Seven-Transmembrane Proteins as Odorant and Chemosensory Receptors

Peter Mombaerts

The olfactory systems of various species solve the challenging problem of general molecular recognition in widely differing ways. Despite this variety, the molecular receptors are invariably G protein–coupled seven-transmembrane proteins, and are encoded by the largest gene families known to exist in a given animal genome. Receptor gene families have been identified in vertebrates and two invertebrate species, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. The complexity of the odorant receptor repertoire is estimated in mouse and rat at 1000 genes, or 1 percent of the genome, surpassing that of the immunoglobulin and T cell receptor genes combined. Two distinct seven-transmembrane gene families may encode in rodents the chemosensory receptors of the vomeronasal organ, which is specialized in the detection of pheromones. Remarkably, these five receptor families have practically no sequence homology among them. Genetic manipulation experiments in mice imply that vertebrate odorant receptors may fulfill a dual role, also serving as address molecules that guide axons of olfactory sensory neurons to their precise target in the brain.

What makes the olfactory system so specific and sensitive? The olfactory mechanisms of vertebrates, *C. elegans*, and *D. melanogaster*, despite their anatomical and physiological differences, have very large chemosensory receptor repertoires. Their genetic basis is explored here.

Vertebrates

Chemosensation in higher organisms consists of taste and smell (*1*). These senses can be distinguished anatomically. Terrestrial animals smell airborne chemicals, typically small hydrophobic organic molecules. In mammals, olfactory sensory neurons (OSNs) are located within the olfactory epithelium of the nasal cavity. An OSN is a bipolar neuron, with its dendrite ending in cilia and its single axon penetrating the skull into the cranial cavity and terminating in the olfactory bulb. Odorants activate specific receptors on the cilia of OSN dendrites. The major signaling pathway in mammals consists of G protein activation, stimulation of adenylyl cyclase, opening of cyclic nucleotide–gated-ion channels, and membrane depolarization (*2*). Action potentials are generated and are transmitted to the olfactory bulb via OSN axons, which synapse with the dendrites of second-order neurons and interneurons within structures called glomeruli.

Cloning of odorant receptor genes. The molecular era in olfaction began in 1991 with the landmark discovery by Buck and Axel of a multigene family in rat (*3*). The experimental design that led to the isolation of odorant receptor genes was based on three assumptions. First, because biochemical evidence had implicated G proteins in olfactory signal transduction (*4*), odorant receptors are likely G protein–coupled receptors. Such receptors invariably have a seven-transmembrane (7TM) spanning topology (*5*). Second, a diverse repertoire of receptors is required to detect and discriminate an immense number of molecules with vastly different chemical structures. Odorant receptors are thus likely encoded by members of a large gene family. Third, odorant receptors are likely expressed selectively in OSNs.

The conservation of certain amino acid motifs within 7TM proteins (*6*) was exploited to identify a large family of 7TM genes selectively expressed in the olfactory epithelium (*3*). These genes are interchangeably referred to as odorant receptor, olfactory receptor, and odor receptor genes, and are abbreviated as OR genes (*7*).

OR gene sequences. The coding region of ORs is ~1 kb long and lacks introns. Conserved amino acid motifs distinguish ORs from other 7TM proteins (*3, 6, 8*). They include the motifs Leu-His-Thr-Pro-Met-Tyr in intracellular loop (IC) 1, Met-Ala-Tyr-Asp-Arg-Tyr-Val-Ala-Ile-Cys at the end of transmembrane domain (TM) 3 and the beginning of IC 2, Ser-Tyr at the end of TM 5, Phe-Ser-Thr-Cys-Ser-Ser-His at the beginning of TM 6, and Pro-Met-Leu-Asn-Pro-Phe in TM 7. Allowing for some degree of degeneracy, the coexistence of these motifs is sufficient to classify a vertebrate 7TM sequence as an OR. By contrast, hypervariable regions can be discerned within TMs 3, 4, and 5 (*8*). These may form the ligand-binding pockets by analogy with other 7TM proteins such as the β_2 adrenergic receptor (*5*). Structural diversity in ligand-binding domains is expected for receptors that interact with chemicals of vastly different structures.

