Olfaction – Update No. 5

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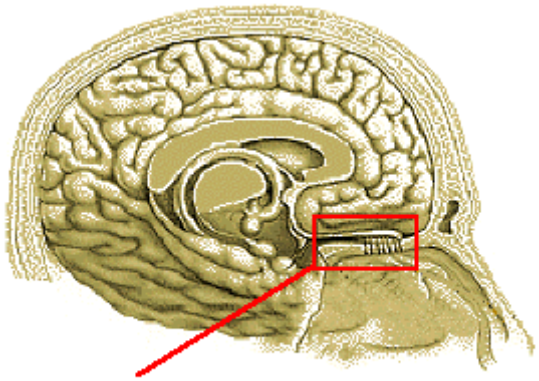
The sense of smell is a primal sense for humans as well as animals. From an evolutionary standpoint it is one of the most ancient of senses. Smell (or Olfaction) allows vertebrates and other organisms with olfactory receptors to identify food, mates, predators, and provides both sensual pleasure (the odor of flowers and perfume) as well as warnings of danger (e.g., spoiled food, chemical dangers). For both humans and animals, it is one of the important means by which our environment communicates with us.

This paper will explore the current status our understanding of olfaction and provide in some detail the possible molecular interactions that specify odorant signaling.

General Physiology of Olfaction

Odorants are volatile chemical compounds that are carried by inhaled air to the Regio olfactoria (olfactory epithelium) located in the roof of the two nasal cavities of the human nose, just below and between the eyes.



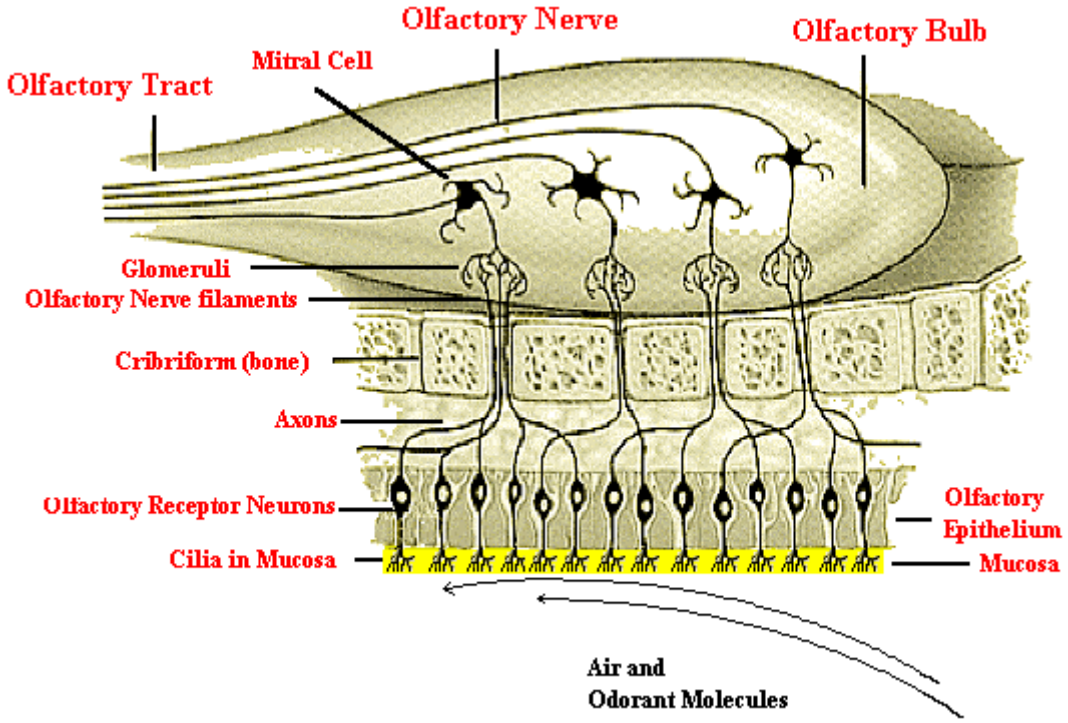
Olfactory Region *(Regio olfactoria)*

The odorant must possess certain molecular properties in order to provide sensory properties. It must have some water solubility, a sufficiently high vapor pressure, low polarity, some ability to dissolve in fat (lipophilicity), and surface activity. And to date, no known odorant possesses a molecular weight greater than 294.[1]

The olfactory sense is able to distinguish among a practically infinite number of chemical compounds at very low concentrations.[2]

The olfactory region of each of the two nasal passages in humans is a small area of about 2.5 square centimeters containing in total approximately 50 million primary sensory receptor cells.

The olfactory region consists of cilia projecting down out of the olfactory epithelium into a layer of mucous which is about 60 microns thick.[2a] This mucous layer is a lipid-rich secretion that bathes the surface of the receptors at the epithelium surface. The mucous layer is produced by the Bowman's glands which reside in the olfactory epithelium. The mucous lipids assist in transporting the odorant molecules as only volatile materials that are soluble in the mucous can interact with the olfactory receptors and produce the signals that our brain interprets as odor. Each olfactory receptor neuron has 8-20 cilia that are whip-like extensions 30-200 microns in length. The olfactory cilia are the sites where molecular reception with the odorant occurs and sensory transduction (i.e., transmission) starts.



Above the mucous layer is the base olfactory epithelium which consists partially of basal cells located in the lowest cellular layer of the olfactory epithelium which are capable of mitotic cell division to form olfactory receptor neurons when functionally mature. The olfactory receptor neurons turnover approximately every 40 days. The epithelium also contains pigmented cells that are light yellow in humans and dark yellow to brown in dogs. The depth of color seems to be correlated with olfactory sensitivity.

While the olfactory receptor neurons extend through the epithelium to contact odorants in the atmosphere, on the opposite side within the epithelium, the neuronal cells form axons that are bundled in groups of 10-100 to penetrate the ethmoidal cribriform plate of bone, reaching the olfactory bulb of the brain where they converge to terminate with post-synaptic cells to form synaptic structures called glomeruli. The glomeruli are connected in groups that converge into mitral cells. (Note that in the picture above this convergence is not clearly depicted). For example, in rabbits, there are 26,000 receptor neurons converging onto 200 glomeruli which

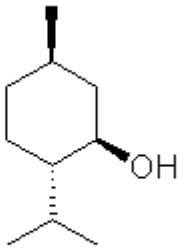
then converge at 25:1 onto each mitral cell. The total convergence is estimated to be about 1000:1.[3]

Physiologically, this convergence increases the sensitivity of the olfactory signal sent to the brain. From the mitral cells the message is sent directly to the higher levels of the central nervous system in the corticomedial amygdala portion of the brain (via the olfactory nerve tract) where the signaling process is decoded and olfactory interpretation and response occurs.

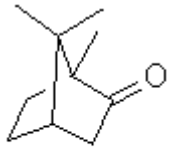
The Trigeminal Sense in the Olfactory Epithelium

It must also be recognized that the olfactory epithelium contains another sensory system in the form of "Trigeminal Nerve" receptors. Whereas, the olfactory receptor system is highly localized in humans, the fifth cranial or trigeminal nerve (which is the largest cranial nerve and is the responsible sensory nerve of the face, teeth, mouth, most of the scalp, and the motor nerve of the muscles of mastication) provides a second set of nerve endings which are responsible for tactile, pressure, pain and temperature sensations in the areas of the mouth, eyes and nasal cavity. A number of chemical trigeminal stimulants produce effects described as hot, cold, tingling or irritating. For example, "leavo-menthol or (-)-menthol" produces the trigeminal feeling of cold at moderate concentrations and "hot" at high concentrations in the nasal cavity. This type of sensory "description" is often not just limited to the areas of the nose, mouth and eyes, but also occurs on skin areas not served by the 5th cranial nerve (especially, the genitalia) and thus such stimulants may effect a variety of nerve endings. Similarly "camphor" which possesses markedly more aroma than menthol, also produces the "cold" sensation via interaction with trigeminal receptors. Ohloff states that "About 70% of all odors are said to stimulate the trigemenal nerve although, in general, they may be several times less sensitive than olfactory receptors".[4]

Other commonly encountered trigeminal stimulants include the chemicals allyl isothiocyanate (mustard, mustard oil), capsiacin (hot Chile powder, mace spray) and Diallyl sulfide (onion).



(-)-1R,3R,4S-MENTHOL

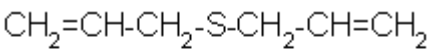


CAMPHOR

CH_2=CH-CH_2-N=C=S

ALLYL ISOTHIOCYANATE

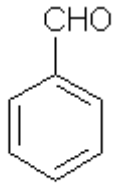
CAPSAICIN



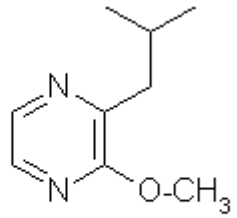
DIALLYL SULFIDE

The Odorant Binding Proteins

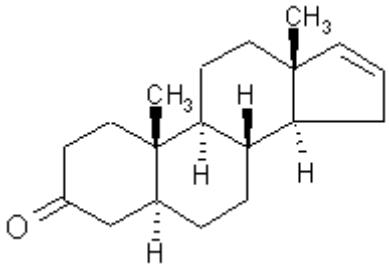
In 1979, Steven Price and coworkers[5] discovered a protein in the olfactory epithelium that bound the chemical "anisole, while Fesenko, et.al.[6], found a camphor binding protein. Since that time a number of other so-called "Odorant Binding Proteins" or "OBP's" have been found for odorant chemicals such as benzaldehyde (cherry-almond odor), 2-Isobutyl-3-methoxypyrazine (green bell pepper odor) and 5-a-androst-16-en-3-one (urine odor).



