Chimia 55 (2001) 453–459

ISSN 0009–4293

The Molecular Basis of Olfaction

Abhay D. Kini and Stuart Firestein*

Abstract: The olfactory sense plays a dominant role in a variety of behaviors across many species ranging from invertebrates to higher mammals. Consequently, there is great interest in understanding how olfactory perception is initiated. The olfactory signaling pathway is triggered *via* a receptor-mediated event and is then conveyed onto to higher order neurons and ultimately to the cortex. The last ten years have witnessed an increased interest and success in understanding olfaction from a receptor perspective. These findings have significantly enhanced our insight of the olfactory system.

Keywords: Odorant receptors · Strucure-activity relationship

The olfactory sense plays a dominant role in a variety of behavioral aspects such as, but not limited to, food detection, social behaviors and reproduction. Two independent olfactory organs, the main olfactory epithelium and the vomeronasal organ, mediate mammalian olfaction. The olfactory epithelium is responsible for detection of low molecular weight molecules while the latter is devoted to pheromone detection and behavioral responses.

Signal Transduction in Olfactory Neurons

The signal transduction cascade in olfactory neurons is initiated by the binding of odor molecules to receptors (R; Fig. 1) in the cilia [1–3]. The first insight into the signal transduction pathway was gained by identification of an odorant sensitive adenylate cyclase (AC; type III), present in isolated dendritic membranes of olfactory sensory neurons (OSNs) [4]. It was subsequently demonstrated that odorant activation of AC is tissue specific, and its activity was guanine triphosphate (GTP) dependent thus implicating heterotrimeric G-proteins and G-protein coupled receptors (GPCRs) in olfactory transduction [5][6]. Single channel recordings from on-cell patches demonstrated that odor induced channels show no voltage-dependent behavior and that channel activity depends on intracellular concentration of cyclic nucleotides [7] [8]. A cyclic nucleotide-gated channel (CNG) has been subsequently cloned and it is composed of three different subunits [9–11]. It is now widely accepted that olfactory transduction pathway begins with the activation of an odorant receptor (Fig. 1) upon ligand binding [12]. which in turn stimulates an olfactory tissue specific stimulatory G-protein G_{olf}. G_{olf} in its active form activates AC type III, resulting in the elevation of intracellular cyclic adenosine mono phosphate (cAMP) [13]. The cAMP then directly binds to a CNG channel, a non-selective cation channel expressed predominantly in ciliary membrane. Opening this channel leads to the influx of sodium and calcium, and depolarization of the plasma membrane. Increased intracellular calcium concentration has a dual role: it activates a calcium-dependent chloride channel and, on the other side, it most likely binds calmodulin, closing the channel by reducing its sensitivity to cyclic nucleotides [14][15].

The existence of another second messenger pathway was demonstrated in olfactory neurons of some vertebrate species. It has been shown that amino acids increase intracellular inositol triphosphate (IP_3) levels in catfish neurons in GTP-dependent manner [16]. In addition, IP_3-gated channels have been found in olfactory cilia of catfish neurons [17][18]. Both cAMP and IP_3 pathways have been implicated in lobster olfactory neurons [19–21]. Signaling in insect olfactory neurons, although not understood as well as in vertebrates, is believed to have both pathways cAMP and IP_3 pathway [22]. There are biochemical data that suggest the existence of the IP_3 pathway in rat olfactory neurons [23], but recordings from a mouse with a targeted deletion of the a subunit of the CNG channel strongly indicated a cAMP pathway as the only second messenger pathway in mammalian olfactory neurons [24].

Signal Processing in the Olfactory System

As per our current understanding, all odors appear to induce an excitatory response in primary OSNs, all of which send their axons to an anterior region of the brain known as the olfactory bulb. The surface of the olfactory bulb is marked by some 2000 spherical structures, about 50–100 microns in diameter, called glomeruli. Each glomerulus consists of incoming fibers from OSNs and the dendrites of the second order projection neurons, the mitral cells. There is a tremendous convergence at this stage with several thousand axons from OSNs forming synapses on the dendrites of fewer than 25 mitral cells [25]. All of the

*Correspondence: Prof. Dr. S. Firestein
Department of Biological Sciences
Columbia University
600 Sherman Fairchild Center
1212 Amsterdam Avenue
New York, NY 10027
Tel.: +1 212 854-4539
E-Mail: sjf24@columbia.edu

neurons expressing a particular odor receptor converge into one or two glomeruli (see below) and a given mitral cell sends its dendrites to only a single glomerulus. The mitral cells send their axons out of the bulb by way of the lateral olfactory tract (LOT) which projects widely in the olfactory cortex. Additional processing occurs in the olfactory bulb through complex interactions between a population of inhibitory interneurons (granule cells) and lateral projections from the mitral cells (Fig. 2).

A critical question is whether the glomerular sheet represents a transformation of the olfactory stimulus into a spatial map. Several recent studies using techniques for imaging brain activity in the intact olfactory bulbs of living animals have begun to reveal some broad organizational patterns correlated with chemical structure of the odor stimuli [26]. Additionally, recordings from single mitral cells in various regions of the olfactory bulb have suggested a degree of tuning that presumably reflects the sensitivity of the primary OSNs projecting to particular glomeruli [25][27–29].

Using a series of aliphatic compounds as ligands, recordings of extracellular spike responses were made from mitral cells of rabbits [28]. In the dorsomedial part of the MOB, individual mitral cells were activated by subsets of n-fatty acids and/or aliphatic aldehydes having similar chain lengths. The extent of the response for the molecules in this group was not a function of the concentration. However, the range of the effective aliphatic compounds increased with increasing concentrations. Furthermore, cells that were activated by branched fatty acids were also activated by subsets of n-fatty acids having similar hydrocarbon chain length. It was also demonstrated that cells that were responsive to n-aliphatic acids were also responsive to n-aliphatic aldehydes. These cells did not respond to n-alcohols and alkanes. Thus demonstrating the rather stringent requirement of the carbonyl group in addition to chain length for activation of these cells. The other observation demonstrated that ketones and esters with the carbonyl group in the middle of the molecule were also able to activate the same mitral cells that were responsive to the n-acids and aldehydes. This established that mitral cells in the MOB display a stereochemical structure-dependent response to odorants.