The complexity of the OR repertoire is estimated at 1000 genes in mouse and rat, 500 to 750 genes in human, and 100 genes in zebrafish and catfish (*9, 10*). OR sequences have been isolated from ~20 vertebrate species ranging from lamprey to human (*10–15*) and are archived in a special database (*16*). Knowledge about OR genes is fragmentary in any species. Sequence information is usually derived from portions of the coding region between TM 2 and TM 7, cloned by subjecting genomic DNA to the polymerase chain reaction (PCR) with degenerate OR-specific primers; few sequences from entire coding regions are available. Because the coding region of OR genes is intronless, genomic DNA is a suitable PCR template; it is trivial to obtain compared to cDNA derived from the olfactory epithelium. The OR repertoire has tentatively been subdivided according to the patterns of sequence conservation (*17*).

A recently reported enigma is the high frequency of pseudogenes in the human OR repertoire (*18*): Because of frameshifts, nonsense mutations, and deletions, between 38 and 76% of the 500 to 750 OR-like sequences do not appear to encode full-length polypeptides (*12, 19*). By contrast, no pseudogenes have been reported among ~200 OR sequences in mouse and rat (*10*), and in other vertebrate species, OR pseudogenes are also scarce. This raises the interesting issue of whether the pseudogenes contribute to perceptual diversity in the human population, with individuals having different pseudogenes. The massive degeneration of the human OR repertoire may be related to our inferior sense of smell relative to other species. Perhaps less selective pressure was exerted on the OR repertoire during the evolution of *Homo sapiens*, who apparently came to rely more on the visual and auditory senses.

Genomic organization and gene regulation. Multigene families are typically arrayed in clusters in the genome, but the OR repertoire is unusual: It is fractionated over a few dozen genomic clusters in mouse (*10, 20*) and human (*12, 21*). Most chromosomes harbor an OR gene cluster, with

The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA. E-mail: pm@rockvax.rockefeller.edu

intergenic distances of 5 to 50 kb. The size of the repertoire and the peculiar genomic organization pose a formidable challenge to OR gene regulation. Not only is expression restricted to mature OSNs, but different OSNs each express different complements of the repertoire, with OR genes spread throughout the genome.

It is often stated that a single OSN expresses only one of the 1000 OR genes, but the evidence is limited and largely circumstantial. Only one direct and comprehensive analysis of this difficult problem has been published, resulting in a single OR identified from 44% of cells, but no OR from the remaining 56% (*22*). Although coexpression of multiple ORs has never been reported, much more testing of the "one neuron, one OR" hypothesis is required. Interestingly, mouse OR genes are expressed in a monoallelic fashion, with transcripts derived from either the paternal or maternal allele in different OSNs within an individual (*23*). The purpose of monoallelic expression may be to ensure that an OSN expresses a single type of OR, rather than two potentially different alleles of the same OR gene (*24*). The cis-acting DNA elements controlling gene expression remain to be characterized, but one study suggests that they may be located unexpectedly close to the coding region (*25*).