BENZALDEHYDE



2-ISOBUTYL-3-METHOXYPYRAZINE

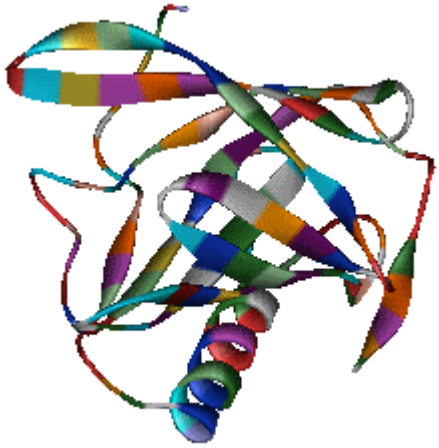


5-a-ANDROST-16-EN-3-ONE

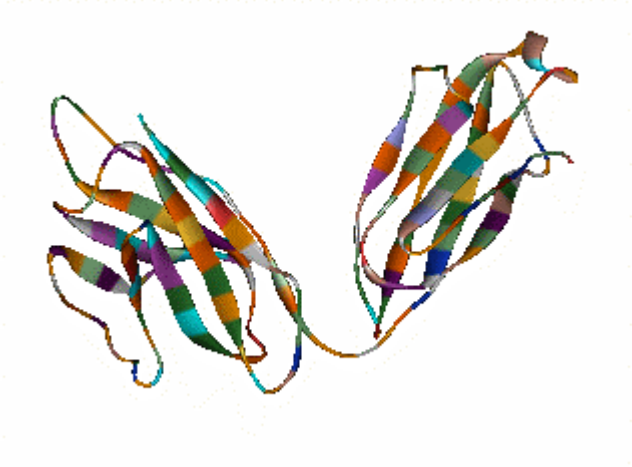
Although the role of Odorant Binding Proteins is not totally clear, one proposed role is that they bind lipophilically to odorants in the aqueous/lipid mucous increasing the concentration and then facilitate transport through the mucous layer to the receptors in the olfactory membrane. Other possibilities suggested are that they bind to the ligand and receptor and

assist in transport across the olfactory membrane, or that they act as a kind of filter to prevent excessive amounts (over saturation) from reaching the receptor.

The role for odorant binding proteins became clearer in 2000 with the characterization of human lipocalins involved in odorant binding. Lipocalins are carrier proteins for hydrophobic molecules in many biological fluids. In the oral sphere (nasal mucus, saliva, tears), they have an environmental biosensor function and are considered involved in the detection of odours and pheromones. Human OBP (IIa) was strongly expressed in the nasal structures, salivary and lachrymal glands, and lung, therefore having an oral sphere profile while hOBP (IIb) was more strongly expressed in genital sphere organs such as the prostate and mammary glands.[6a] Similarly, although monoclonal antibodies (MCAB) had been shown to bind odorants (anisole & benzaldehyde) by Price in 1988[6b], in 1999 workers at the Pluckthun lab[6c] (Univ. Zurich) showed monoclonal antibodies were elicited against the small hydrophobic hapten traseolide (a commercially available musk, 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (Traseolide) . Antibody variable region sequences were found to belong to different sequence groups, and the binding characteristics of the corresponding antibody fragments were studied. To elucidate the structural basis for the fine specificity of binding, they determined the crystal structure of the fragment complexed with the Traseolide at 2.6 A resolution. The crystal structure showed that only van der Waals interactions are involved in binding. The structural understanding of odorant specificity in an antibody gives a degree of insight in the physical principles on how specificity for such hydrophobic molecules may be achieved.



Partial structure of Human OPB IIa modeled against 1EW3A (CRYSTAL STRUCTURE OF THE MAJOR HORSE ALLERGEN EQU C 1)

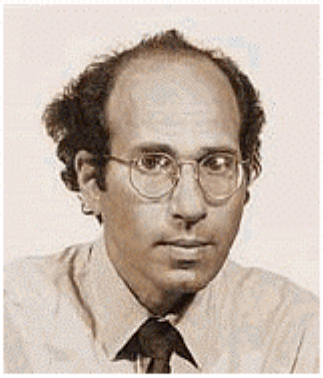


Mouse MCAB Chain B
Structure 1C12B.pdb from Swiss-Model

Odorant receptors.

In 1991, Linda Buck[7] and Richard Axel[8] discovered both the family of transmembrane proteins that were believed to be the odor receptors and some of the genes that encode them. The cloned and characterized 18 different members of an extremely large multigene family that encodes the seven transmembrane proteins whose expression was restricted to the olfactory epithelium. This was a seminal breakthrough in our potential understanding of the olfactory system.[9] The proteins found all contained the 7 helical transmembrane structure and contained sequence similarity to other members of the "G-protein" linked receptor family. [See below for details on membranes and G-protein coupled receptors]. It is now known that there are about 350 odorant receptor genes and about 560 odorant receptor pseudogenes in humans.[7, 12f, 12c, 12h] This number of genes and pseudogenes, specific to the olfactory system, comprises nearly 2% of the 50,000 or so genes of the human genome. This number is second only to the receptors of the immune system.

Linda Buck



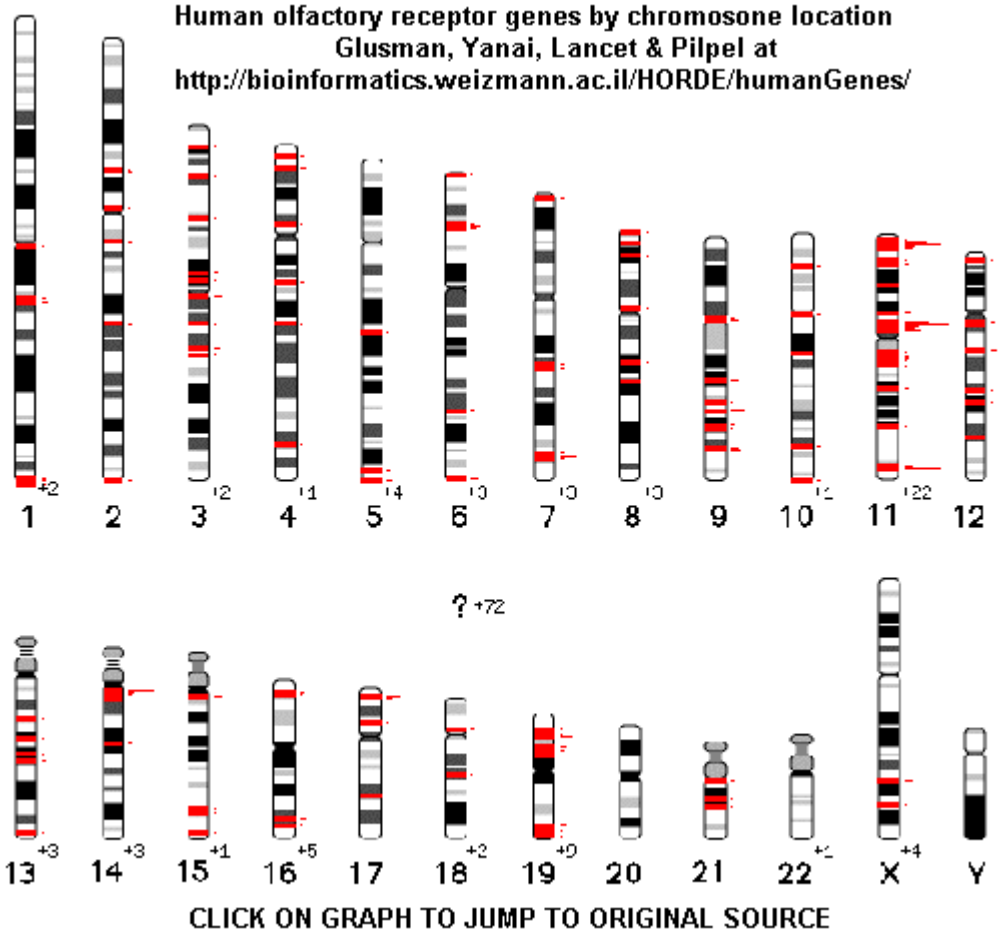
Richard Axel

Stuart Firestein

Until early 1998, however, there was no direct proof that functionally these were actually odor receptors. In January, 1998, Firestein[10] and coworkers at Columbia University effectively demonstrated that genes coded to produce olfactory receptors could be inserted into the rat olfactory system and that specific odorant chemicals would generate significantly higher signaling as measured by the electrical activity in the neurons.[11] "The Columbia team inserted two linked genes, one that codes for a rat olfactory receptor, called rat I7, and a gene for green fluorescent protein (GFP), a substance found normally in fluorescent jellyfish but now used by molecular biologists to mark genetically altered cells, into a disabled adenovirus - the same virus that causes colds. The modified adenovirus was in turn introduced into rat olfactory neurons. Cells that carried the rat I7 gene also carried the GFP gene, and could be discerned because they glowed bright green when exposed to blue light. Firestein's graduate student, Haiqing Zhao, now at Johns Hopkins Medical School, treated rats with the modified adenovirus and then exposed their olfactory neurons to various odorants. He monitored the electrical activity in the neurons, producing a chart called an electro-olfactogram. Electrical activity was highest when the nerve cells were exposed to octanal, an aldehyde that smells meaty to humans".[12] {Note that most flavorists & perfumers would not describe octanal as being "meaty", but as being "fatty-fruity with citrus-orange notes"] In this work researchers evaluated 74 odorants on a specific odor receptor. A long standing question as to whether

individual receptors recognize multiple odorants, or do single neurons have multiple receptors now appears clarified.[12a] It appears that to reconcile the ability of organisms to detect far more than 1,000 discrete odors, the odors must participate in some kind of combinatorial processing: i.e., one receptor must be able to interact with several discrete odorants. Conversely, an odor molecule must be capable of interacting with multiple receptors. By inference, an individual odor will activate multiple glomeruli in the olfactory bulb.[12b] This is discussed in more detail at the end of this review.

Recently (2000), Doron Lancet & co-workers at the Weizmann Institute of Science Crown Human Genome Center have constructed a database of human olfactory receptor genes by a highly automated data mining system[12c, 12h] to detect all OR genes present in the public databases. This non-redundant dataset includes 906 human olfactory receptor genes, of which >60% appear to be pseudogenes. For details and an interactive chromosomal distribution graph of OR gene distribution, click on the graph below.



The authors of HORDE (The Human Olfactory Receptor Data Exploratorium), where this graph originates from, indicate that olfactory receptor genes are present in practically all human chromosomes, with only chromosomes 20 and Y being apparently devoid of ORs.

Chromosome 11 is by far the richest in OR genes. This type data also may help elucidate the human evolutionary tree.[12d, 12i]

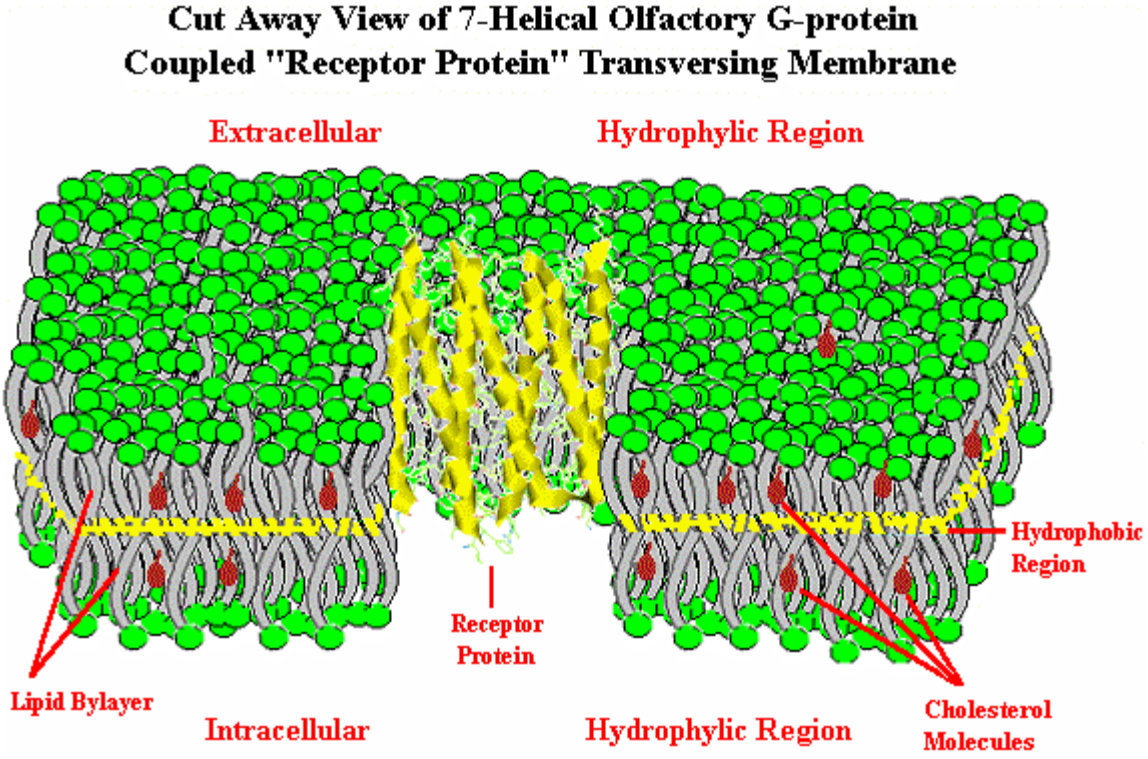
In June, 2001, Zozulya, Echeverri & Nguyen of Senomyx published a paper entitled "The human olfactory receptor repertoire" in which they reported the identification and physical cloning of 347 putative human full-length odorant receptor genes. Comparative sequence analysis of the predicted gene products allowed them to identify and define a number of consensus sequence motifs and structural features of this vast family of receptors. They believe that these sequences represent the essentially complete repertoire of functional human odorant receptors.[12f] They found some differences between their data and those in the HORDE database, however. These include 29 "human OR" (hOR) genes that were apparently identified as pseudogenes in the HORDE database, but encoded as functional hOR candidates their analysis, as well as 10 hORs not found in HORDE. This online article includes a downloadable file with all 347 hOR's in FASTA format.[12g]

In the January 22, 2002 issue of Nature Neuroscience[12j], Stuart Firestein with Xinmin Zhang at Columbia University identified the mouse OR genes from the nearly complete Celera mouse genome by a comprehensive data mining strategy. They found 1,296 mouse OR genes (including 20% pseudogenes). Human ORs cover a similar 'receptor space' as the mouse ORs, suggesting that the human olfactory system has retained the ability to recognize a broad spectrum of chemicals even though humans have lost nearly two-thirds of the OR genes as compared to mice.

With elucidation of the general classification of olfactory receptors as being G-protein coupled receptors, let us now examine how such receptors work (in general) and whether such receptors can be influenced. In order to set the stage, however, we must first examine the structure of the cellular membranes in which the receptors reside.

The Cellular Membrane

Below is depicted a biological membrane transversed by an olfactory receptor protein. The membrane consists of a phosholipid bilayer. At the both the extracellular and intracellular sides depicted in green are the hydrophilic negatively charged phosphate groups. The "tails" of the phospholipids consist of hydrocarbon chains (fat) which orient towards each other (pointing inward to the middle of the membrane), creating a hydrophobic environment. These hydrocarbon "tails" are depicted in gray. Such membrane lipid bilayer are virtually impermeable to large molecules and relatively impermeable to charged ionic molecules and yet quite permeable to lipid soluble low molecular weight molecules.



The "Fluid Mosaic Model" of the biological membrane accounts for the fact that the membrane includes proteins and cholesterol, as well as phospholipids and other molecules, and is fluid…not static. The membrane is about 5 nanometers thick, yet individual phospholipids diffuse rapidly throughout the two dimensional surface of the membrane. It is known that phospholipids can move to the opposite side of the membrane within a few minutes at room temperature in bacterial cells even though the distance is several thousand times the size of the phospholipid. Proteins also diffuse (or move fluidly) within the membrane, but at a much slower rate due to their massive size.

Cholesterol is a necessary component of these cellular membranes helping to break up Van der Waals interactions and the close packing of the lipid portion of the phospholipids, which is integral to making the membrane more fluid.

Membranes serve to (1.) define and compartmentalize the cell, (2.) serve as the locus of specific functions, (3.) control movement of substances into and out of the cell and its compartments and (4.) play a pivotal role in cell-to-cell communication and detection of external signals (e.g., olfaction).

G-Protein Coupled Receptors

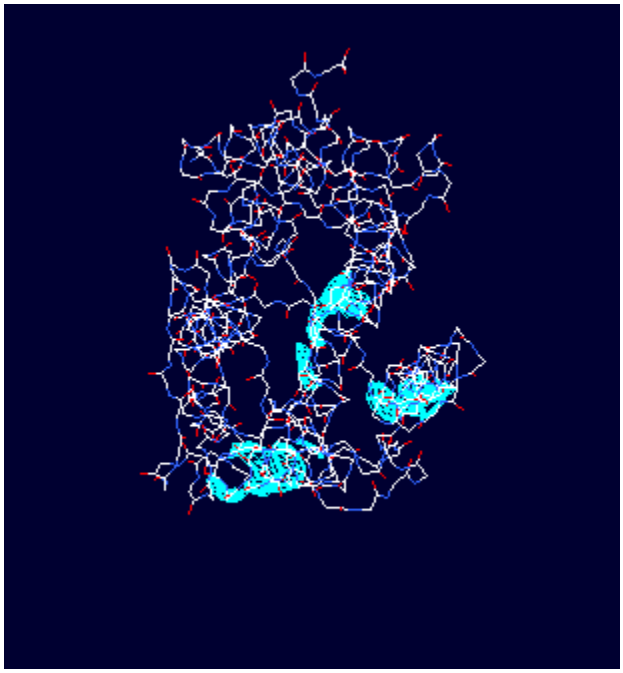
G-protein coupled receptors are known to have been present in the acoelomate flatworms of the Precambrian era over 800 million years ago. This ancient organism (believed to be the ancestor of all bilaterally symmetric metazoans) contained a surprisingly rich collection of

cellular signaling mechanisms.[13] G-protein - coupled receptors are a pharmacologically important protein family with approximately 450 genes identified to date.

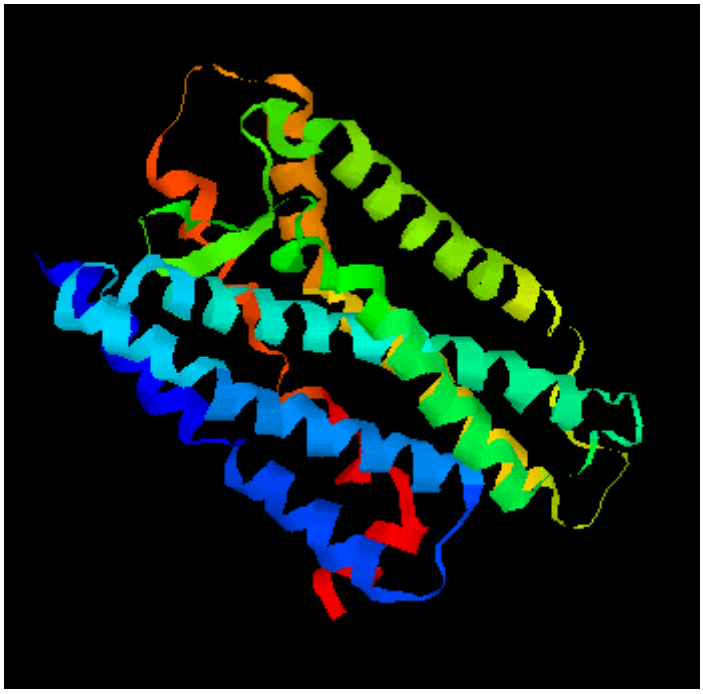
The olfactory receptors are one of the largest groups of G-protein coupled receptors described to date.

Olfactory G-protein linked receptors trigger the biochemical synthesis of neurotransmitters, including cAMP and inositol triphosphate, which open cation channels to that ultimately lead to action potentials and signaling.[14]

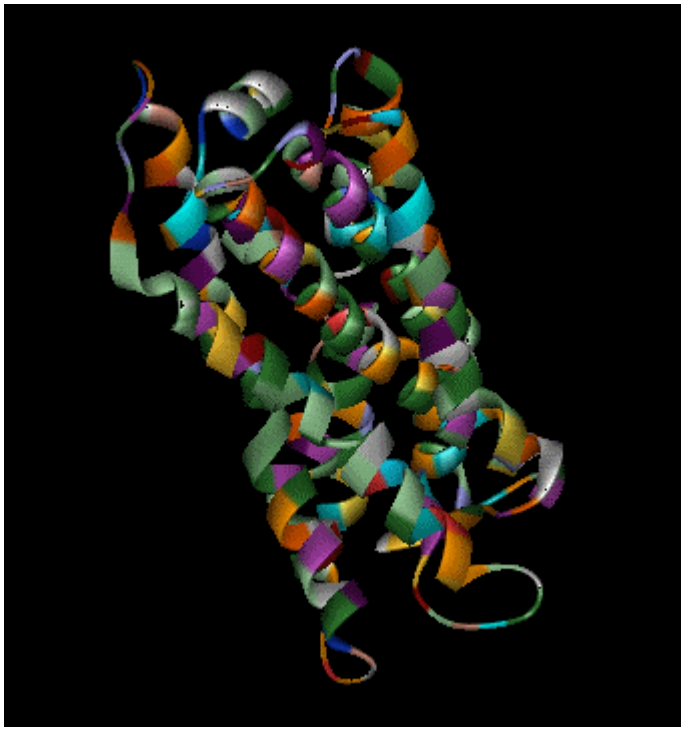
In order to visualize the helical nature of the olfactory receptor, we have constructed the following views of the human olfactory receptor OR1.01.05 (Senomyx nomenclature) by homologous modeling against rhodopsin.



OR1.01.05 Receptor Protein as viewed in the Swiss-PdbViewer with predicted odorant complimentary binding regions on helices 3,4 & 5 highlighted (using the technique of Pipel & Lancet).



OR1.01.05 Receptor Protein as viewed in Chime

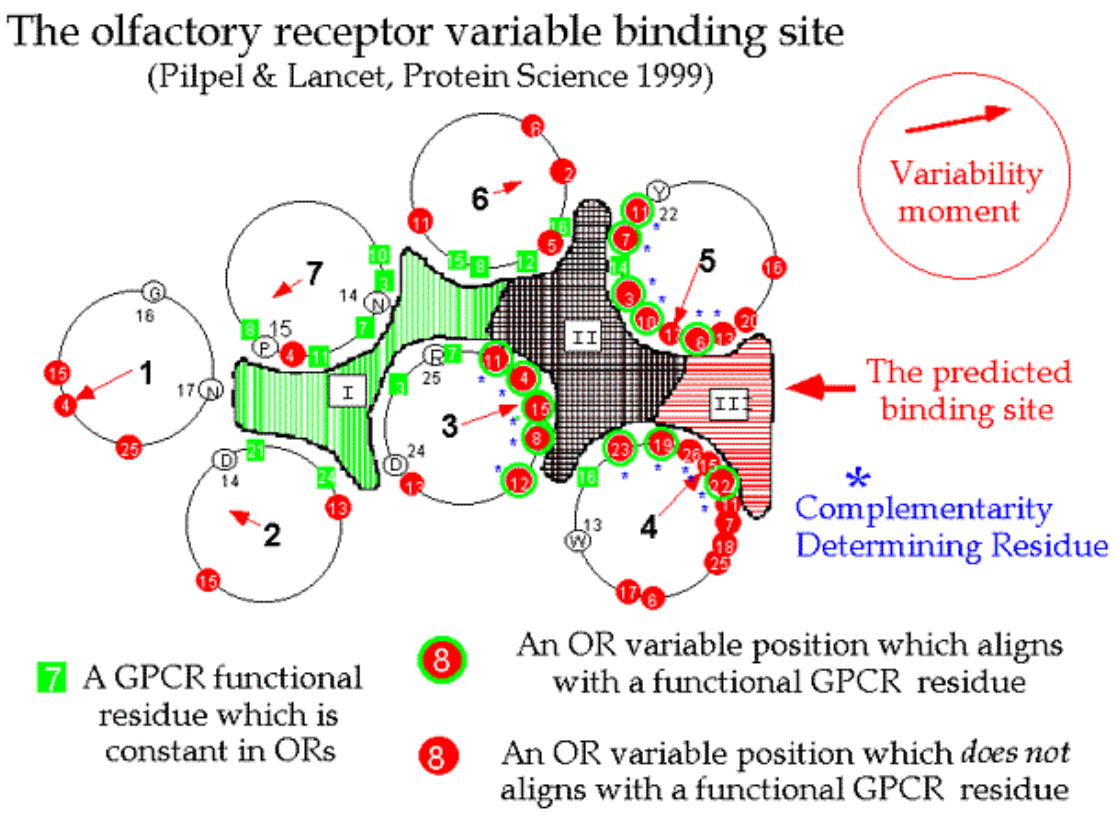


OR1.01.05 Receptor Protein as viewed in Weblab Viewer

The following general principles appear to hold for such receptor proteins:

- 1. The helical axes of the first, third, fifth and seventh helices are quasi-antiparallel to those of the second, fourth and sixth helices.
- 2. The hydrophobic side of each helix faces the lipid phase and the hydrophilic side of each helix was facing either another helix or the pore formed by the helical bundle.
- 3. Conserved residues, either identical throughout a subset or highly homologous, determine the orientation of each helix relative to the other helices.
- 4. The assembly of helices maintain a clockwise order, when seen from the intracellular side, as argued by Baldwin.[14a]
- 5. None of the helices are intersecting.