In a separate study responses to aromatic compounds were recorded from rabbit mitral cells in the ventromedial region of the olfactory bulb [30]. Analysis

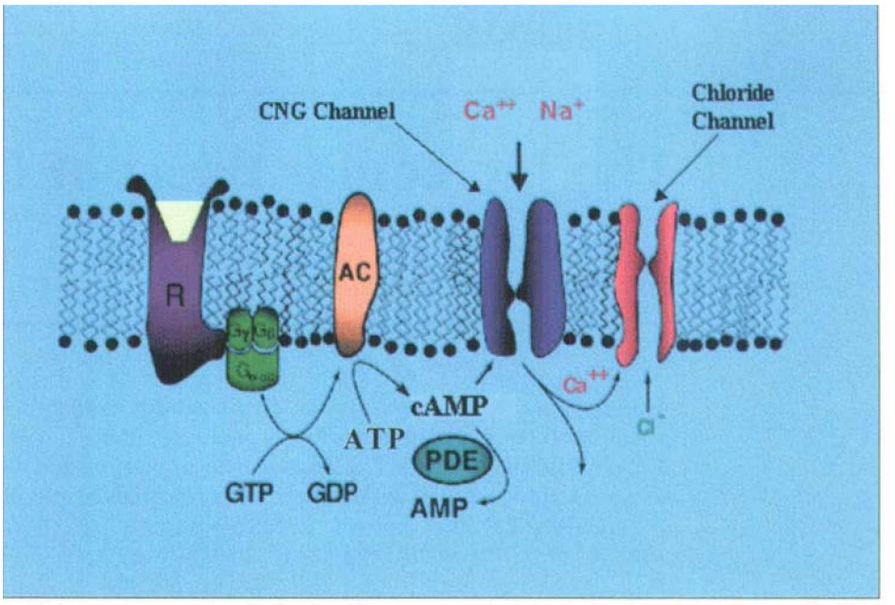


Fig. 1. Schematic representation of olfactory signal transduction cascade: Binding of an odorant (yellow) to the OR initiates the pathway. The pathway consists of the olfactory receptor (R); G_{olf}, G_{β} and G_{γ}, the three subunits of the heterotrimeric G-protein; adenylate cyclase (AC); CNG channel (blue); chloride channel (red). The phosphodiesterase (PDE) is responsible for the degradation of cAMP and thus downregulating the signaling cascade.

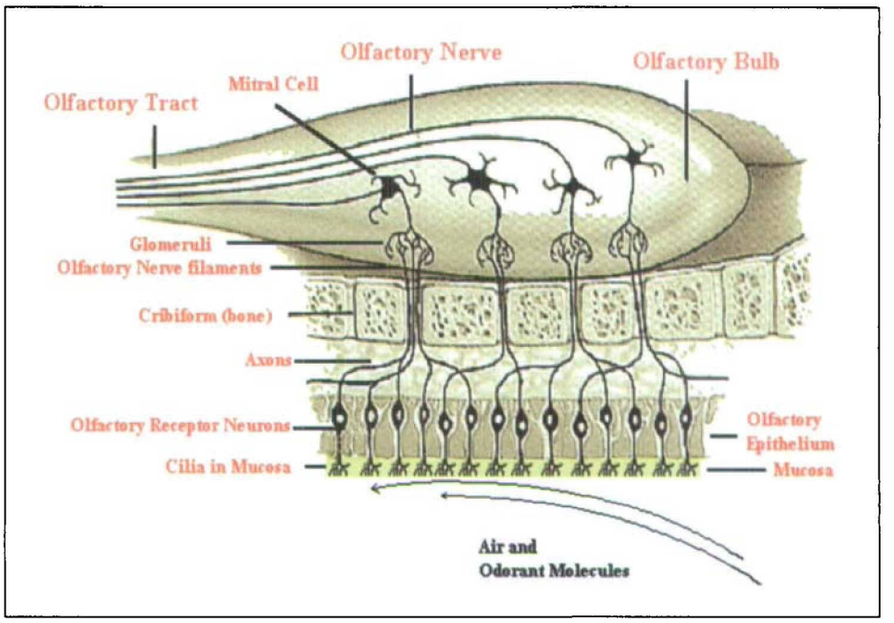


Fig. 2. Graphical representation of the mammalian olfactory system.

of these responses showed that the length of the hydrocarbon chain length attached to the aromatic ring played an important role in determining the specificity of the responses. Analysis of a panel of alkyl benzenes and disubstituted benzenes showed that a subset of both these groups, bearing similar conformations were capable of activating single mitral/tufted cells [30]. The mitral cells activated by alkyl-substituted benzenes were also activated by benzene rings substituted with methoxy (-OMe) or bromine (-Br) groups. In comparison, aromatic molecules bearing the amino ($-NH_2$), hydroxyl (-OH) or carboxyl groups did not activate the same cells. In addition to activating single mitral cells, some molecules were found to cause strong inhibition of these cells as well. Molecules that were responsible for inhibition of spike responses from mitral cells were all

found to be benzene molecules (mono or di-substituted) bearing side chains containing two or more carbons atoms.

Both these studies demonstrated that different mitral/tufted cells have dissimilar excitatory molecular receptive ranges with respect to the molecular conformation [28][30]. The dorsomedial and ventromedial parts have characteristic features specific to each of them. In contrast to the ventromedial part which very rarely involved activation by fatty acids the dorsomedial part in most cases involved activation by fatty acids. For example the cells in the dorsomedial part responded with increased spikes to benzoic acid, whereas no response was exhibited to the same compound in the ventromedial region. This established that the conformational features of the odor molecules were one of the important parameters in the spatial representation of the olfactory bulb [25].

In a more recent study, intrinsic optical imaging was used to examine how structural features are represented spatially in the sensory map of the rat OB [29]. It was found that the dorsal OB contained two topographically fixed domains; glomeruli in each domain could be activated by odorants with particular functional groups. The various functional groups used in these studies were as follows; primary alcohols (R-OH; *e.g.* CH_3-CH_2-OH), secondary alcohols (R-OH; *e.g.* $(CH_3)_2$-CH-OH), carboxylic acids (R-COOH; *e.g.* CH_3-CH_2-COOH), aldehydes (R-CHO; *e.g.* CH_3-CH_2-CHO), ketones (R^1-CO-R^2; *e.g.* CH_3-CO-CH_3), esters (R^1-CO-OR^2; CH_3-CH_2-CO-OCH_3), and phenol (Ph-OH). Using a large panel of odors, the structural features of odorants were mapped into two classes that differentially affected the spatial map [29]. Carboxylic acids (carbon chain length, n = 3 to 8) and aliphatic aldehydes (n = 2 to 8) were found to activate glomeruli that were clustered in the anteromedial domain of the dorsal OB. Primary alcohols (n = 3 to 9) and phenol on the other hand were able to evoke modest to strong responses in the lateral part of the dorsal OB, with little overlap with the activation pattern of the carboxylic acid responsive anteromedial domain. Esters, which are formed by the combination of carboxylic acids and alcohols demonstrated glomerular activation depending on the number of carbon atoms attached to the carboxylic acid part of the ester. Esters with more than four carbons in the acid part (C_4-COO-R) activated the anteromedial domain, while esters with one or two carbons in the acid part and four or five carbons in the alcohol part (C_1/C_2-COO-C_4/C_5) activated the lateral domain. Propyl propionate (C_2-COO-C_3), with three carbons in each part, activated glomeruli in both anteromedial and lateral domains. Thus in this series, changing the position of the functional group (–COO–) from one end of the molecule (hexanoic acid (C_5-COOH)) to the other end (n-pentyl formate (HCOO-C_5); switched the activated glomeruli from the anteromedial to lateral domain, with an intermediate pattern for propyl propionate. Alcohols on the other hand activated the lateral domain. Aliphatic compounds with other functional groups evoked little activation in the region imaged. The only exception was 1-hexanethiol and 1-hexylamine; the first one exhibited very weak activity in the lateral domain whereas the latter exhibited activity in the anteromedial domain. Aliphatic hydrocarbons evoked little or no odor responses in either of the two domains. The authors were able to demonstrate that within each domain, structural features such as functional groups, other structural features such as chain length and branching were represented by local differences in patterns. These results suggest that structural features are categorized into two classes, primary features (functional groups) that characterize each domain, and secondary features that are represented by local positions within each domain. Such hierarchical representations of different structural features correlate well with psychophysical structure–odor relationships [29].

While these results paint an intriguing picture of stimulus processing in the bulb, they provide only a partial view. A complete understanding can only be gained by appreciating the nature of the incoming signal from the primary neurons of the sensory epithelium. And this signal is dependent on the particular tuning curve of those receptor neurons, *i.e.* the range of sensitivity to different odorous compounds. This leads to the fundamental question of the nature of the molecular receptors that bind ambient odor molecules.

Odorant Receptors are G-Protein Coupled Receptors

Although the existence of a receptor protein family involved in odorant binding was postulated long before the signaling cascade was elucidated, the discovery of G-protein activated adenylate cyclase made it evident that olfactory signaling probably involved GPCRs (Fig. 3). The structure of odorant receptors (ORs) remained unknown until 1991, when the first eighteen members of putative odorant receptor family were cloned [31]. The effort to clone odorant receptors was based on the following assumptions: 1) It was expected that they belonged to a large family of receptors; 2) ligand diversity suggested a substantial receptor di-

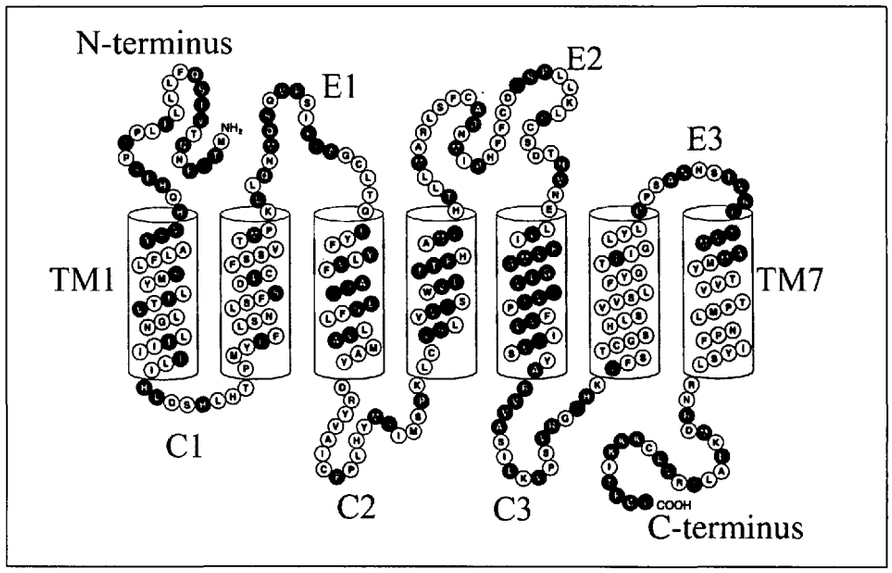


Fig. 3. Graphical representation of a 7TM GPCR. This figure represents the rat odorant receptor I15, showing seven transmembrane domains. The extra-cellular, intra-cellular loops and transmembrane regions are labeled E1, E2, E3; C1, C2, C3, and TM1 – TM7 respectively. More conserved regions are shown as white balls and variable regions are shown as black balls (adapted from [32]).

versity and existence of a multigene family; 3) the expression of these receptors should be restricted to the olfactory epithelium. Sequence analysis of the first ten cloned receptors revealed the presence of seven transmembrane (7TM) domains as well as motifs conserved among members of the GPCR superfamily.

They also have unique motifs shared only within the odorant receptor family. These include motifs Leu-His-Thr-Pro-Met-Tyr in intracellular loop 1 (C1), Met-Ala-Tyr-Asp-Arg-Tyr-Val-Ala-Ile-Cys at the end of TM3 and beginning of C2, Ser-Tyr at the end of TM5, Phe-Ser-Thr-Cys-Ser-Ser-His- in TM6 and Pro-Met-Leu-Asn-Pro-Phe in TM7 [31-33]. Particularly striking were hypervariable regions in third, fourth and fifth transmembrane domains of the receptors, believed to form much of the ligand-binding site. The coding region of ORs is about 1 kb long and it is intronless. The odorant receptor multigene family can be divided into subfamilies based on their sequence similarity and location within the genome. Northern blot analysis performed with a mix of seven divergent receptor clones suggested that receptor mRNA was restricted to the olfactory epithelium, thus demonstrating specificity of receptor expression.

Early estimates suggested the existence of about 1000 genes encoding odorant receptors [34–36], making this the largest family among vertebrate genomes. Experiments that ensued the discovery of the odorant receptor family showed *via in situ* hybridization, that sensory neurons expressing distinct receptors are segregated in clearly defined zones within the olfactory epithelium and that within a zone they appear to be randomly distributed [37][38]. The functional significance of this zonal expression remains unknown.

Since then, a number of odorant receptor families have been cloned from other species. Mouse OR genes were quite similar to rat OR genes, both in size and sequence identity [39]. The human OR family [40], has an unusually high number of pseudo genes [41][42]. A family of ORs examined in the channel catfish was estimated to have about 100 genes [43]. Goldfish olfactory receptors [44][45] exhibit significant homology to the mGluR family of receptors [46] and to the family of vomeronasal V2R receptors [47]. Interestingly, cloning of *C. elegans* chemosensory receptors demonstrated the existence of yet another highly divergent GPCR family, with approximately 500 functional receptors [48][49]. A single chemosensory neuron in *C. elegans* can express more than one receptor, contrary to mammalian OSNs which most likely express only one OR per neuron [34], – although some studies have suggested that a few mammalian neurons may express two ORs [50]. The family of ancient vertebrate receptors, cloned from lamprey (*Lampetra fluviatilis*) shows similarities to modern ORs up to the second intracellular loop, where it loses similarity, especially in the third intracellular loop [51]. The long search for insect odorant receptors was successfully accomplished by the application of specially designed computer algorithms to search the Drosophila genome database [52][53]. In Drosophila, the OR family is encoded by 61 genes and their expression has been mapped within Drosophila olfactory system [53]. Individual olfactory neurons express a single OR gene with exception of OR83b (known also as A45), which is expressed in all OSNs, indicating a special (still undetermined) function for this gene.

The observation that olfactory receptors are randomly distributed within olfactory zones, and the fact that the number of glomeruli in the olfactory bulb corresponds to the number of OR genes suggests that, in order to encode an odor quality, OSNs expressing the same type of receptor should project to one, or a small subset, of spatially defined glomeruli. To test this hypothesis, Vassar *et al.* employed an *in situ* hybridization method to detect mRNA in the terminals of sensory axons in the olfactory bulb [54]. Their experiments provide evidence that neurons expressing a given receptor project their axons to one or a small number of glomeruli within olfactory bulb. These data were consistent with earlier studies demonstrating the odor-induced activity detected in different glomeruli in response to different odor stimuli, by 2-deoxyglucose metabolic labeling [55–58], optical recording [59], or electrophysiological recordings [28] [30] [60]. The most compelling evidence for specific targeting of axonal projections from OSNs expressing the same OR was provided by gene targeting in mice [61]. Transgenic mice had a reporter gene targeted to the coding region of a specific receptor (P2), resulting in labeled axons in every OSN expressing this particular receptor.

It is now generally accepted that OSNs expressing a given OR send their axons to two symmetrical glomeruli. The zonal organization observed in olfactory epithelium is preserved in the olfactory bulb [37][54][62]. Each glomerulus represents a convergence site for axonal inputs coming from several thousand OSNs, and in each glomerulus information is transmitted to about 20 mitral and tufted cells [63]. The information is processed within the olfactory bulb and propagated to the olfactory cortex.

Function of Odorant Receptors

The cloning of a multigene family of olfactory receptors provided the necessary tools for answering many questions regarding the expression pattern of ORs in the olfactory epithelium and their projections to the olfactory bulb [31]. However, critical questions regarding odor binding and pharmacology of ORs remained unanswered mainly due to inability to express these receptors in heterologous systems. Functional expression of ORs would not only prove that ORs can be activated by odorant molecules, but would also provide information about the range and specificity of ligands that bind a particular receptor. Eventually, the identification of numerous receptor-ligand(s) pairs will allow precise identification and analysis of ligand binding domains within receptor proteins. Many attempts to express ORs in various heterologous systems failed for two likely reasons: the inability of host cells to properly target ORs to the plasma membrane and the inability of ORs to couple to the transduction machinery present in the heterologous cells [64].

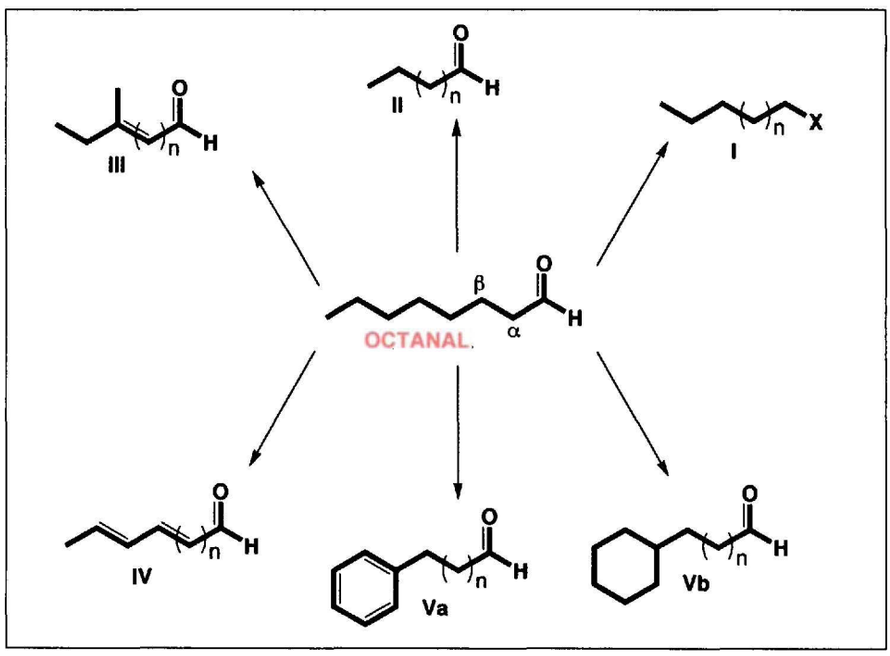
The first study reporting successful functional expression of odorant receptors used the baculovirus system to functionally express rat OR5 in Sf9 cells [65]. The results obtained in this study indicate a rather non specific nature of the OR5 receptor, since activation of the receptor by five chemically unrelated odor mixtures lead to an increase in intracellular level of IP_3. The response profile of OR5 does not seem to fit the expected specificity of odorant receptors. The other receptor tested in the study, OR12, did not show activation with any of the tested odors. Although no specific ligand was identified, the zebrafish odorant receptors demonstrated functional expression but only after fusion with an import sequence from guinea-pig serotonin receptor [66]. The *C. elegans* chemosensory receptor ODR10 showed a low level of expression in HEK 293 cells, allowing determination of its ligands [66][67].

Problems associated with expressing ORs in traditional expression system

such as yeast, bacteria or mammalian cell lines have precluded the study of these receptors in a manner analogous to other GPCRs. In an attempt to circumvent this problem, Zhao *et al*, developed an adenovirus driven expression system, which made it possible to express an olfactory receptor *in vivo*, and then study the receptor using an electrophysiological technique [68]. Since the binding of an odor molecule and activation of the second messenger cascade leads to depolarization of the OSNs, the activation of the OSNs can be measured by placing a recording electrode directly on the olfactory epithelium. This method, called an electroolfactogram (EOG), was used to record odorant-induced responses in this study. This was the first study in which a mammalian OR was paired with its cognate ligand. This study identified octanal as the primary ligand for the rat I7 receptor. These results were confirmed in a study from Krautwurst *et al* [69]. in which they developed a system to express chimeric ORs in the HEK 293 cell line. The chimeric constructs contained a rhodopsin N-terminal extension to facilitate the expression and translocation of the protein in HEK 293 cells. The most striking data in this study was the difference in selectivity between the rat and mouse I7 receptor. The I7 receptor in mouse and rat exhibits ~95% sequence identity and represent orthologous genes in the two species. Despite this high degree of identity, it was determined that the mouse I7 receptor preferred heptanal to octanal whereas the rat receptor had the opposite preference. This difference was attributed to a single amino acid difference in TM5 ($V_{206}I$) between the rat and mouse I7 receptor. Besides the change in selectivity of the I7 receptor, the system was used to screen 26 odors and identify receptor-ligand pairs for three more chimeric receptors. The results obtained by both these groups demonstrate a successful expression of the full-length rat I7 receptor as well as a large number of chimeric constructs.

Since a robust functional expression system for ORs is still elusive, two laboratories have paired some receptors with their cognate ligands using an approach that combined the use of single cell PCR and calcium imaging [70][71]. In this method olfactory neurons are stimulated with different odorants at varying concentrations. The cells that respond to an odorant exhibit an increase in intracellular level of calcium, which can be detected by a calcium sensitive dye. The cells that respond to any odor are then subject to single cell RT-PCR with degenerate primers matching conserved amino acid sequence motifs in mammalian ORs. Using this approach Malnic *et al* [71], were able to link odorant receptors to some alcohols (-OH), acids (-COOH) and diacids (-HOOC-R-COOH). The authors were able to demonstrate that a single OR can recognize multiple odorants and a single odorant can be recognized by multiple ORs, but that different odorants are recognized by different combinations of ORs. Their experiments further demonstrated that highly related ORs as well as divergent ORs can recognize a single odorant, and that odorants that are similar in structure are recognizable by different, but often overlapping sets of ORs. They also document that small change in the structure or concentration of an odorant can alter the combination of receptors that recognize that odorant. This study provides evidence for the use of a combinatorial code by the mammalian olfactory system.

In an attempt to define the receptive range for an ORs, Araneda *et al* recently screened over 275 compounds and were able to define the ligand binding characteristics of the rat I7 receptor [28][72]. The authors employed the use of rational drug design, a strategy employed by medicinal chemists to screen molecules, using octanal as a template (Scheme) [73]. In doing so molecules with varying degrees of unsaturation, functional groups and side chains (cyclic and acyclic) were used to define the ligand binding characteristics of the rat I7 receptor. Using EOG as a measure of receptor activation, it was demonstrated that besides the aldehyde moiety, no other functional group was capable of activating the receptor. In addition, molecules longer than 12Å (more than eleven carbon atoms) or shorter than 8Å (less than seven carbon atoms) were unable to activate the receptor, a relationship also observed in mitral cell recording described earlier [25]. Interestingly, within the acceptable length range for active molecules (8Å–12Å), structural variations towards the tail end were more tolerated, suggesting the presence of a flexible hydrophobic binding pocket where, provided that the length of the ligand is correct, the tail could assume various conformations. While the degree of unsaturation (*e.g. trans*-2-octenal) did not seem to alter the activity of the molecules to any appreciable extent, a combination α,β unsaturation with methyl substitutions at the α- or β- carbons atom abolished activity of the molecule (*e.g.* 3-methyl-*trans*-2-octenal). Using a combination of molecular modeling and experimental techniques, it was demonstrated that molecules bearing double bonds at the α,β position of the aldehyde molecule



Scheme. Strategy used in screening ligands for the rat olfactory receptor, I7. The Scheme shows the five main classes of compounds screened in this study. Group I, compounds with different functional groups where X = COOH , OH, *etc.* Group II, III and IV represent groups of molecules that contained varying degrees of unsaturations and substitutions along the carbon bone of the parent compound (octanal). Molecules containing aromatic and aliphatic rings are pooled together in Group V.

(sp^2 hybridized), forces the methyl group at the α or β position into the plane of the carbonyl, rendering the molecule inactive (Fig. 4). These results thus define a very stringent binding pocket at the aldehydic end (head) of the molecule.

A comparison of the data from Mori's research and this study brings out some similarities and differences [28][72]. In both cases aliphatic hydrocarbons (saturated and unsaturated) did not evoke any appreciable response, indicating the significance of functional groups in the perception of smell. Also the carbonyl group along with the length of the molecule was found to be critical in both cases. However, data from our laboratory indicates that in addition to this there exists a very rigid steric requirement for the rat I7 receptor around the front end of the molecule, while the tail of the molecule appears fairly liberal in its ability to accommodate different and at times bulky substitutions [72]. This comparison brings out the possibility that ORs could be characterized as broadly or narrowly tuned (Fig. 5). For example, the broadly tuned receptors would be able to recognize ligands with a particular functional moiety (carbonyl group from carboxylic acid or an aldehyde) [28][29], while the narrowly tuned ones would recognize a specific functional group regardless of the moiety it shares with any other functional group [72].

Considering the current state of experimental protocols, the ability to test 1000 odors on each receptor is not a trivial task. However, the use of molecular modeling as an *in silico* equivalent of high-throughput screening (HTS) could be a viable alternative to *in vitro* or *in vivo* screening [74]. Very few studies on OR models have been reported so far [32][75][76]. Pilpel and Lancet have predicted an odor-binding pocket comprised of TM3 – TM6 [32], whereas two other studies have developed models based on the 7.7 Å crystal structure of rhodopsin [77]. The availability of the high resolution (2.8 Å) crystal structure of rhodopsin [78] now makes it possible to develop OR models based on rhodopsin, enabling one to conduct binding studies and test a large number of compounds for any OR receptor for which even a single ligand has been identified. These studies could, in all probability, not only allow one to define the amino acid residues involved in the binding region, but eventually allow one to predict the type of compounds that would be ligands for any given OR. While this is not a trivial task, it is certainly a reasonable alternative to crystallizing over a thousand ORs.

The olfactory system is a remarkable chemical detector and discriminator that has evolved to allow animals to perceive thousands of complex odors in their environment. Recent advances in understanding this sensory system have resulted from the application of molecular biology, genetics, physiology, pharmacology and medicinal chemistry techniques. As a result we now have a rudimentary understanding of how the binding of a chemical compound to a receptor can be transformed into a neural perception of an odor. Critical questions in the field that remain include, how a thousand receptors can cover the olfactory spectrum of the entire spectrum and how GPCR structure determines ligand affinity and signaling. Finally, we hope to understand the olfactory code in the brain that leads to the captivating world of odors.

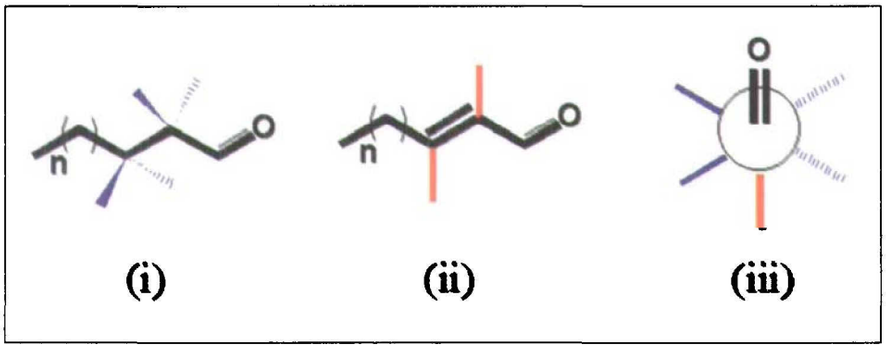


Fig. 4. (i) In the absence of the double bond the α,β carbons are sp hybridized thereby allowing the methyl groups (blue) rotate out of plane. (ii) In the presence of the α+β double bond, the α,β carbons are sp2 hybridized forcing the methyl groups (red) on these carbon atoms into the plane thereby interfering with the ability of the molecule to activate the receptor. (iii) Shows the top down view for the combination of (i) and (ii).

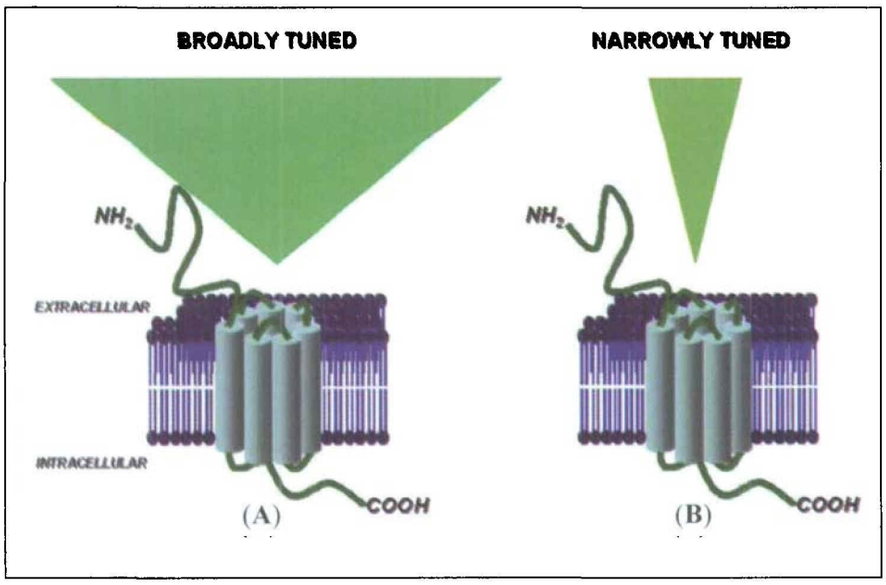


Fig. 5. Schematic representation of ORs that are broadly and narrowly tuned receptors. (**A**) Ones that might recognize the carbonyl functionality (broad); (**B**) receptors that recognize one functional group (*e.g.* aldehyde).

Acknowledgements

We would like to thank the McKnight Foundations and the NIDCD for funding our research over the years. We would also like to thank John Leffingwell for permission to use a figure (Fig. 2) from his website (*www.leffingwell.com*). Acknowledgements are also due to the past and present members of the Firestein lab, who have contributed to the projects discussed here.

Received: March 28, 2001

[1] A.A. Bronshtein, A.V. Minor, *Tsitologiia* **1977**, *19*, 33–39.
[2] D.G. Moulton, L.M. Beidler, *Physiol. Rev.* **1967**, *47*, 1–52.
[3] D.G. Moulton, *Am. Zool.* **1967**, *7*, 421–429.

[4] U. Pace, E. Hanski, Y. Salomon, D. Lancet, *Nature* **1985**, *316*, 255–258.
[5] P.B. Sklar, R.R.H. Anholt, S.H. Snyder, *J. Biol. Chem.* **1986**, *261*, 15538–15543.
[6] U. Pace, D. Lancet, *Proc. Nat. Acad. Sci. USA* **1986**, *83*, 4947–4951.
[7] S. Firestein, B. Darrow, G.M. Shepherd, *Neuron* **1991**, *6*, 825–835.
[8] S. Firestein, F. Zufall, G.M. Shepherd, *J. Neurosci.* **1991**, *11*, 3565–3572.
[9] W. Bonigk, J. Bradley, F. Muller, F. Sesti, I. Boekhoff, G.V. Ronnett, U.B. Kaupp, S. Frings, *J. Neurosci.* **1999**, *19*, 5332–5347.
[10] R.S. Dhallan, K.W. Yau, K.A. Schrader, R.R. Reed, *Nature* **1990**, *347*, 184–187.
[11] E.R. Liman, L.B. Buck, *Neuron* **1994**, *13*, 611–621.
[12] D.T. Jones, R.R. Reed, *Science* **1989**, *244*, 790–795.
[13] H.A. Bakalyar, R.R. Reed, *Science* **1990**, *250*, 1403–1406.
[14] T.-Y. Chen, K.-W. Yau, *Nature* **1994**, *368*, 545–548.
[15] M. Liu, T.-Y. Chen, B. Ahamed, J. Li, K.-W. Yau, *Science* **1994**, *266*, 1348–1354.
[16] T. Huque, R.C. Bruch, *Biochem. & Biophys. Res. Comm.* **1986**, *137*, 36–42.
[17] T. Miyamoto, D. Restrepo, E.J. Cragoe Jr., J.H. Teeter, *J. Membr. Biol.* **1992**, *127*, 173–183.
[18] D. Restrepo, T. Miyammoto, B.P. Bryant, J.H. Teeter, *Science* **1990**, *249*, 1166–1168.
[19] D.A. Fadool, B.W. Ache, *Neuron* **1992**, *9*, 907–918.
[20] W.C. Michel, T.S. McClintock, B.W. Ache, *J. Neurophysiol.* **1991**, *65*, 446–453.
[21] W.C. Michel, B.W. Ache, *J. Neurosci.* **1992**, *12*, 3979–3984.
[22] J. Krieger, H. Breer, *Science* **1999**, *286*, 720–723.
[23] I. Boekhoff, E. Tareilus, J. Strotmann, H. Breer, *EMBO* **1990**, *9*, 2453–2458.
[24] L.J. Brunet, G.H. Gold, J. Ngai, *Neuron* **1996**, *17*, 681–693.
[25] K. Mori, H. Nagao, Y. Yoshihara, *Science* **1999**, *286*, 711–715.
[26] P. Mombaerts, *Ann. Rev. Neurosci.* **1999**, *22*, 487–509.
[27] K. Katoh,H. Koshimoto, A. Tani, K. Mori, *J. Neurophysiol.* **1993**, *70*, 2161–2175.
[28] K. Imamura, N. Mataga, K. Mori, *J. Neurophysiol.* **1992**, *68*, 1986–2002.
[29] K. Mori, *Nature Neurosci.* **2000**, *3*, 1035–1043.
[30] K. Katoh, H. Koshimoto, A. Tani, K. Mori, *J. Neurophysiol.* **1993**, *70*, 2161–2175.
[31] L. Buck, R. Axel, *Cell* **1991**, *65*, 175–187.
[32] Y. Pilpel, D. Lancet, *Prot. Sci.* **1999**, *8*, 969–977.
[33] W.C. Probst, L.A. Snyder, D.I. Schuster, J. Brosius, S.C. Sealfon, *DNA Cell Biology* **1992**, *11*, 1–20.
[34] R. Axel, *Scientific American* **1995**, *273*, 154–159.
[35] L.B. Buck, *Current Opinion in Neurobiology* **1992**, *2*, 282–288.
[36] L.B. Buck, in 'Sensory Transduction', vol. 47, Eds. D.P. Corey, S.D. Roper, Rockefeller Univ Press, New York, **1992**, pp. 39–51.
[37] K.J. Ressler, S.L. Sullivan, L.B. Buck, *Cell* **1993**, *73*, 597–609.
[38] R. Vassar, J. Ngai, R. Axel, *Cell* **1993**, *74*, 309–318.
[39] P. Nef, I. Hermans-Borgmeyer, H. Artières-Pin, L. Beasley, V.E. Dionne, S.F. Heinemann, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8948–8952.
[40] M. Parmentier, F. Libert, S. Schurmans, S. Schiffmann, A. Lefort, D. Eggerickx, C. Ledent, C Mollereau, C. Gerard, A. Grootegoed, G. Vassart, *Nature* **1992**, *355*, 453–455.
[41] G. Glusman, S. Clifton, B. Roe, D. Lancet, *Genomics* **1996**, *37*, 147–160.
[42] S. Rouquier, S. Taviaux, B.J. Trask, V. Brand-Arpon, G. van den Engh, J. Demaille, D. Giorgi, *Nat. Genet.* **1998**, *18*, 243–250.
[43] J. Ngai, M.M. Dowling, L. Buck, R. Axel, A. Chess, *Cell* **1993**, *72*, 657–666.
[44] Y. Cao, B.C. Oh, L. Stryer, *PNAS* **1998**, *95*, 11987–11992.
[45] D.J. Speca, D.M. Lin, P.W. Sorenson, E.Y. Isacoff, J. Ngai, A.H. Dittman, *Neuron* **1999**, *23*, 487–498.
[46] Y. Tanabe, M. Masu, T. Ishii, R. Shigemoto, S. Nakanishi, *Neuron* **1992**, *8*, 169–179.
[47] G. Herrada, C. Dulac, *Cell* **1997**, *90*, 763–774.
[48] C.I. Bargmann, H.R. Horvitz, *Cell* **1993**, *74*, 515–527.
[49] E. Troemel, J. Chou, N. Dwyer, H. Colbert, C. Bargmann, *Cell* **1995**, *83*, 207–218.
[50] N.E. Rawson, J. Eberwine, R. Dotson, J. Jackson, P. Ulrich, D. Restrepo, *J. Neurochem.* **2000**, *75*, 185–195.
[51] A. Berghard, L. Dryer, *J. Neurobiol.* **1998**, *37*, 383–392.
[52] P.J. Clyne, C.G. Warr, M.R. Freeman, D. Lessing, J. Kim, J.R. Carlson, *Neuron* **1999**, *22*, 327–338.
[53] L.B. Vosshall, H. Amrein, P.S. Morozov, A. Rzhetsky, R. Axel, *Cell* **1999**, *96*, 725–736.
[54] R. Vassar, S.K. Chao, R. Sitcheran, J.M. Nunez, L.B. Vosshall, R. Axel, *Cell* **1994**, *79*, 981–991.
[55] L. Astic, D. Saucier, *Brain Res.* **1983**, *312*, 257–263.
[56] F. Jourdan, A. Duveau, L. Astic, A. Holley, *Brain Res.* **1980**, *188*, 139–154.
[57] L.C. Skeen, *Brain Res.* **1977**, *124*, 147–153.
[58] W.B. Stewart, J.S. Kauer, G.M. Shepherd, *J. Comp. Neurol.* **1979**, *185*, 715–734.
[59] J.S. Kauer, *Nature* **1988**, *331*, 166–168.
[60] K. Mori, N. Mataga, K. Imamura, *J. Neurophysiol.* **1992**, *67*, 786–789.
[61] P. Mombaerts, *Curr. Opin. Genet. Dev.* **1999**, *9*, 315–320.
[62] S.L. Sullivan, S. Bohm, K.J. Ressler, L.F. Horowitz, L.B. Buck, *Neuron* **1995**, *15*, 779–789.
[63] J.P. Royet, C. Souchier, F. Jourdan, H. Ploye, *J. Comp. Neurol.* **1988**, *270*.
[64] T.S. McClintock, M.R. Lerner, *Chem. Senses* **1994**, *19*, 517–517.
[65] K. Raming, J. Krieger, J. Strotmann, I. Boekhoff, S. Kubick, C. Baumstark, H. Breer, *Nature* **1993**, *361*, 353–356.
[66] C. Wellerdieck, M. Oles, L. Pott, S. Korsching, G. Gisselmann, H. Hatt, *Chem. Senses* **1997**, *22*, 468–476.
[67] Y. Zhang, J.H. Chou, J. Bradley, C.I. Bargmann, K. Zinn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12162–12167.
[68] H. Zhao, L. Iviv, J.M. Otaki, M. Hashimoto, K. Mikoshiba, S. Firestein, *Science* **1998**, *279*, 327–242.
[69] D. Krautwurst, K.-W. Yau, R.R. Reed, *Cell* **1998**, *95*, 917–926.
[70] K. Touhara, S. Sengoku, K. Inaki, A. Tsuboi, J. Hirono, T. Sato, H. Sakano, T. Haga, *Proc. Natl. Sci. USA* **1999**, *96*, 4040–4045.
[71] B. Malnic, J. Hirono, T. Sato, L. Buck, *Cell* **1999**, *96*, 713–723.
[72] R.C. Araneda, A.D. Kini, S. Firestein, *Nat. Neurosci.* **2000**, *3*, 1248–1255.
[73] D.P. Marriott, I.G. Dougall, P. Meghani, Y.J. Liu, D.R. Flower, *J. Med. Chem.* **1999**, *42*, 3210–3216.
[74] M. Afshar, R.E. Hubbard, J. Demaille, *Biochimie* **1998**, *80*, 129–135.
[75] M.S. Singer, *Chem. Senses* **2000**, *25*, 155–165.
[76] M.S. Singer, G.M. Sheperd, *NeuroReport* **1994**, *5*, 1297–1300.
[77] G.F. Schertler, *Eye* **1998**, *12*, 504–510.
[78] K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739–745.