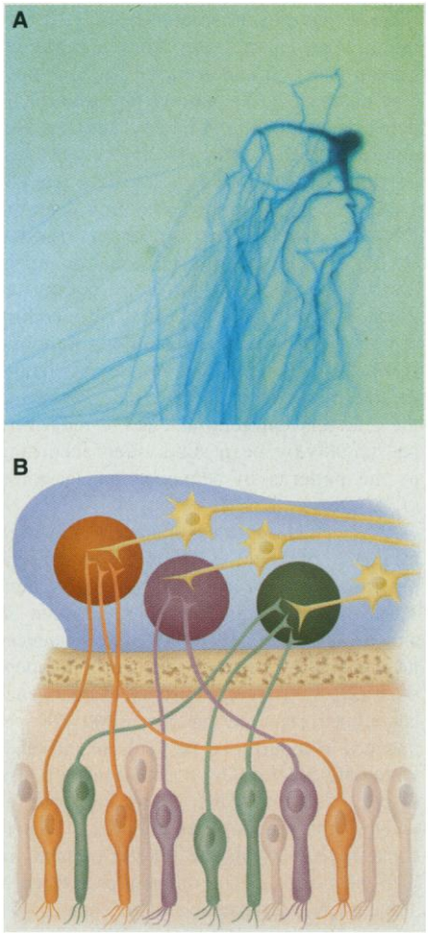

Fig. 1. Axonal convergence to a glomerulus. **(A)** Gene targeting technology was used to generate a strain of mice in which expression of a particular odorant receptor gene is coupled to that of an axonal marker, which is revealed as a blue stain. **(B)** Olfactory sensory neurons that express the same odorant receptor gene project their axons to either of two glomeruli in the olfactory bulb. Three populations of OSNs, each expressing a different OR, are depicted in different colors. Their axons converge on specific glomeruli, where they synapse with the dendrites of the second-order neurons (in yellow).

I speculate that by analogy with the regulation of antigen receptor gene expression in B and T lymphocytes (*26*), OR gene expression may depend on a DNA rearrangement event in OSNs or their precursors, basal cells. This genomic alteration would not affect the coding region and would not create sequence diversity from a limited germ line repertoire. Rather, the recombination event would restrict expression of the repertoire to a single OR gene from a single allele per OSN. The overwhelming size of the repertoire and the lack of clonal OR-expressing cell lines have thus far precluded exploration of this hypothesis. It may now be tested by cloning mice through nuclear transfer (*27*) from OSNs expressing defined ORs: In the simplest outcome, the genome of all cells of the cloned mice should contain the identical DNA rearrangement.

Patterns of OR gene expression and axonal projections. A given OR gene is expressed in a very small subset of OSNs (*11, 14, 15, 28*) within one of four stripes or "zones" of the olfactory epithelium (*11, 28*). An exception to the zonal expression rule is a small subfamily of ORs that are expressed in a patch crossing multiple zones (*29*). Within a zone, a punctate expression pattern is discerned, with OSNs expressing a given OR interspersed with OSNs expressing other ORs in a mosaic fashion. The zonal topography has been documented in mouse (*11*), rat (*28*), and zebrafish (*14*), but may have been overlooked in catfish (*15*); no information is available for other species, including human. The biological importance of the zones remains unclear. Perhaps this compartmentalization is a primary determinant of the organization of the axonal projections: Rather than assorting axons from 1000 distinct populations of OSNs, each expressing a different OR, the wiring problem may be rendered less complex by segregation into four subsets.

The zonal expression of OR genes is apparent in the earliest phase of the development of the olfactory system, and does not depend on the presence of the olfactory bulb (*30*). The first axons reach the bulb after the onset of OR gene expression. Glomeruli are formed in the perinatal period and continue to develop for several weeks. This sequence of events fulfills an important prerequisite of the hypothesis that ORs are implicated in axon guidance and convergence to glomeruli (see below).

The targets of axonal projections from OSNs expressing the same OR have been visualized by two different methods in mouse and rat. One approach used ultrasensitive imaging techniques to detect the presence of minute amounts of OR mRNA within axon terminals, and identified a small number of spatially defined glomeruli for an OR (*31*). The other approach used gene targeting in the mouse to visualize, by histochemical means, individual axons from OSNs expressing a particular OR (*32, 33*). Strains of mice were created in which expression of a defined OR is coupled to that of the axonal marker taulacZ (*34*). In these mutant mice, labeled axons project typically to either of two glomeruli, which reside at stereotyped and symmetrical positions in each bulb (Fig. 1). There are ~1800 glomeruli in an adult mouse bulb (*35*) and ~1000 OR genes in the mouse genome (*10*); thus, each OR may correspond to two specific glomeruli. These findings define the glomerulus as a convergent site of axonal projections from OSNs that express a given OR. This modern concept, which arose from molecular and genetic studies, is consistent with anatomical and physiological investigations carried out over the past 50 years that collectively have generated the notion of the glomerulus as a functional unit for integrating olfactory information (*9, 36*).