Recently, Doron Lancet and co-workers have inferred, that for olfactory receptors, the odorant complementarity determining regions reside in the transmembranal segments 3, 4, and 5. [14b]

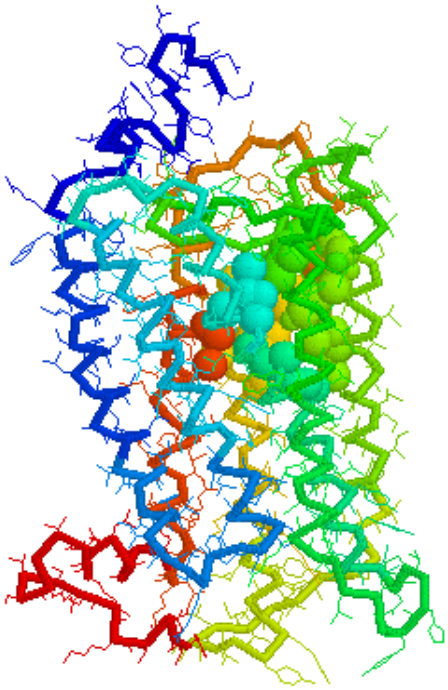


However, Singer[14c] in an Analysis of the Molecular Basis for Octanal Interactions in the Expressed Rat I7 Olfactory Receptor makes a strong case that octanal binds with OR-I7 in a pocket ~10 Å from the extracellular surface **formed by transmembrane domains 3–7.** In addition, Goddard, et.al.[14d], have modeled the mouse receptor ORL466 (OR S25) and in docking studies predicted the binding pocket for the compounds hexanol and heptanol. Docking results show that **TMs 3, 5, and 6** have residues directly involved in binding and that TM4 may have an important role in binding as it packs against TM3 and TM5 and therefore can alter their relative position if key residues of TM4 are mutated. The presence of a critical Lys on TM7 is similar to the related rhodopsin, where Lys-296 (TM7) binds the retinal chromophore[14e]. Substitutions in this residue may switch receptor specificity toward other functional groups.

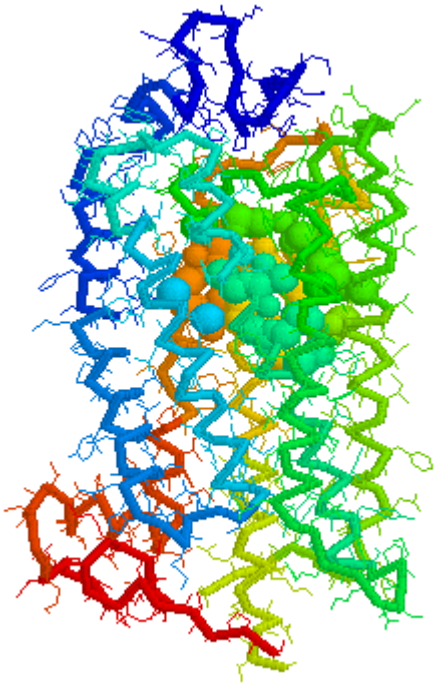
CastP for determining predicted ligand binding sites

Similarly, Leffingwell[14f], in a study of the predicted ligand binding sites for certain Human OR's of chromosone 1, has found that the largest cavity (as derived by CastP[14i]) in the complimentary region also falls in the transmembrane domains **TM 3–7**, especially in the region of TM3-TM5-TM6. This is depicted below for Human OR1.04.06[12f-g] as modeled by Leffingwell. As the CastP technique for determining putative binding cavities and pockets predicts the binding site for retinal in the bovine rhodopsin models 1HXZ (chain A)[14g] and

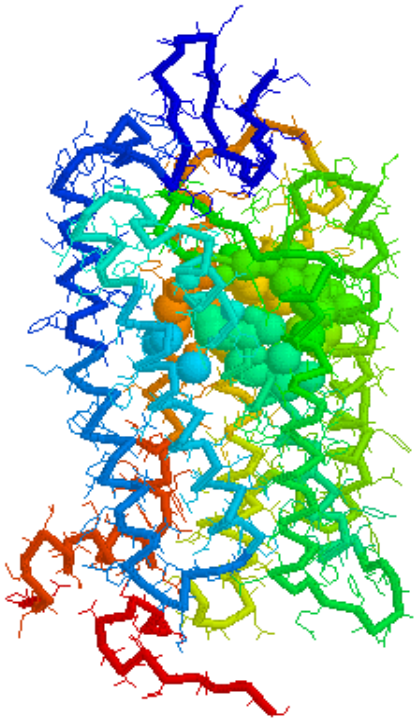
1F88 (chain A)[14h] with an excellent correlation[14f] to that previously found, CastP[14i] may provide a simple and fast method for predicting binding sites in olfactory receptors.



Putative Binding cavity in Human OR1.04.06 derived using CastP



Binding cavity for retinal in Bovine rhodopsin 1HZX Chain A derived using CastP

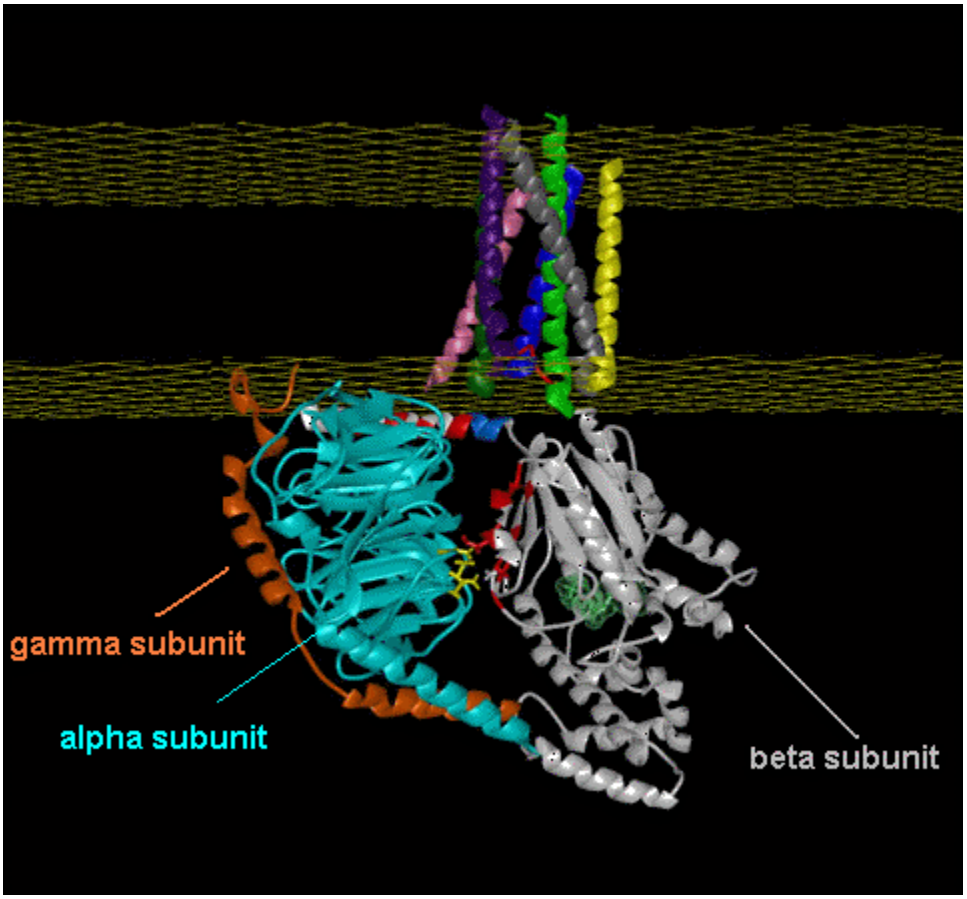


Binding cavity for retinal in Bovine rhodopsin 1F88 Chain A derived using CastP

Leffingwell has also used the publicly available Human OR PDB models[14f] to construct homologous models of the Mouse olfactory receptor OR S25 (Mouse ORL466) and the Rat I7 receptor (Rat ORL11) in order to test the CastP methodology for determining binding sites. These receptors have been previously modeled by Singer[14c] and Goddard, et.al.[14d] as mentioned above.

G-Proteins

The following model illustrates both the transmembranal 7-helical receptor protein (at the top) and the G-protein (at the bottom) within the cell.



Trimeric G proteins are signaling machines that transduce messages from receptors for extracellular stimuli into cellular responses mediated by effector enzymes or ion channels. Responding to G protein-coupled receptors (GPCRs) for example, odorants, these G proteins relay signals to adenylyl cyclase, phospholipase C, K+ channels, and other effectors.

This model (adapted from work at the Bourne lab) of the G protein trimer (bottom), highlights an interaction (red and yellow residues) between the beta and alpha subunits (cyan and gray, respectively) which are postulated to play an important role in mediating GPCR-triggered release of bound GDP (green, near center of alpha subunit). The plasma membrane is indicated by a yellow grid at the top. The gamma subunit is orange.

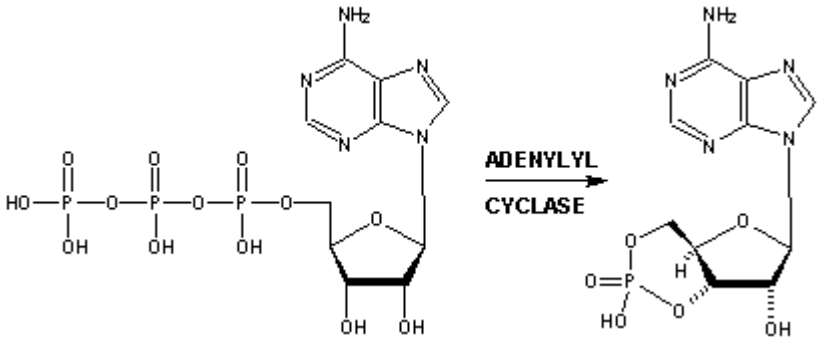
An excellent interactive chime visualization of the action of a G Protein "molecular nanomachine" has been created by Edward K. O'Neil and Charles M. Grisham at the University of Virginia.

The cAMP Transduction Cascade

In simple terms for the cAMP mediated transduction process:

- Studies suggest that the olfactory protein receptors bind small ligands which possibly causes the receptor to change shape and couple to…

- the G-proteins (G-s and/or G-olf). [These proteins are called a G-proteins because they bind Guanosine TriPhosphate (GTP). G-proteins are comprised of three subunits; an alpha subunit that can be considered the active portion, and beta and gamma subunits that regulate.] At rest the alpha subunit binds GDP (Guanosine DiPhosphate) - the OFF state.
- (Remember that the G-proteins are not the receptors, but represent the second step in the signaling process). An olfactory cyclic Adenosine MonoPhosphate (cAMP) gated ion channel controls the electrophysiological state of these neurons' response to olfactory stimuli.
- When the olfactory receptor couples to the G-protein, the GDP in the alpha subunit is replaced by GTP. - the ON state.
- Once the GTP is bound, the alpha subunit protein disassociates from the beta and gamma subunits
- the alpha subunit then associates with and activates the enzyme adenylyl cyclase
- Once activated, adenylyl cyclase cyclizes Adenosine TriPhosphate (ATP) into cyclic-3',5'-AdenosylMonoPhosphate (cAMP) [this involves the release of the beta and gamma phosphates from the ATP and the linking of the surviving phosphate (which is attached to the 5'-hydroxyl of the ribose portion to the 3'-hydroxyl) forming the cyclic neurotransmitter, cAMP.]



- The neurotransmitter cAMP, whose intracellular concentration has greatly increased, now acts as an intracellular hormone [often termed "second messengers"] which can move throughout the cell cytoplasm and and activate gated ion protein channels (or pumps) allowing the flow of extracellular inorganic ions (here the Ca++ ion)….
- Across the cellular membrane into the olfactory neuronal cell which generates a membrane signal potential leading to an electrical signal (spike generation) that represents transfer of the chemosensory information to the olfactory bulb via the axons. The cAMP

concentration then diminishes as it hydrolyses to AMP (Adenosine MonoPhosphate).

- Before discussing ion protein channels, however, we need to know how the G-protein deactivates. The alpha subunit of the G-protein is an enzyme that hydrolyses the GTP to GDP in the process of activating the adenylyl cyclase. Thus , the alpha subunit effectively terminates its own activity. Once the GTP has been hydrolyzed to GDP, the alpha subunit rejoins the beta and gamma subunits, and the G-protein returns to its resting state (OFF state).

Ion Protein Channels

As mentioned, receptor interaction with an odorant triggers activation of the G-protein (i.e., G-olf) that triggers adenylyl cyclase resulting in an increase in intracellular cAMP in the olfactory cells. This sequence of events is referred to as as a multi-step "cascade". The direct activation of a cation permeable channel by cAMP is the final step in producing the odor induced ionic current. In the presence of normal physiological extracellular Ca^{++}, the second messengers (i.e., cAMP) elicits the opening of the channel allowing Ca^{++} to flow in, and the increase in intracellular Ca^{++} concentration appears to activate a chloride current that helps depolarize the olfactory cell. Thus the cyclic nucleotide gated channels plus the Cl^- evoked whole cell current results in the signal transduction.[15-16]

Other Second Messengers in Olfaction

In some vertebrate species, odorants have been shown to elicit rapid increases in IP3, as well as in cAMP, implicating that two separate signal transduction pathways exist in olfactory neurons (Breer and Boekhoff, 1992; Schild and Restrepo, 1998). The odorants citralva and eugenol appear to increase cAMP, whereas some other odorants such as lyral, lilial, and ethyl vanillin have been shown to elicit IP3 increases in biochemical assays (Breer and Boekhoff, 1992; Schild and Restrepo, 1998).

IP3 (Inositol TriPhosphate)

Another second messenger, Inositol TriPhosphate (IP3) is also known to actively participate in olfactory transduction within certain mammals. While the general cascade process is similar to that described for cAMP above, chemically it is quite different.

There is evidence that another category of G-protein is involved in the activation of the intracellular membrane-bound enzyme phospholipase C (PLC). PLC hydrolyses a lipid phosphatidylinositol-4,5-bisphosphate (PIP2) in the plasma membrane, producing inositol trisphosphate (IP3) and diacyl glycerol (DAG). In 1998, Breer and co-workers[18] identified particular individual G protein subtypes that were activated upon stimulation of isolated olfactory cilia by chemically distinct odorants. These results indicate that different subsets of odorants selectively trigger distinct reaction cascades and provide evidence for dual transduction pathways in olfactory signaling (i.e. a cAMP path and a IP3/DAG path).[19]

Both IP3 and DAG can act directly on ion channels and also on intracellular Ca2+ levels. It thus appears that both cAMP and IP3/DAG systems may coexist in the same cell and may be activated by different odorants.[20] They may even have anti effects, since a rise in calcium levels might activate Ca2+-dependent K+ channels which may hyperpolarize the cell and slow or terminate signaling.

cGMP

Recent evidence that the cAMP transduction cascade is mediated by another cyclic nucleotide cGMP (cyclic Guanosine MonoPhosphate) has been published.[17] It appears that odorants cause a delayed and sustained elevation of cGMP. It has been suggested that at least part of this response is due to activation of one of two ciliar receptor guanylyl cyclases by calcium and a guanylyl cyclase activating protein (G-cap). The cGMP formed serves to augment the cAMP signal by activation of adenylyl cyclase. cAMP, which in turn, negatively regulates guanylyl cyclase, limiting the cGMP signal. [See also the role of Carbon monoxide].

The Roles of Nitric Oxide and Carbon Monoxide

Both Nitric oxide and Carbon monoxide also appear to play roles in the olfactory system. Nitric oxide (NO) has been implicated in synaptic plasticity in other regions of the brain as a result of its modulation of cyclic GMP levels. In sheep, the neuronal enzyme nitric oxide synthase (nNOS) is expressed in both mitral and granule cells, whereas the guanylyl cyclase subunits that are required for NO stimulation of cGMP formation are expressed only in mitral cells. Nitric oxide therefore seems to act as a retrograde and/or intracellular messenger, being released from both mitral and granule cells to potentiate glutamate release from mitral cells by modulating cGMP concentrations. Kendrick[21] therefore has proposed that the resulting changes in the functional circuitry of the olfactory bulb underlie the formation of olfactory memories . This is consistent with the report of Okere, et.al.[22], who demonstrated that exogenous administration of nitric oxide into the female mouse accessory olfactory bulb (whose synaptic activities are modified by pheromonal inputs after mating) can induce a pheromone-specific olfactory memory without mating.

In addition, data suggests a prominent function for NO in activity-dependent establishment of connections within both developing and regenerating olfactory neurons.[23]

Similarly, results indicate that Carbon monoxide (CO) may serve as a gaseous neuronal messenger linked to cyclic GMP production suggesting its involvement in the developmental processes of the olfactory receptor neuron.[24] Further, it has been found that endogenous CO/cGMP signals contribute to olfactory adaptation and underlie the control of gain and sensitivity of odor transduction. The findings offer a mechanism by which a single, brief odor stimulus can be translated into long-lasting intracellular changes that could play an important role in the perceptual adaptation to odors, and explain the long-standing puzzle that the olfactory CNG channels can be gated by both cAMP and cGMP.[25]

Chemical Olfactory Stimulation - Theories on Olfaction

Over the years a number of theories relating odorant quality to molecular structure have been proposed. Here we will review the two most prominent theories and add another involving the direct participation of certain neurotransmitters or their hydrolysates in assisting the docking of odorant molecules with the olfactory receptor protein.

- **The Steric Theory of Odor**

In 1946, future Nobel laureate, Linus Pauling[26] indicated that a specific odor quality is due to the molecular shape and size of the chemical. Similarly, in the book, "Molecular Basis of Odor" by John Amoore[27], he extended the idea of a "Steric Theory of Odor" originally proposed by R.W. Moncrieff in 1949[28] that stated air borne chemical molecules are smelled when they fit into certain complimentary receptor sites on the olfactory nervous system. This "lock and key" approach was an extension from enzyme kinetics. Amoore proposed primary odors (ethereal, camphoraceous, musky, floral, minty, pungent and putrid). The molecular volume and shape similarity of various odor chemicals were compared (by making hand prepared molecular models and physically measuring volume and creating silhouette patterns - there were no computer molecular modeling programs in that era).

The steric theory is well suited to the idea that the odorant receptor proteins accept only certain odorants at a specific receptor sites. The receptor is then activated (by conformation deformation?) and couples to the G-protein and the signal transduction cascade begins.

