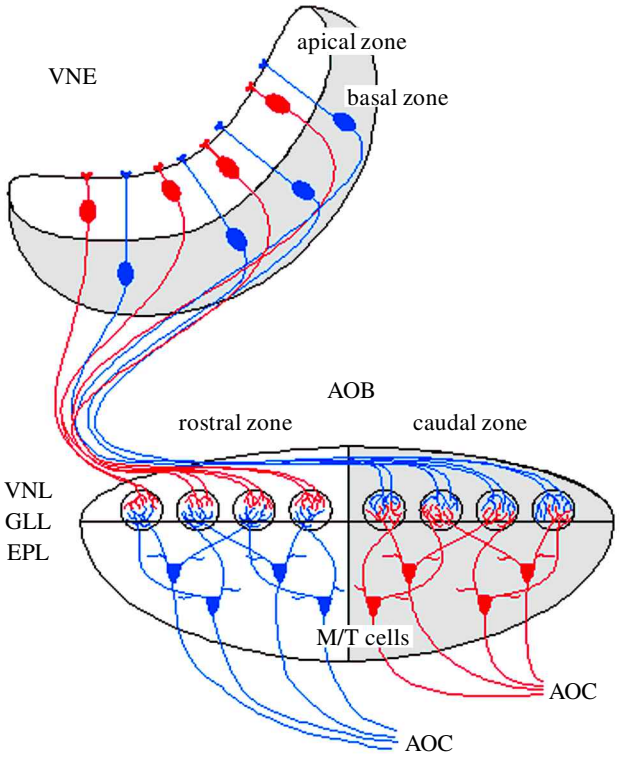


Figure 6. Zonal organization in the accessory olfactory pathways. Apical zone sensory neurons (OCAM positive, red) project their axons selectively to glomeruli in the rostral zone of the AOB and make synaptic connections with the rostral zone mitral–tufted cells (OCAM negative, blue). Basal zone sensory neurons (OCAM negative, blue) project their axons to the caudal zone of the AOB and make synaptic connections with caudal zone mitral–tufted cells (OCAM positive, red). VNL, vomeronasal nerve layer; GLL, glomerular layer; EPL, external plexiform layer; M/T cells, mitral–tufted cells.

neurons expressing a specific VR project to multiple glomeruli that reside within spatially restricted domains in either the rostral or the caudal zone of the AOB. This result indicated that the organization of the accessory olfactory system is similar to the main olfactory system with respect to the zone-to-zone projection, but is different in terms of the number of glomeruli onto which a given type of afferent axons converge.

(c) *Zonal organization within the accessory olfactory bulb*

Mitral–tufted cells in the AOB extend primary dendrites into glomeruli and receive inputs from axons of vomeronasal sensory neurons. Unlike the mitral–tufted cells in the MOB, AOB mitral–tufted cells send multiple primary dendrites to different glomeruli (Takami & Graziadei 1990; Mori 1987). Innervation to multiple glomeruli is also present in mitral–tufted cells in the MOB of lower vertebrates (Dryer & Graziadei 1994).

During development of the mouse AOB, mitral–tufted cells in the caudal zone express OCAM strongly whereas mitral–tufted cells in the rostral zone do not express OCAM (von Campenhausen *et al*. 1997). Mitral–tufted cells located in the rostral zone of mouse AOB receive innervation exclusively from apical zone sensory neurons, whereas basal zone sensory neurons send their signals selectively to mitral–tufted cells in the caudal zone, a finding that was independently confirmed by intracellular labelling of mitral–tufted cell dendrites in the opossum (Jia & Halpern 1996, 1997) and by electrical signal propagation studies in slices of the guinea pig AOB (Sugai *et al*. 1997). Therefore mitral–tufted cells of a given zone integrate signals detected exclusively by VRs of the same family. The segregation of the vomeronasal axonal projection is preserved at the level of mitral–tufted cells, which further strengthens the functional significance of the zonal classification of the VRs.