Function of ORs in olfaction. Functional evidence that ORs mediate responses to particular odorants was provided more than 6 years after their discovery (*37*). The difficulty in functionally expressing ORs in heterologous cell systems partly explains this long delay. The first proof was delivered in vivo by adenovirus-mediated gene transfer of a cloned OR, I7, into rat OSNs (*37*). Elevated responses were recorded in infected epithelium to octyl aldehyde and other short-chain aliphatic aldehydes. The most obvious interpretation is that rat I7 is a receptor for these molecules, but binding studies have not yet been reported.

Further evidence was provided by overexpression of hybrid ORs in human embryonic kidney (HEK293) cells along with G proteins that couple to many 7TM proteins (*38*). Transient elevations in intracellular calcium were detected upon application of certain odorants. Not only was the association between octyl (C_8) aldehyde and rat I7 confirmed, but expression of the equivalent OR from mouse resulted in maximum responses to heptyl (C_7) aldehyde. Site-

directed mutagenesis of the rat and mouse I7 sequences indicated that a single residue in TM 5, either valine (rat) or isoleucine (mouse), influences differential responsiveness to octyl aldehyde and heptyl aldehyde. This is the first report of structure-function relations for ORs. Testing 80 hybrid mouse ORs against a panel of 26 odorants (2080 combinations) resulted in three pairwise associations (*38*). This heterologous expression system provides an opportunity for a systematic and rapid analysis of ligand-OR interactions.

Another approach is to record responses from individual OSNs exposed to defined odorants, and to identify retrospectively the OR gene(s) that they express (*22, 39*). The logic is that the cloned OR must be a receptor for those odorants, relying on the belief that a single OR is expressed per cell. However, to verify that the cloned OR is responsive for the functional properties of the OSN from which it was isolated, it is necessary to demonstrate that these responses can be recapitulated by functional gene transfer, for instance by either of the above-described assays. This critical control has only been provided in a single case, in which the mouse *MOR23* gene was implicated in responsiveness to the odorant lyral (*39*).

These and similar assays (*40*), including our method using genetically marked, intact OSNs (*41*), and the recently developed technique of imaging odorant responses in living rodents simultaneously over many glomeruli (*42*), may provide definitive answers to the complex and long-standing question of the breadth of tuning of vertebrate OSNs and their ORs. It has been amply documented in many species that OSNs respond broadly but differentially to panels of odorants (*1, 2*). How the vertebrate olfactory system strikes a balance between the opposing requirements of sensitivity and specificity at the receptor level is a controversial issue for which satisfying solutions have now become feasible. The olfactory bulb may have a major role in sharpening olfactory percepts by physiological interactions between glomeruli.

Function of ORs in axon guidance. The axonal convergence of OSNs expressing a given OR to a pair of spatially defined glomeruli in the bulb creates a daunting wiring problem: 1000 neuronal subsets must be sorted reproducibly onto 1800 targets in the bulb during development (*32, 43*). Moreover, because the map stays constant while OSNs are being replaced throughout adult life, every day correct synaptic connections must be made by axons of newly generated OSNs.

Genetic studies have unveiled an unexpected role of ORs in the axon guidance mechanisms that underlie the formation of this stereotyped map. When the coding region of the OR P2 was replaced with that of M12 by targeted mutagenesis in the mouse, the axons projected neither to the endogenous P2 or M12 glomeruli, but to glomeruli at invariant positions in the vicinity of the P2 glomeruli (*33*). This receptor replacement has raised the tantalizing concept that ORs may have a dual role: in odorant reception at the level of the dendrite, and in pathfinding at the level of the axon (*44*). This notion has been extended by additional genetic manipulations of P2 (*45*). It must be emphasized that ORs cannot be the exclusive determinants of axon guidance; additional molecules must be involved (*46*).

Involving the ORs in these distinct but related functions would be biologically parsimonious. The OR would thus simultaneously control two critical properties of an OSN that determine its contribution to olfactory function: the spectrum of odorants to which it responds, and the site in the bulb to which its axon projects. Genetic experiments have yet to provide clues about mechanisms. In one embodiment of the hypothesis, OR proteins are expressed in axons or growth cones, but this has not yet been demonstrated. In an extreme version, ORs or OR fragments interact directly with proteins encoded by an equally large family of genes that are expressed differentially in cells of the bulb.

Vomeronasal and putative taste receptors. Chemosensation in higher species comprises additional sensory functions, the vomeronasal and gustatory systems. Interestingly, their molecular receptors are also probably 7TM proteins.

The vomeronasal system (*47*) is specialized in the perception of pheromones. Two gene families encoding a total of >240 7TM proteins have been discovered in mouse and rat that are each expressed in small subsets of sensory neurons of the vomeronasal organ (*48, 49*). These genes encode candidate pheromone receptors, but the evidence is far from definitive. Thus, an appropriate term is vomeronasal receptors. Neurons expressing a vomeronasal receptor from the first identified family (*48*) project their axons to multiple glomeruli in the mouse brain (*50*), in sharp contrast to the wiring diagram in the main olfactory system (*31, 33*).

Taste can be distinguished anatomically from smell: The neural pathway projects from the oral cavity to distinct regions in the brain. Paradoxically, although taste is much less complex than smell, our knowledge of the molecular basis of taste lags far behind. Two 7TM genes, *TR1* and *TR2*, have recently been isolated from taste receptor cells in mouse and rat (*51*). They have limited sequence homology with the second family of vomeronasal receptors, and can be regarded as candidate taste receptors.

Caenorhabditis elegans

This multicellular organism has been proposed as a simpler model for olfaction, but it is becoming increasingly clear that its chemosensory system is organized in fundamentally different ways.

Chemosensation is the prime sensory modality of nematodes; they cannot see or hear. The nematode as a model system lends itself to sophisticated genetic screens based on chemotaxis assays (*52*). Chemosensation is mediated by 32 neurons of 14 types, which are mostly arranged in bilateral pairs on the left and right sides. Individual cells are referred to by name and can be identified in the light microscope and killed with a laser microbeam, thus allowing for an assessment of their function in the context of a living organism. The ASE neuron, for instance, mediates chemotaxis to water-soluble substances. The AWA and AWC neurons are responsible for chemotaxis to volatile molecules, respectively diacetyl or pyrazine, and benzaldehyde or butanone. Adaptation is odorant-selective: Nematodes can adapt specifically and independently to different odorants sensed by a single neuron type (*53*). The mechanisms of signal transduction are complex (*54*).

Because it is not meaningful to apply the concepts of taste and smell to these simple organisms, the receptors are preferably not called odorant receptors, but chemosensory receptors (CRs).

Identification of CR genes. CR genes were cloned following a bioinformatics approach (*55*) rather than in the test tube. Inspection of the nucleotide sequence of the *C. elegans* genome at an intermediate stage of completion in 1995 resulted in the identification of more than 40 highly divergent 7TM genes arranged in small clusters. Most of them were found to be expressed in restricted subsets of chemosensory neurons. Thus, the same criteria used for the cloning of rat OR genes (*3*) were used to classify these genes as potentially encoding receptors for chemosensation.

CR genes have no sequence homology to vertebrate OR genes; they are evolutionarily unrelated. In contrast to vertebrate ORs, CR genes have multiple introns within the coding region. Another distinguishing feature is that CR genes display limited sequence similarity to each other (*55*). When the essentially complete nucleotide sequence of the 97-megabase genome of *C. elegans* became available in 1998, among the 19,099 genes, ~1000 orphan 7TM receptors were identified that could be CRs (*56*). As many as 30% of these sequences are

pseudogenes; another 20% are not expressed in chemosensory neurons. The estimate is thus a CR repertoire of 500 functional receptors (*56*), clearly the largest gene family in the nematode. On the basis of this and other considerations, a single chemosensory neuron likely expresses on average ~20 CR genes. Olfactory coding in this organism is thus qualitatively different from vertebrates, where only one or a few OR genes are expressed per neuron.

ODR-10 and diacetyl. The wild-type *C. elegans* is attracted by diacetyl. A genetic screen for chemotaxis mutants led to the cloning of *odr-10*, which encodes a CR (*57*). The defect is caused by a point mutation substituting a tyrosine for a histidine in TM 3; the phenotype is indistinguishable from a complete deletion of *odr-10*. The gene is strongly expressed in the two AWA neurons, and the protein is present on their cilia. When ODR-10 is ectopically expressed in the AWB neurons, which normally mediate detection of repulsive odorants such as 2-nonanone, transgenic nematodes avoid diacetyl instead of being attracted to it (*58*). This shows that the behavioral response to a chemical is determined not by the CR but by the neurons that express it. That *odr-10* encodes a receptor for diacetyl was functionally demonstrated by gene transfer into human HEK293 cells, which respond to diacetyl (and also pyruvate and citrate) with a transient calcium increase (*59*). It is the first chemosensory or odorant receptor from any species for which ligands have been characterized; interestingly, it also remains the only CR from *C. elegans* with identified ligands.

Drosophila melanogaster

Earlier this year, candidate ORs were identified by a computational approach (*60*). Two groups devised algorithms to scan the available 16% of the *D. melanogaster* genome sequence for 7TM proteins [see also (*61*)]. Candidate sequences were further screened individually for expression in OSNs. Some 7TM genes were found to be expressed in small groups of OSNs in stereotyped patterns. A combined total of 17 candidate OR genes were identified at multiple genomic locations, suggesting that the fruit fly genome may harbor 100 OR genes. Again, this would be the largest known gene family in the genome of this species.

As is the case in *C. elegans*, but in contrast to vertebrates, *D. melanogaster* OR genes have multiple introns in their coding regions. The sequence similarity among OR genes is very low. It appears that, as in vertebrates but in contrast to *C. elegans*, a single OSN expresses a small number of OR genes, perhaps only a single OR gene. But numerical considerations suggest that some of the 43 glomeruli (*62*) may receive mixed input from OSNs expressing different ORs.

Given the extreme divergence of receptors within the fruit fly, it may prove impossible to use homology-based approaches to isolate mosquito, moth, and bee odorant receptor genes.

A Cautionary Note

The breakthrough of Buck and Axel (*3*) relied on strong biochemical evidence that olfactory signal transduction in vertebrates occurs via a G protein–coupled pathway (*4*) and thus must use receptors of the 7TM type. Since then, four novel and large repertoires of 7TM proteins (the two families of vomeronasal receptors, nematode CRs, and fruit fly ORs) have been identified and proposed as candidate chemosensory receptors, with varying degrees of evidence for a G protein–coupled pathway. It remains possible that some of these receptors are not involved in chemosensation but in other functions such as axon guidance. In addition, it is a formal possibility that receptors of a non-7TM type also contribute to olfactory reception (*63*).

