A molecular dissection of spatial patterning in the olfactory system

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The identification and cloning of genes encoding odorant receptors has provided molecular probes with which to examine the molecular mechanisms and organizational strategies underlying olfactory information processing. Recent studies using odorant receptor genes have revealed unexpected patterns of expression that provide new insights into how information may be organized in the nose and in the axonal projection from the nose to the brain.

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Introduction

The mammalian olfactory system is able to perceive and discriminate a wide variety of structurally diverse odorous molecules (odorants) [1]. It is estimated, for example, that humans are able to detect more than ten thousand different odorants and to discriminate among as many as five thousand. Only the immune system surpasses the capacity of the olfactory system to recognize such an array of ligands. The identification of a large multigene family encoding odorant receptors has recently provided new tools with which to explore the molecular mechanisms and organizational strategies employed by the olfactory system to achieve a high level of perceptual acuity [2]. This article will review the findings of recent studies that have used the odorant receptor gene family to examine how sensory information might be organized in the nose and in the axonal projection from the nose to the olfactory bulb.

Information flow in the olfactory system

Olfactory perception involves a series of neural structures in which olfactory sensory input is thought to be increasingly sharpened or refined to achieve a high level of acuity [1,3]. The first of these structures is the olfactory epithelium of the nose (Fig. 1a), which, in mammals, contains between one and ten million olfactory sensory neurons (OSNs) that extend cilia into the mucus that lines the nasal cavity. Volatile odorants dissolve in the nasal mucus and bind to odorant receptors on the protruding cilia of OSNs. This initiates a cascade of transduction events that culminate in the generation of action potentials in the axons of the OSNs [4–6] and the transmission of signals to the olfactory bulb (Fig. 1b) [7]. In the olfactory bulb, the axons of OSNs form synapses, with the dendrites of olfactory bulb relay neurons and interneurons, within specialized regions of neuropil called glomeruli [7]. Sensory input is thought to be processed in the bulb and then transmitted by the relay neurons to the olfactory cortex where further processing may occur. Over the past forty years, numerous studies have sought to gain information about the mechanisms by which olfactory information is processed [7–14]. Although these inquiries have provided valuable insights, the mechanisms underlying olfactory information processing and odor discrimination have remained obscure.

Odor discrimination involves a multitude of different odorant receptors

In 1991 a multigene family was discovered that codes for odorant receptors on rat OSNs [2] (Fig. 2). Two highly unusual features of the odorant receptor gene family were immediately apparent. First, it is enormous, containing hundreds of individual genes. Second, it is extremely diverse. Although odorant receptors share some sequence motifs, they are extremely heterogeneous in amino acid sequence. This diversity is consistent with the ability of odorant receptors to recognize a wide variety of structurally diverse odorous ligands. Families of odorant receptor genes homologous to those initially described in the rat have now been found in a variety of different species, including human [15–18], mouse [19•], salamander (A Jerusum, D Chikarai, J Kaver, abstract 53.2, 23rd Annual Meeting of Soc for Neurosci, Washington DC, November 1993), catfish [20•], and zebrafish (S Korsching, A Baier, R Welker, S Schwarz, F Weth,

Abbreviation
OSN—olfactory sensory neuron.

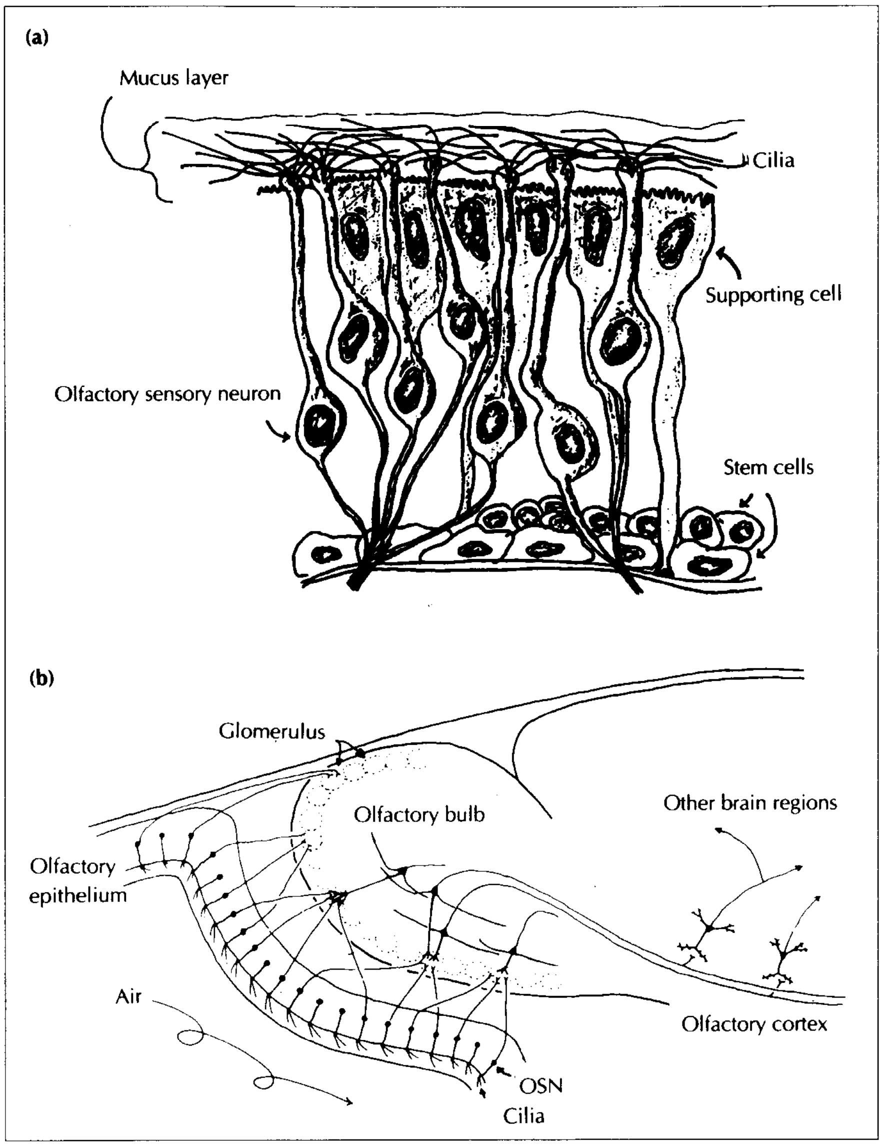

Fig. 1. The anatomical organization of the mammalian olfactory system. **(a)** The olfactory epithelium. The initial events in olfactory perception occur in the posterior nasal cavity in this specialized neuroepithelium. Odorants interact with odorant receptors (ORs) located on the cilia of olfactory sensory neurons (OSNs). The signals generated by these events are propagated along OSN axons, which project to the olfactory bulb. **(b)** Relationship between the peripheral and central olfactory system. Air entering the nasal cavity carries odorants to the cilia of OSNs in the mucus layer of the olfactory epithelium. The axons of OSNs project to the olfactory bulb. Within glomeruli (specialized regions of neuropil), the OSN axons branch several times and synapse on local interneurons (not shown) and relay neurons. The relay neurons (mitral and tufted cells) transmit signals to the olfactory cortex. Pyramidal cells of the olfactory cortex project to a variety of brain regions. Adapted from [7].

abstract 12.10, 23rd Annual Meeting of Soc for Neurosci, Washington DC, November 1993). Interestingly, although human and mouse odorant receptor gene families contain 500–1000 genes (L Dryer, J Brenman, E Liman, L Buck, unpublished data), the catfish appears to have only about 100 odorant receptor genes [20•].

Consistent with previous studies that had implicated G proteins in olfactory signal transduction [4–6], the odorant receptors were found to resemble a variety of G protein coupled receptors characterized by having seven, potentially membrane-spanning, alpha helices [2,16,20•]. Studies of other G protein coupled receptors have suggested that some of these receptors bind ligand via a ligand-binding pocket that is formed in the plane of the membrane by a combination of the transmembrane domains [21,22]. Interestingly, the odorant receptors appear to be particularly variable in three of these domains. Despite their tremendous diversity, odorant receptor genes can be grouped into subfamilies on the basis of their ability to hybridize to one another [2,19•,20•]. Members of the same subfamily encode receptors that are highly related in amino acid sequence and may therefore recognize the same, or similar, odorants.

The unprecedented size and diversity of the odorant receptor family suggest that each odorant receptor might be highly specific for one, or a few, odorants. Although the specificity of individual odorant receptors has not yet been assessed, studies of perceptual variations among humans are consistent with the notion that individual receptors could be highly specific. Many individuals lack the ability to detect one or another odorant at a concentration perceived by most people [23–25]. Such 'specific anosmias' may well result from the absence of specific odorant receptors. However, specific anosmias are only detectable at low odorant concentration, suggesting that at higher concentrations, other receptors might be engaged that interact at lower affinity with the odorant. The extent to which such low-affinity interactions might

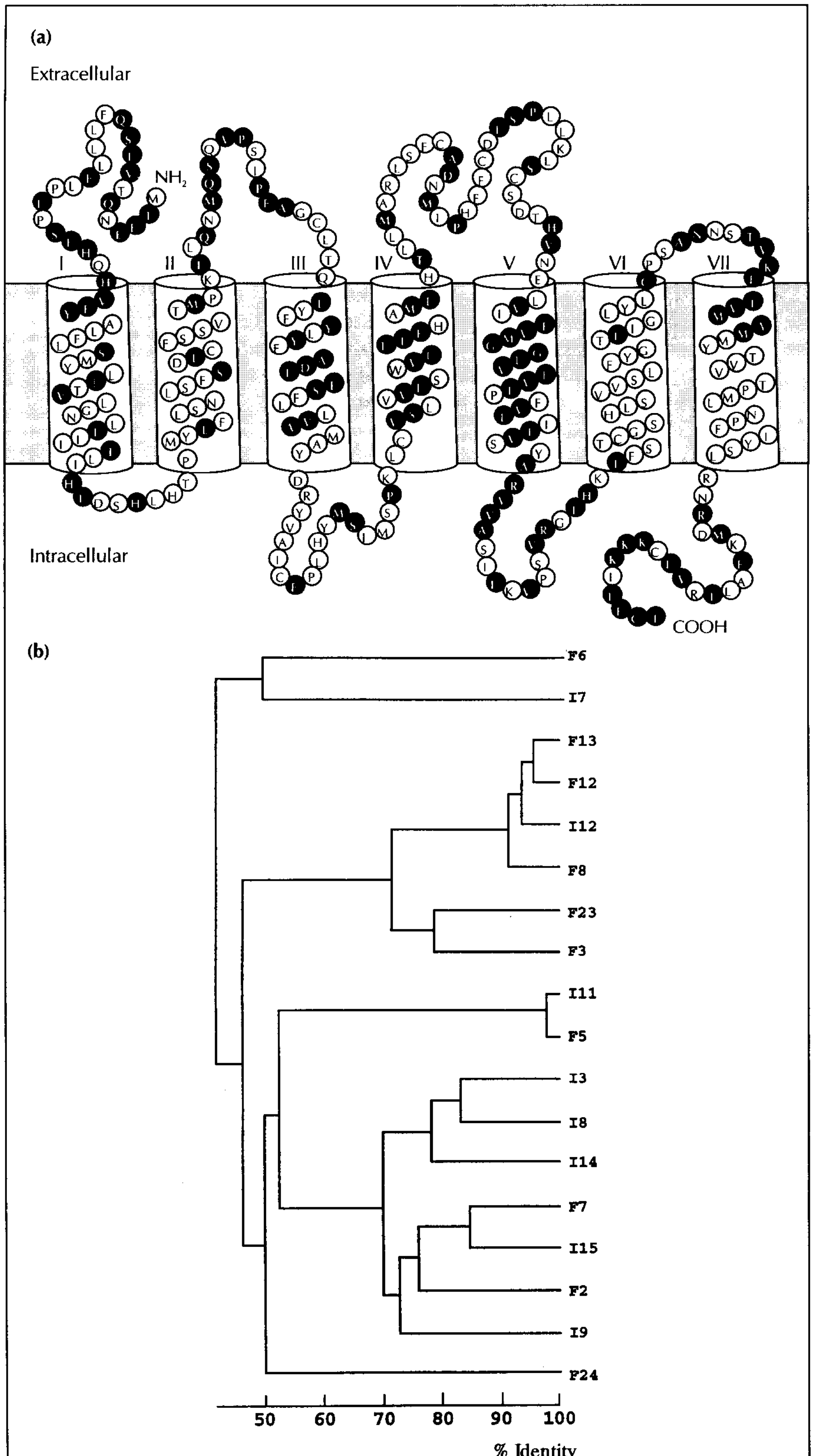

Fig. 2. Diversity within the odorant receptor multigene family. **(a)** Amino acid sequence of a typical member of the odorant receptor gene family is shown in its presumed configuration within the membrane, outlining positions of greatest variability in the odorant receptor proteins. The vertical cylinders delineate the seven putative alpha helices spanning the membrane (Roman numerals). Positions at which 60% or more of 10 rat odorant receptors are identical [2] are shown as white balls. More variable regions are shown as black balls. The extreme variability in transmembrane regions III, IV, and V is evident. Adapted from [2]. **(b)** Percent nucleotide identity in transmembrane domain V and its flanking regions among a group of rat odorant receptor genes (F2–I15). The odorant receptor gene family is extremely diverse; however, some odorant receptor genes are closely related in nucleotide sequence. Groups of receptors that are more than 70–80% identical in nucleotide sequence are generally defined as belonging to the same subfamily, by hybridization criteria. The receptors encoded by subfamily members are highly related in amino acid sequence and may recognize the same or similar odorants, whereas different subfamilies might recognize different classes of odorants.

contribute to olfactory discrimination under normal environmental conditions is not clear. A comparison of the total number of odorant receptor genes in the human genome (about 500) with the estimated number of odors that can be discriminated (about 5000) suggests, however, that each odorant receptor probably interacts with a small number of different odorants.

Each OSN may express only one or a few odorant receptors

The cloning of genes encoding odorant receptors has provided a set of molecular probes that are now being used to investigate a number of questions concerning how the olfactory system organizes sensory information. Several important pieces of information have come from *in situ* hybridization studies in which individual odorant receptor gene probes have been hybridized to sections of the olfactory epithelium. These studies have shown that both in catfish [26•] and rodents [19•,27,28,29•], each odorant receptor gene is expressed in only a small fraction of OSNs.

In one study conducted in the mouse, quantitative analysis of the percentage of neurons that hybridized to three different odorant receptor probes indicated that the three distinct odorant receptor gene subfamilies recognized by these probes were expressed in only 0.1–0.2% of OSNs [19•]. The patterns of hybridization obtained with numerous other odorant receptor gene probes, some of which recognized only a single gene, indicates that many, or most, odorant receptor genes are expressed in a similarly low percentage of neurons, but that some odorant receptor genes are consistently expressed at a higher frequency (S Sullivan, K Ressler, L Buck, unpublished data). When the frequency of expression of individual genes versus mixtures of genes are examined, it appears that the different genes are not co-expressed by individual neurons [26•].

These results suggest that, firstly, each OSN may express only one or, at most, a few odorant receptor genes. Secondly, if each OSN expresses only a single type of odorant receptor and if that odorant receptor is highly specialized for a few odorants, then each OSN might recognize only a few different odorants. Alternatively, each OSN may express a small number of broadly tuned receptors, and thus recognize many different odorants. Electrophysiological studies have shown that in the presence of rather high concentrations of odorant, each OSN may recognize a number of different odorants, but that each recognizes a different set [30,31]. When lower concentrations are used, however, fewer neurons respond to each odorant (S Firestein, personal communication).

Together, these findings suggest that individual OSNs may respond broadly to a number of odorants at high concentrations, but at concentrations of odorant nearer to threshold detection levels, individual OSNs may be highly specific for a small number of odors.

The olfactory epithelium is a mosaic of OSNs expressing different receptors

In addition to providing information about the diversity of OSNs, the cloning of odorant receptors has permitted an examination of how information might be organized in the olfactory epithelium and in the axonal projection from the olfactory epithelium to the olfactory bulb. Recent studies conducted on several species have examined the possibility that in the olfactory epithelium there may be odor-specific spatial maps that result from the differential patterns of expression of different odorant receptor genes [19•,26•,27,28,29•].

In situ hybridization studies performed in catfish, rat, and mouse have now demonstrated that OSNs that express the same odorant receptor are broadly distributed in the olfactory epithelium [19•,26•,29•]. Each neuron that expresses a particular receptor gene or gene subfamily is surrounded by many neurons that do not. In the catfish, neurons that express the same receptor gene appear to be randomly distributed throughout the entire olfactory epithelium [26•]. In the mouse and rat, as will be discussed below, there is a broad organization of odorant receptor gene expression into spatial zones [19•,29•]. However, within a single zone, neurons expressing the same receptor gene are randomly distributed. These studies demonstrate that the olfactory epithelium is not composed of patches of OSNs that all respond to the same odorants. Rather, it is a mosaic of OSNs that are all likely to respond to different odorants (Fig. 3).

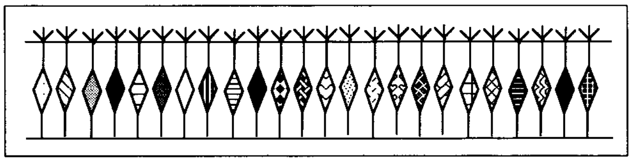

Fig. 3. The olfactory epithelium is composed of a mosaic of OSNs expressing hundreds of different odorant receptors. The schematic diagram illustrates the low frequency at which individual odorant receptor genes are expressed in the OSN population. *In situ* hybridization experiments indicate that each odorant receptor gene or gene subfamily is expressed in only 0.1%–0.2% of OSNs. Moreover, neurons that express the same odorant receptor gene are not clustered in one region of the epithelium, but instead appear to be randomly distributed within a given region.

Interestingly, although neurons expressing the same odorant receptor gene are spaced many cell bodies apart from each other, they are close enough so that their cilia, which can be up to 200 microns long [3], overlap somewhat. Therefore, within a zone, the cilial meshwork may contain a complex assortment of cilia with every receptor from that zone. An odorant molecule coming in contact with the epithelium would thus have ample opportunity to interact with cilia expressing receptors specific for it.

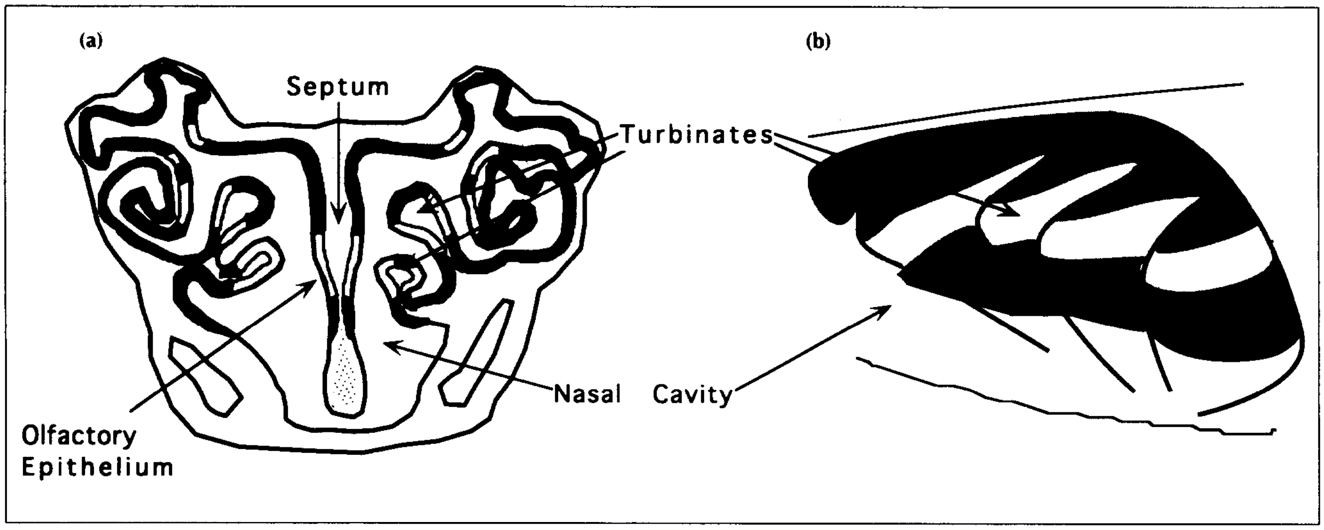

Fig. 4. Spatial zones of odorant receptor gene expression in the olfactory epithelium. **(a)** A schematic diagram of a coronal section through the mouse nasal cavity illustrates the different zones of odorant receptor gene expression. The olfactory epithelium covers the nasal septum and the bony turbinates that protrude from the back and side walls into the cavity. The odorant receptor gene family is segregated in its expression into at least four distinct spatial zones, which are shown here in different shades of gray. The zones are bilaterally symmetric. In coronal section, the zones appear to radiate symmetrically from the dorso-medial to the ventro-lateral regions of the cavity. **(b)** A schematic lateral view of the nasal cavity shows the nasal turbinates as they might appear if viewed from inside the cavity. Each odorant receptor gene expression zone consists of a series of elongated bands that extend along the anterior–posterior axis of the nasal cavity. The zones exhibit a characteristic dorsal–ventral relationship to one another.

Multiple spatial zones of odorant receptor gene expression in the nose

Initial *in situ* hybridization studies to examine odorant receptor gene expression in rodents showed that a single odorant receptor gene probe hybridized to neurons only in restricted regions of the olfactory epithelium, but that these regions were the same in the two nasal cavities [19•,27,28,29•]. This was the first indication that there might be at least a rough spatial mapping of odorant receptor gene expression in the olfactory epithelium.

Subsequent studies using a large number of different odorant receptor genes as probes, revealed that the olfactory epithelium is divided into a series of spatial zones in which different sets of odorant receptor genes are expressed [19•,29•]. Thus far, four distinct zones have been identified in the mouse [19•]. Each zone consists of a series of elongated bands or stripes that extend along the anterior–posterior axis of the nasal cavity. The zones exhibit a characteristic dorsal–ventral relationship to one another. Individual bands are evident on various structures in the nasal cavity, including the nasal septum and the turbinates, which are bony structures that protrude into the nasal cavity. In coronal sections, which are cut perpendicular to the anterior–posterior axis, this organization presents as a mosaic with dissociated patches of olfactory epithelium belonging to the same zone (Fig. 4a). However, in schematics of saggital views of the nasal cavity (Fig. 4b) and in whole-mount preparations, the zonal bands and their dorsal–ventral relationships are obvious.

Detailed analyses of the expression zones indicate that they are distinct, but that they overlap to a small degree at shared boundaries. These analyses also indicate that the zones are virtually identical in the two nasal cavities and in different individuals, regardless of gender [19•]. Quantitative analyses of patterns observed with several different odorant receptor probes have shown that 93–99% of OSNs that express a particular odorant receptor gene are located within zonal boundaries, most of the remainder being located just beyond the boundaries [19•].

Although the vast majority of odorant receptor gene probes tested hybridize to OSNs in one of the four characterized zones, several atypical patterns have also been observed that deserve comment. It has been reported that one rat odorant receptor gene probe hybridizes to neurons that are confined to small patches present on the tips of two turbinates [28]. There is also suggestive evidence that there may be another spatial zone in the mouse that is partially overlapping with two adjacent zones, but does not include all of either zone [19•]. Finally, a small proportion of probes have been shown to hybridize to more than one zone [19•]. As each of these probes recognizes a large subfamily of highly homologous receptor genes, it is possible that this is due to the expression of different subfamily members in different zones, rather than the expression of a single gene in more than one zone; but this issue remains to be clarified. Nonetheless, at least in the mouse, the majority of individual odorant receptor genes and gene subfamilies examined are expressed in only a single zone [19•]. Furthermore, both the topographical features of the zones and the genes expressed in these zones appear to be identical in different individuals [19•,29•].

The zonal assignment of each odorant receptor gene appears to be strict. Within each zone, however, OSNs that express a particular receptor gene are broadly distributed.

Clustering or other obvious spatial ordering has not been observed within a zone. Furthermore, similar densities of neurons that hybridize to an odorant receptor probe are found throughout a zone, thus excluding the possibility that there are subtle, subzonal gradients of expression. It thus appears that when an OSN or its progenitor chooses which odorant receptor gene(s) to express from among the 500–1000 possibilities, its choice may be restricted to a single zonal gene set, but that it may select a gene (or set of genes) to express from that set via a stochastic mechanism.

As mentioned previously, fish do not appear to have zones, and they have many fewer odorant receptors [20•,26•]. It is interesting to speculate that the different zones in the mammal may represent duplications of a more primitive single zone [29•]. As the number of odorant receptors increased from fish to mammal, the domains of epithelium to bulb organization may have been duplicated to allow an increase in receptor number without increasing the complexity of the encoding capacity of the duplicated compartments. Thus, the zones in mammals may reflect an increase in complexity in the repertoire of odorants to which they can respond.

A zonal segregation of information in the nose extends to the olfactory bulb

The *in situ* hybridization studies described above indicate that neurons that express the same odorant receptor gene, and which, therefore, presumably recognize the same odorants, are confined to a single spatial zone in the olfactory epithelium. Zonal patterns of odorant receptor gene expression have been observed with receptor probes that appear to recognize a single gene, as well as with those that recognize a subfamily of closely related receptor genes [19•]. As members of the same receptor subfamily probably recognize the same or related odorants, these observations imply that neurons that recognize related odorants are also confined to the same spatial zone. These findings suggest that the zonal patterning observed in the nose may serve to broadly organize incoming sensory information before its transmission to the olfactory bulb.

Numerous studies of the axonal projection from the olfactory epithelium to the olfactory bulb have shown that this projection, like the odorant receptor expression zones, is organized along the dorsal–ventral axis [32–40,41•]. In some of these studies, retrograde tracers were deposited in different regions of the olfactory bulb, and the resulting patterns of retrogradely labeled neurons in the olfactory epithelium were examined [36,41•]. The patterns of labeled neurons seen in these studies bear an uncanny resemblance to the zonal patterning of odorant receptor gene expression [19•]. This correspondence suggests that neurons in different odorant receptor expression zones project axons to different regions of the olfactory bulb. Thus, it appears that the zonal organization of sensory information in the epithelium is maintained in the transmission of this information to the bulb (Fig. 5). OSNs that express the same odorant receptor are all located in the same zone in the nose and they project axons to the same broad region of the olfactory bulb.

A further subzonal refinement of sensory information in the olfactory bulb

From the above discussion, it is apparent that there is a broad zonal organization of information in the olfactory epithelium that is maintained when that information is transmitted to the olfactory bulb. What happens when that information reaches the olfactory bulb? Is it further organized via the formation of more discrete maps for different odors?

Over the years, numerous studies have suggested that there may be a spatial mapping of sensory information in the olfactory bulb that involves the glomeruli and, consequently, the afferents from the OSNs innervating the glomeruli (reviewed in [1,7,42•]). Early studies indicated that prolonged exposure to different odorants would lead to different patterns of degeneration in the glomerular layer of the bulb [43]. 2-deoxyglucose metabolic labeling techniques allowed for a more refined analysis of odor-induced activity in the olfactory bulb and provided evidence that exposure to different odorants led to activity in different glomeruli [44–48]. The patterning observed in these earlier studies has now been observed at higher resolution using elevations in the expression of the immediate early gene, c-*fos*, as an indicator of neuronal activity [49•]. Single-unit recordings from mitral cells in different bulb regions also suggest that responsiveness to particular odorants may be segregated within the bulb [50]. What forms this spatial map of odorant information in the bulb?

There appears to be a convergence of information that occurs in the projection from the nose to the olfactory bulb (Fig. 5). In the mouse, there are about 4–5 million OSNs in each nasal cavity, whereas there are only 1–2 thousand glomeruli in each olfactory bulb [51–53]. Given that there may be 1000 different odorant receptor genes in the mouse, is it possible that axons of OSNs that express the same odorant receptor might converge on precisely the same glomeruli? Preliminary studies using antibodies specific for a subset of odorant receptors suggests that there may be such a convergence (L Horowitz, L Buck, unpublished data). However, more sophisticated approaches are needed to clearly define the patterns of synapses formed in the olfactory bulb by OSNs that express the same odorant receptor and, conversely, to define the diversity of sensory input received by each glomerulus.

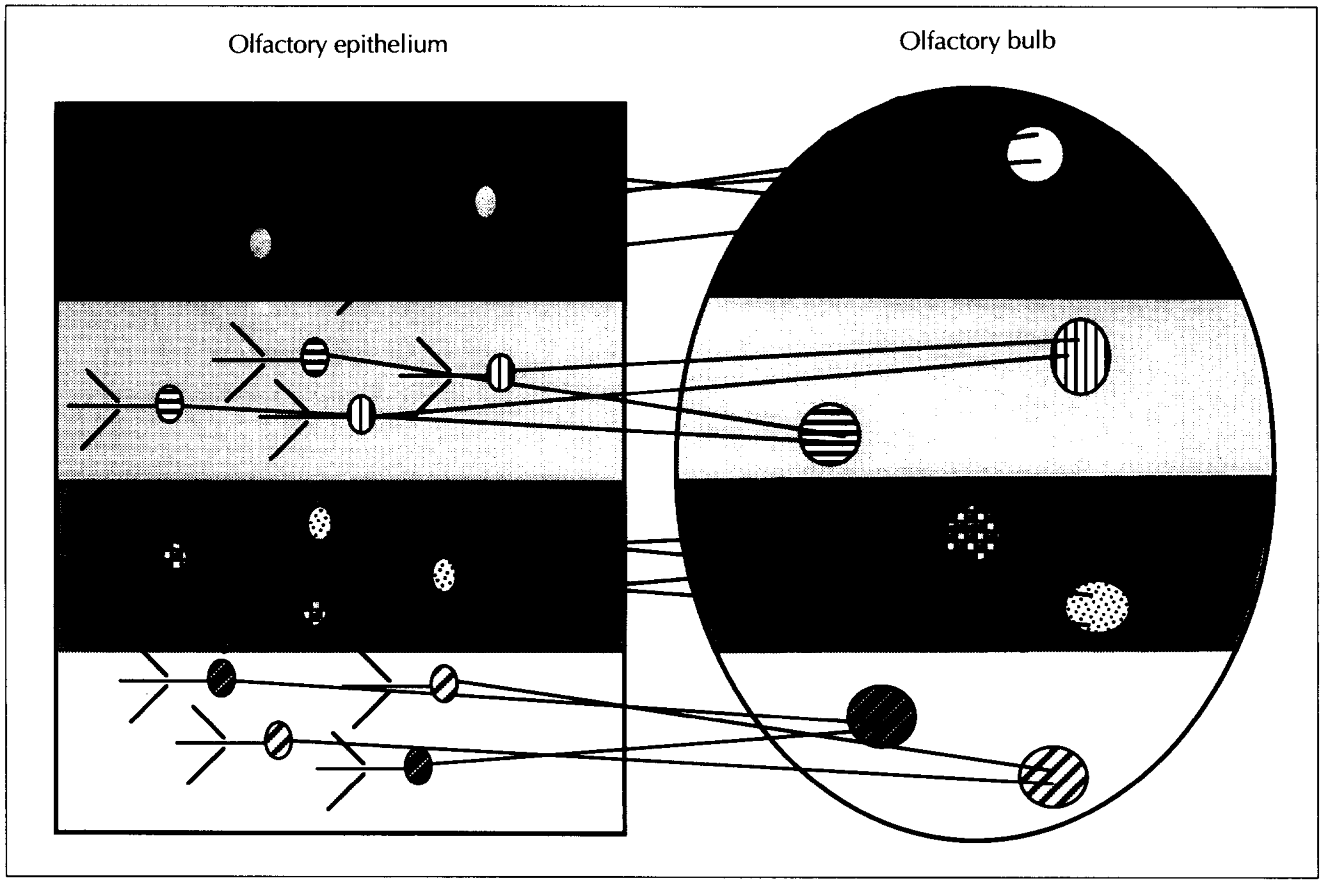

Fig. 5. Zonal organization of the axonal projection from the olfactory epithelium to the olfactory bulb. OSNs expressing the same odorant receptor (those filled with identical patterns) are located in the same zone of the olfactory epithelium. OSNs in each zone project in a segregated fashion to a corresponding region of the olfactory bulb. There is some evidence that within the olfactory bulb, sensory afferents are rearranged such that the projections of OSNs responding to the same odorants, and expressing the same or related receptors may converge onto a small number of glomeruli ([42•,44–48,49•,50]; L Horowitz, L Buck, unpublished data).

Conclusion

The identification and cloning of genes encoding odorant receptors on OSNs have provided a means of addressing a number of questions concerning the mechanisms and organizational strategies underlying olfactory information processing. The tremendous size and diversity of the odorant receptor gene family have indicated that odor discrimination is accomplished via the differential odorant binding properties of as many as 500–1000 different receptors.

As we have discussed, *in situ* hybridization studies conducted in several different species have provided new insights into how sensory information might be organized in the olfactory epithelium and its axonal projection to the olfactory bulb. These studies suggest that each OSN might express only one out of the 500–1000 possible odorant receptor genes. Furthermore, the olfactory epithelium appears to be a mosaic of neurons expressing a variety of different odorant receptor genes. One of the most surprising findings to come out of these studies has been the identification of spatial zones of odorant receptor gene expression in the nose. The observed spatial patterns indicate that there may be a broad organization of sensory information in the nose and, further, that this organization is maintained in the transmission of information to the olfactory bulb. Finally, preliminary experiments have provided tantalizing hints that there may be an extreme convergence of information at the level of bulb glomeruli whereby the axons of neurons that express the same odorant receptor gene synapse in only a subset of glomeruli.

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