- **The Vibrational Theory of Odor**

In 1938, Dyson[29] suggested that the infrared resonance (IR) which is a measurement of a molecules vibration might be associated with odor. This idea was popularized by R.H. Wright in the mid 1950's as infrared spectrophotometers became generally available for such spectral measurements which Wright was able to correlate with certain odorants.[30]

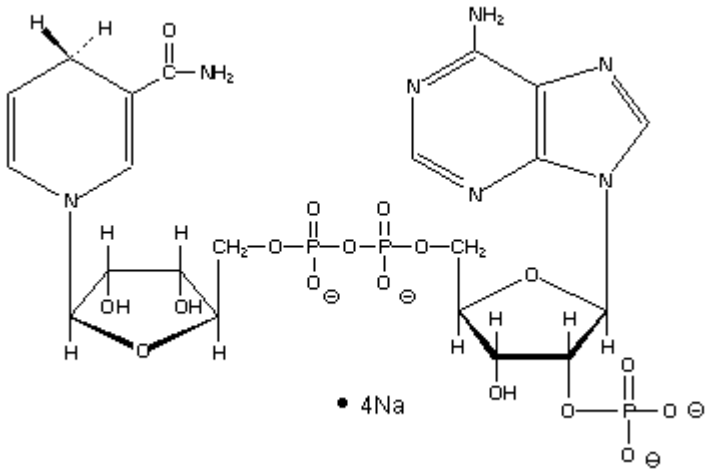
During the 60's and early 70's, vigorous debate raged as to the validity of each theory for classifying chemical odorants.

By the mid-70's, it appeared that Wright's theory had failed a critical test. The optical enantiomers of Menthol[31] and of Carvone[32] smelled distinctly different, although the corresponding infrared spectra were identical. And this theory fell from favor. Recently (August 2001), Leffingwell has published on the internet an extensive site that provides over 100 enantiomeric pairs of odorants that have differing odor properties. This site provides both 2-D and 3-D molecular structures along with odor descriptors, odor thresholds and original references.

- **Vibrational Induced Electron Tunneling Spectroscope Theory**

Until the seminal dissertation of Luca Turin[33] in 1996, the vibrational theory had been placed under a very dark cloud. Turin, however, has attempted to provide a detailed and plausible mechanism for the biological transduction of molecular vibrations that, while not accepting the mechanical vibrational spectroscopy theory previously proposed, replaces it with a theory that the receptor proteins act as a "biological spectroscope". What was proposed is a process called "inelastic electron tunneling". Since this paper, which appeared in Chemical Senses in 1996 is available for downloading off the Internet [at http://www.physiol.ucl.ac.uk/research/turin_l/], I will only outline the process of electron transfer proposed.

Suffice it to say that the receptor is triggered by an odorant in the presence of NADPH (ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form), which is formed by the enzymatic reduction of ß-Nicotinamide Adenine Dinucleotide Phosphate (NADP). NADPH is widely distributed in living matter and acts as an enzyme cofactor. ß-NADPH is a product of the pentose phosphate pathway, a multifunctional pathway whose primary purpose is to generate reducing power in the form of ß-NADPH. ß-NADPH transfers H+ and 2e- to oxidized precursors in the reduction reactions of biosynthesis. Thus, ß-NADPH cycles between catabolic and biosynthetic reactions and serves as the carrier of reducing power in the same way that ATP serves as the carrier of energy.[34]



NADPH (as Sodium salt)

Since according to Turin's theory the receptor functions as an "NADPH diaphorase", it may be significant that high levels of diaphorase activity have been detected in olfactory receptor neurons.[35]

In order for such electron transfer to occur, Turin proposes from molecular modeling that a zinc binding site is present both on the odorant receptor protein and the G-protein. Zinc's involvement in olfaction, its ability to form bridges between proteins, its presence in electron transfer enzymes, such as alcohol dehydrogenase and the presence of a the redox-active amino acid cysteine in the receptor's zinc-binding motif all point to a possible link between electron flow and G-protein transduction. "Suppose the zinc-binding motif on the olfactory receptor is involved in docking to the olfactory G-protein g(olf), and that the docking involves formation of a disulfide bridge between receptor and G-protein. One would expect to find on g(olf) the other half of a zinc coordination site, for example two histidines in close proximity, and a cysteine nearby." A search in the primary sequence of g(olf) finds the motif (His-Tyr-Cys-Tyr-Pro-His). This motif has the requisite properties for docking. It is exposed on the surface of the G-protein and is known to interact with G-protein coupled receptors. In the closely-related adrenergic receptors, a role for cyclic reduction and oxidation of disulfide bridges has been suggested (Kuhl, 1985)[36] . It involves cross-linking of the G-protein to the receptor by an S-S bridge which is then reduced upon binding of the (redox-active) catecholamine to the receptor, thereby releasing the G-protein. Turin proposes that a similar mechanism may be at work in olfaction.

Electron tunneling basically is the transfer of electrons down the backbone of the protein and here this would only occur as follows:

"When the (olfactory) receptor binding site is empty, electrons are unable to tunnel across the binding site because no empty levels are available at the appropriate energy. The disulfide bridge between the receptor and its associated G-protein remains in the oxidized state. When an odorant (here represented as an elastic dipole) occupies the binding site, electrons can lose energy during tunneling by exciting its vibrational mode. This only happens if the energy of the vibrational mode equals the energy gap between the filled and empty levels. Electrons then flow through the protein and reduce the disulfide bridge via a zinc ion, thus releasing the G-protein for further transduction steps.

If there is a molecule between the electron source and electron sink, and if that molecule vibrates then (taking the energy of the vibrational quantum as E) indirect tunneling can occur by an additional channel if there is an energy level in the source with energy E above that in the sink. After tunneling, the molecule will have a vibrational energy higher by E. In other words, tunneling occurs only when a molecular vibrational energy E matches the energy difference between the energy level of the donor and the energy level of the acceptor. The receptor then operates as a spectrometer which allows it to detect a single well-defined energy, E . If the change in energy between donor and acceptor levels is sufficiently large, tunneling current flows across the device only when a molecule with the appropriate vibrational energy is present in the gap. If there are several vibrational modes, which one(s) get excited will depend on the relative strengths of the coupling, and that may be expected to depend, among other things on the partial charges on the atoms and the relative orientation of the charge movements with respect to the electron tunneling path."[33]

While Turin's theory has not been validated, it seems quite plausible. However, even if generally valid, it does not necessarily mean that the "Steric Theory" doesn't play a role.

[The so-called electron tunneling concept in proteins is a major topic of debate as to the exact mechanism of electron transfer. This stems from the work of Jacqueline Barton (Electron transfer between metal complexes bound to DNA: is DNA a wire?) at the California Institute of Technology.[37]][37a]

While both the "Steric" and "Vibrational Induced Electron Tunneling Spectroscope" theories answer many of the questions posed, as one is solved, others arise.

Ribonucleotides as the Odorant carrier?

It is now obvious, perhaps, that a multiplicity of events occur in olfaction. But several major questions that have not been addressed remain to be answered.

> 1. Are certain neurotransmitters (or their hydrolysates) involved not just as so-called "second messengers" in the transduction cascade, but are they also involved as "Amplifiers" that help to capture the odorant molecules and direct them to the receptor sites?
>
> 2. Are the ribonucleotides (AMP, cAMP, GMP and cGMP), [as well as possibly IP3], the glue that helps to bind odorants into the odorant receptor sites?

While there are a few intriguing clues in the literature relative to these questions, it appears that the potentially powerful electrostatic affinity properties of some of these neurotransmitters (or their hydrolysates) may possibly play a significant role early in the olfactory process.

In the chemoreception of "taste", it has long been known that certain ribonucleotides (especially 5'-guanosine monophosphate [5'-GMP] and 5'-inosine monophosphate [5'-IMP]) have potent synergistic effects with MSG (monosodium glutamate)[38] including a significant lowering of the MSG threshold level. In 1980, Torii and Kagan showed that a several-fold enhancement of binding of glutamate occurred with bovine taste papillae in the presence of certain 5'-ribonucleotides (e.g., 5'-GMP, 5'-IMP) but not with others (e.g., 5'AMP).[39]

Now it should be noted that 5'-IMP and 5'-GMP (as their sodium salts) commercially are used extensively as flavor enhancers, especially for meat and fish products to enhance meaty, brothy and the "uamani" character (often in conjunction with MSG to take advantage of the synergistic flavor enhancement).

It has also been observed that there is a large synergism was observed between MSG and two species of nucleotides (GMP and IMP) in most mongrel dogs[40] and between MSG and three species of nucleotides (GMP, IMP, and AMP) in beagles. This has also been observed for GMP and glutamate in mice.[41]

Chemically, it should also be noted that 5'-inosine monophosphate is the product of enzymatic deamination of 5'-adenosine monophosphate. For example, In meat extracts, after slaughter, there is as rapid transformation of 5'-ATP to 5'-AMP to 5'-IMP.

In olfaction, very few studies are available that show activity of these ribonucleotides in enhancing olfaction. However, Getchell has demonstrated that 8-Bromo-cAMP applied to the ciliated side of the mucosa of the bullfrog caused a concentration-dependent, reversible increase in the basal short-circuit current, but not when it was applied to the submucosal side. Pulses of 8-Bromo-cAMP and odorant presented simultaneously resulted in currents that added nonlinearly.[42]

In addition, 5'AMP odorant binding sites on the dendrites of the olfactory receptor neurons in the sensilla of the spiny lobster are distributed along the entire dendritic region that is exposed to odorants. The distribution of these 5'AMP binding sites is considered much more extensive than that of enzymes that inactivate 5'-AMP.[43]

The few implications of ribonucleotides to playing an active part in what I will refer to as the extracellular side if the receptor neurons in the mucosa has largely been overlooked probably due to two factors: (1.) the ribonucleotides are largely water soluble and have not been examined as possibly complexing with lipid odorants and (2.) researchers have focused on the intracellular second messenger activity of such compounds.

Results in our laboratory using molecular modeling and molecular fitting programs, however, show that 5'AMP, 5'cAMP, 5'GMP, 5'cGMP and IP3 all demonstrate dramatic electrostatic affinity for fitting with many odorants. For example, when compared to fitting with 5'-ATP, 5'-ADP, 5'-GTP or 5'GDP, the [computed] electrostatic fitting energy is of an order of magnitude 10^5 - 10^6 more favored. In addition, with certain odorants in a related series that have similar odor properties, we see similar fitting patterns for certain conformers [An explanation of conformers will follow in a subsequent update]. These observations are intriguing, since, if such electrostatic forces assist the odorant via some sort of complex in fitting into the receptor…this may be a "first" step in the transduction process.

Recent Events in Olfactory Understanding

- **A Combinatorial Process for odor Interpretation:**

In March 1999, Linda Buck and Bettina Malnic at Harvard Medical School, and Junzo Hirono and Takaaki Sato at the Life Electronics Research Center in Amagasaki, Japan appear to have unravelled the mystery of how can the nose can interpret a plethora of different odors.[44]

It appears that the sense of smell in mammals is based on a combinatorial approach to recognizing and processing odors. Instead of dedicating an individual odor receptor to a specific odor, the olfactory system uses an "alphabet" of receptors to create a specific smell

response within the neurons of the brain. As in language (or music), the olfactory system appears to use combinations of receptors (analogous to words or or musical notes, or to the way that computers process code) to greatly reduce the number of actual receptor types actually required to convey a broad range of odors.

As in genetic code where the four nucleotides (adenine, cytosine, guanine and thymine) allow the creation of a nearly infinite number of genetic combinatorial sequences, the findings of Buck et. al. provides the first confirmation that the nerves that constitute the mammalian olfactory system also use a combinatorial approach.

When an odor excites a neuron, the signal travels along the nerve cell's axon and is transferred to the neurons in the olfactory bulb. This structure, located in the very front of the brain, is the clearinghouse for the sense of smell. From the olfactory bulb, odor signals are relayed to both the brain's higher cortex, which handles conscious thought processes, and to the limbic system, which generates emotional feelings.

In the reported study, individual mouse neurons were exposed to a range of odorants. Using a technique called calcium imaging, the researchers detected which nerve cells were stimulated by a particular odor. (When an odorant molecule binds to its odor receptor, calcium channels in the membranes of the nerves open and calcium ions pour inside. This generates an electrical charge that travels down the axon as a nerve signal. Calcium imaging measures this influx of calcium ions). Using this technique, it was shown that (1) single receptors can recognize multiple odorants (2) a single odorant is typically recognized by multiple receptors and (3) that different odorants are recognized by different combinations of receptors thus indicating that the olfactory system uses a combinatorial coding scheme to encode the identities of odors. This explains how 1,000 or so receptors can describe many thousands of different odors. Buck and her colleagues also demonstrated that even slight changes in chemical structure activate different combinations of receptors. Thus, octanol smells like oranges, but the similar compound octanoic acid smells like sweat. Similarly, it was found that large amounts of a chemical bind to a wider variety of receptors than do small amounts of the same chemical. This may explain why a large whiff of the chemical indole smells putrid, while a trace of the same chemical smells flowery.

- **Combinatorial Process Visualization**

For a novel "Shockwave" visualization of the "Combinatorial Process" that illustrates how odor molecules fit into scent receptors. See http://www.leffingwell.com/combi.htm. Note that, as with a chord played on a piano, some smells are triggered by a combination of different parts of the same odor molecule fitting into different receptors.

Human Olfactory Receptor Genes

On page 2 of this review, we describe briefly the recent identification & structural elucidation of human olfactory receptor genes by Lancet and co-workers[12h] at the Weizmann Institute of Science Crown Human Genome Center in Israel which is now publicly availabe in the HORDE online database and the sophistcated work of Zozulya and co-workers at

Senomyx in which the latter describe the identification and physical cloning of 347 putative human full-length odorant receptor genes that they believe represent essentially the complete repertoire of functional human odorant receptors.[12f] Peter Mombaerts has recently also reviewed this subject[67].

The Human Vomeronasal Organ

The VNO has been known to be present in human fetuses and has been reported sporadically in adults since the eighteenth century, although many find this improbable. Most of the work on vomeronasal function has been in rodents, snakes and insects where pheromonic chemicals play a communication role in attraction & reproduction. Its presence and function (if it, indeed, functions) in humans has been a matter of debate. Recently, however, Savic et. al. have shown that women smelling an androgen-like compound activate the hypothalamus, with the center of gravity in the preoptic and ventromedial nuclei. Men, in contrast, activate the hypothalamus (center of gravity in paraventricular and dorsomedial nuclei) when smelling an estrogen-like substance. This sex-dissociated hypothalamic activation suggests a potential physiological substrate for a sex-differentiated behavioral response in humans.[69] Whether this provides indirect (or direct) evidence of VNO like descrimination in humans remains to be seen.

Recently, Mombaerts, Greer and co-workers[70], showed that the human genome contains at least one gene found in epithelial tissue in the nasal that closely resembles a family of mouse pheromone receptors—genes that are primarily involved in detecting odorless chemicals such as pheromones. "Until this report," Greer states, "the consensus was that humans do not have receptors that belong to this family of genes. Now the door is open to reconsidering the functional organization of the human olfactory system." Mombaerts doesn't rule out the possibility that more pheromone receptors will turn up in sequence data in the future, but he is confident that only a few more, if any, will emerge.

Enantiomeric Specificity in the Olfactory bulb

It is well accepted that in humans certain specific chemical enantiomers (optical anti-podes) (such as carvone, menthol, limonene, linalool, citronellol, 7-hydroxy citronellol, 1-octen-3-ol, delta-decalactone, gamma-decalactone, 2-methyl-4-propyl-1,3-oxathiane, p-menthene-8-thiol, nootakatone, patchoulol, alpha-damascone, alpha-ionone, 3-mercapto-2-methylpentanol, (E)- & (Z)-nerolidols, alpha-phellandrene, alpha-terpineol, the theaspiranes, the 2 isomeric & 4 chiral forms of whiskey lactone, 2-ethylhexanoic acid, cis-rose oxide, nerol oxide, ethyl 2-methylbutyrate, methyl 2-methylbutyrate, Jasmine lactone, ethyl 2-oxo-3-methylpentanoate, 2-methylbutyric acid, 2,4,6-trimethyl-4-phenyl-1,3-dioxane, methyl dihydrojasmonate, the1-(2',2',6'-trimethyl-1'-cyclohexyl)-3-hexanols, 2-ethyl-4,4-dimethyl-1-cyclohexanone, 2,5,6-trimethyl-2-heptanol, 2-methyl-4-(2',2',3'-trimethyl-3'-cyclopenten-1'-yl)-4-pentenenitrile, the 2-methyl-4-(2',2',3'-trimethyl-3'-cyclopenten-1'-yl)-4-penten-1-ols, the 3,3-dimethyl-5-(2',2',3'-trimethyl-3'-cyclopenten-1'-yl)-4-pen ten-2-ols, the 5,6,7,8-tetrahydro-3,5,5,6,7,8,8-heptamethyl-2-naphthalenecarbaldehydes, the 5,6,7,8-tetrahydro-3,5,5,6,7,8,8-heptamethyl-2-naphthalenecarbonitriles, 2-ethyl-4-(2,2,3-trimethylcyclopent-3-en-1-yl)-2-buten-1-ol , the ambroxides) can be distinguished as they possess varying degrees

of olfactory differences.[54] Recently, Rubin & Katz have shown that apparently the rat is able to discriminate a wide variety of enantiomers that are indistinguishable to humans.[55] Enantioselectivity of odor perception in honeybees has also recently been studied, but gave results more similar to human discrimination.[56] Recently (August 2001), Leffingwell has published on the internet an extensive site that provides over 100 enantiomeric pairs of odorants that have differing odor properties. This site provides both 2-D and 3-D molecular structures along with odor descriptors, odor thresholds and original references See http://www.leffingwell.com/chirality/chirality.htm.

The olfactory receptor gene superfamily of the mouse

As mentioned on page 2 of this review, in the January 22, 2002 issue of Nature Neuroscience[12j], Stuart Firestein with Xinmin Zhang at Columbia University identified the mouse OR genes from the nearly complete Celera mouse genome by a comprehensive data mining strategy. They found 1,296 mouse OR genes (including 20% pseudogenes). Human ORs cover a similar 'receptor space' as the mouse ORs, suggesting that the human olfactory system has retained the ability to recognize a broad spectrum of chemicals even though humans have lost nearly two-thirds of the OR genes as compared to mice.

3-D Models of selected Human olfactory receptors - determination of the putative odorant binding cavities

In February 2002, Leffingwell & Associates announced the release of theoretical 3-D Models of selected Human Olfactory receptors and a rapid and simple methodology for determining the putative odorant binding cavity. The press release may be viewed at http://www.leffingwell.com/or_press_release.htm

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