Conclusions

The olfactory system is akin to the immune system. Both are molecular recognition devices that detect an extremely broad range of qualitatively different chemical stimuli. Both are probabilistic systems, recognizing unpredictable combinations of molecules that each have no intrinsic biological meaning. Among the millions of molecular species that are floating in air or are dissolved in water, a substantial fraction can be smelled. It is thus not surprising that, like immune defense, olfaction relies on numerous molecular receptors, but it was not anticipated that the receptor genes would form the largest families known to exist in a given animal genome (Table 1). The receptor repertoires identified so far encode 7TM proteins, but there is negligible sequence conservation between them. This receptor structure appears to provide a sufficient number of variations on a theme. The typical G protein–second messenger cascade affords an enormous degree of amplification; it is also used in visual transduction, where opsins are 7TM proteins.

Further understanding of olfaction will depend on integration of molecular biology and genetics with anatomy, physiology, and behavioral biology. I believe that olfactory research is poised to blossom for those species for which genetic manipulation is possible and a complete catalog of OR sequences is or will become available through genome projects: the mouse, nematode, and fruit fly.

Table 1. The five odorant and chemosensory receptor gene families.

	Rodent ORs	Vomeronasal receptors (1st family)	Vomeronasal receptors (2nd family)	Nematode CRs	Fruit fly ORs
Year of discovery	1991	1995	1997	1995	1999
Size of family, including pseudogenes	1000	100	140	800	100
Number of genes expressed per neuron	1?	1?	1?	20	1?
Receptors with identified ligands	A few receptors	None	None	ODR-10/diacetyl	None

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REVIEW

The Olfactory Bulb: Coding and Processing of Odor Molecule Information

Kensaku Mori,[1,3]* Hiroshi Nagao,[1] Yoshihiro Yoshihara[2]

Olfactory sensory neurons detect a large variety of odor molecules and send information through their axons to the olfactory bulb, the first site for the processing of olfactory information in the brain. The axonal connection is precisely organized so that signals from 1000 different types of odorant receptors are sorted out in 1800 glomeruli in the mouse olfactory bulb. Individual glomerular modules presumably represent a single type of receptor and are thus tuned to specific molecular features of odorants. Local neuronal circuits in the bulb mediate lateral inhibition among glomerular modules to sharpen the tuning specificity of output neurons. They also mediate synchronized oscillatory discharges among specific combinations of output neurons and may contribute to the integration of signals from distinct odorant receptors in the olfactory cortex.

The sensory input to the olfactory system is mediated by odor molecules that represent an amazingly diverse range of structure. How can the mammalian olfactory system detect and discriminate such a large variety of odor molecules? Recent studies have begun to elucidate the molecular and cellular mechanisms for the reception of odor molecules at the level of olfactory sensory neurons in the nose (*1–5*). To cope with the diverse odor molecules, mammals have developed up to 1000 odorant receptors (*3, 4, 6*), which are expressed on the cilial membrane surface of sensory neurons in the olfactory epithelium (OE).

The central olfactory system receives the odor molecule information through axons of sensory neurons. The information is processed and integrated as the olfactory quality of objects. The human perception of the olfactory image is characteristic in that it usually associates with pleasant or unpleasant emotions. Because a single object, such as the flower of jasmine, emits a specific combination of dozens of different odor molecules, the central olfactory system has to integrate signals from a large variety of odorant receptors. This poses an interesting but daunting question as to how the central olfactory system combines or compares signals among 1000 types of odorant receptors. Recent progress has begun to unravel the basic cellular mechanisms for processing the molecular information at the first relay station of the central olfactory system, the main olfactory bulb (MOB) (*7*).

The mammalian MOB has a relatively simple cortical structure, containing thousands of signal-processing modules called

[1]Laboratory for Neuronal Recognition Molecules, [2]Laboratory for Neurobiology of Synapse, Brain Science Institute, RIKEN, Wako, Saitama 351–0198, Japan. [3]Department of Physiology, Graduate School of Medicine, University of Tokyo, Bunkyo-Ku, Tokyo 113–0033, Japan.

*To whom correspondence should be addressed. E-mail: moriken@postman.riken.go.jp

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