(d) *Functional implications of the zonal organization of the accessory olfactory system*

What is the functional significance of the zonal segregation of the VNE–AOB pathways that represent signals from different families of VRs? Recently, Krieger *et al*. (1999) suggested that the sensory neurons in different zones are activated by non-overlapping sets of urine fractions of different molecular weight. Previous studies reported two classes of urine-derived molecules possessing pheromonal activity: the major urinary proteins and small volatile molecules such as 2,3-dihydro-*exo*-brevicomin and 2-*sec*-butyl-4,5-dihydro-thiazole. Interestingly, these two groups of urine compounds trigger different behavioural responses (Bacchini *et al*. 1992; Marchlewska-Koj 1981; Jemiolo *et al*. 1986; Novotny *et al*. 1985; Mucignat-Caretta *et al*. 1995) and differentially activate mitral–tufted cells in the two AOB zones (Brennan *et al*. 1999). Taken together these studies suggest that the two zonal VNE–AOB pathways process signals from structurally different sets of pheromone molecules and represent information related to different aspects of the pheromonal responses.

The accessory olfactory system is vital for maturational process during puberty, the integrity of reproductive behaviour, kin recognition and parenting. The zonal segregation of mitral–tufted cells responsive to different categories of pheromonal molecules might facilitate the processing of stimuli involved in different behavioural contexts.

Another way to assess the significance of the segregated VNE–AOB pathways is to analyse how the two zones of mitral–tufted cells connect to the accessory olfactory cortex (AOC), i.e. the medial amygdaloid nucleus (MeA), the bed nucleus of the stria terminalis (BST), the bed nucleus of the accessory olfactory tract (BAOT) and the posteromedial cortical amygdaloid nucleus (PMCoA). Interestingly, some of the AOC regions mediate different behavioural responses, like maternal behaviour (BAOT, Del Cerro *et al*. 1991) or copulatory behaviour (BST, Emery & Sachs 1976). There is also some overlap in functions across AOC regions: lesioning of the male rat BST (Emery & Sachs 1976), for example, results in perturbation of the copulatory behaviour, which is also apparent in MeA-lesioned male hamsters (Lehman *et al*. 1980).

One can imagine that these functional differences among the AOC regions may be attributable to differential axonal projection patterns of mitral–tufted cells in the two zones. A given AOC region might receive selective input from one zonal AOB pathway whereas other AOC regions might combine inputs from the two zonal AOB pathways. We addressed this question by tracing

axonal projections of zonally confined mitral–tufted cells (von Campenhausen & Mori 1999). We could not find any evidence indicating that the segregation of pathways observed at the level of the VNE and the AOB is maintained in the AOC. Labelling of rostral or caudal zone mitral–tufted cells resulted in indistinguishable projection patterns of axons and in a similar distribution of axonal boutons in the AOC. Our results suggest that in all AOC regions ensembles of cortical neurons receive axonal inputs from both subsets of mitral–tufted cells. Thus the convergence of axons from the two AOB zones may allow AOC neurons to combine signals from different families of VRs. Our results support the idea that all parts of the four AOC regions may require inputs from both AOB zones for proper functioning.

Why do ensembles of neurons of all AOC regions sample pheromonal information across zones and thus represent combinations of structurally diverse VRs? The integration of signals across VRs in the AOC regions may reflect the notion that mammalian pheromones are presented in blends of structurally diverse pheromone molecules (Sorensen 1996) detected by the two VR families. Such a blend of distinct pheromone molecules may constitute a stimulus unit and trigger a particular behavioural and/or hormonal response such as pregnancy block in female mice (Bruce 1959; Brennan *et al*. 1990). The combination of signals across VR families may thus enable AOC neurons to integrate pheromonal information associated with a particular pheromone-triggered behaviour.

The convergence of segregated zonal pathways in the cortical regions may be a feature common to both main and accessory olfactory systems. Detailed analysis of axonal projection patterns of zonally restricted mitral–tufted cells in the MOB have not been reported yet. However, injection of tracers into different regions of the main olfactory cortex results in retrograde labelling of mitral–tufted cells distributed over large portions of the MOB (De Olmos *et al*. 1978; Luskin & Price 1983; Price 1973; Scalia & Winans 1975). This implies that also in the main olfactory system olfactory cortical regions combine signals across mitral–tufted cells of different MOB zones.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES