Biol. Rev. (2007), **82**, pp. 455–479.
doi:10.1111/j.1469-185X.2007.00019.x

The sense of smell: molecular basis of odorant recognition

Manuel Zarzo*

Department of Applied Statistics, Technical University of Valencia, Camino de Vera s/n, 46022 *Valencia, Spain*

(*Received* 23 *May* 2006; *revised* 12 *April* 2007; *accepted* 2 *May* 2007)

ABSTRACT

Most animal species rely on odorant compounds to locate food, predators, or toxins. The sense of smell is also involved in animal communication, and revealing the underlying mechanisms will therefore facilitate a deeper understanding of animal behaviour. Since the 1940s different theories have speculated on the fundamental basis of olfaction. It was assumed that odorant molecules were recognized by selective protein receptors in the nose, triggering a nervous signal processed by the brain. The discovery of these receptors in the early 1990s allowed great progress in understanding the physiological and biochemical principles of olfaction. An overview of the different mechanisms involved in the coding of odour character as well as odour intensity is presented here, focusing on the biochemical basis of odorant recognition. Despite the enormous progress achieved in recent years, details of odorant-receptor interaction at the molecular level and the mechanisms of olfactory receptor activation are poorly understood. The likely role of metal ions in odorant recognition is discussed, and also the perireceptor events involved in odorant transport and biotransformation, with a view to providing a comprehensive overview of mammalian olfaction to guide future computational structural models and the design of functional experiments. Recent studies have analysed the olfactory genome of several species, providing information about the evolution of olfaction. The role of the olfactory system in animal communication is also described.

Key words: olfactory receptors, metalloprotein, ligand, GPCR, olfaction.

CONTENTS

* Address for correspondence: E-mail: mazarcas@eio.upv.es ; Tel.: +34 - 963877490 ; Fax: +34 - 963877499

I. INTRODUCTION

The sense of smell allows the perception and discrimination of a large number of volatile environmental chemicals in living organisms ranging from invertebrates to mammals. Such chemical signaling modulates the social behaviour of many species which rely on odorant compounds to locate food, recognize territory, and identify kin, predators, and toxic compounds. Olfaction also plays a role in mate choice, mother-infant recognition, and signaling among members of a group. Thus, clarifying the mechanisms involved in olfaction will allow a better understanding of animal behaviour. For humans, olfaction influences our quality of life. Smell is involved in the perceived quality of food, and scents are used as a means of social communication. Hence, the basis of perception of odour intensity, character and preference is of commercial interest. Olfactory perception is based on the activation by odorant molecules of olfactory receptors (ORs) located at the cilia of olfactory neuronal endings. Since the discovery of OR genes (Buck & Axel, 1991), great progress has been achieved in understanding the physiological and biochemical basis of olfaction. However, the mechanisms involved in odorant recognition at the molecular level are still poorly understood.

II. EARLY THEORIES ABOUT THE MECHANISMS INVOLVED IN ODORANT RECOGNITION

(1) The 'profile-functional group' theory of olfaction

Chemists noticed long ago that the presence of certain chemical groups in a molecule is frequently correlated with a particular odour. The best known example is the thiol moiety (-SH), which gives any molecule, regardless of its shape, a unique sulphuraceous odour character connected with the smell of rotten eggs or garlic. Structure-odour correlation studies have provided a better understanding of odour properties for sulphur-containing compounds (Bersuker *et al.*, 1989; Goeke, 2002). Other chemical groups also confer a particular odour that can be detected by trained observers. Hence, when nitriles (-CN) are used as a chemically stable replacement for aldehydes, they impart an oily-metallic character to any odorant; isonitriles (-NC) produce a flat metallic character of great power and unpleasantness; oximes (-NOH) give a green-camphoraceous character; nitro groups ($-NO_2$) produce a sweet-ethereal odour; isothiocyanate groups (-NS) result in a mustardy smell; amine groups ($-NH_2$) produce a fishy-urinous odour; arsine groups ($-AsH_2$) smell like cabbage, and esters [-(C=O)-O-] usually smell fruity (Burr, 2002). The inherent odour of the functional group is particularly apparent in small molecules, since large alkyl or phenyl groups may hinder the functional group and result in a different odour (Klopping, 1971; Brower & Schafer, 1975).

The correlation observed between functional group and odour character led in the 1920s to the idea that the functional group determines odour quality, whereas the overall structure has a secondary influence. Based on this concept, Beets (1957) proposed the so-called 'profile-functional group' theory. He suggested that odour is determined by two separate contributions: one from the form, size and bulk shape of the molecule, the other from its functional group or groups, that determine the molecular orientation at the receptor site. The efficiency of the functional group, that is, its ability to orient effectively the odorant at the site, was supposed to be at least partly determined by its tendency to participate in hydrogen bonding interactions (Beets, 1957).

(2) The vibrational theory of olfaction

Spectroscopic studies conducted by Dyson in the 1930s revealed a correlation between particular odours and the vibration frequencies of molecules (Dyson, 1938). According to these results, it was suggested that olfactory organs might somehow detect molecular vibrations, and certain odours were assigned to particular Raman frequencies in the 1500–3000 cm^{-1} range. This theory was revised by Wright (1954), who pointed out that in this region, Raman lines are strongly correlated with particular functional groups (OH, SH, CO, *etc.*). If odour could be correlated with these frequencies it could just as well be correlated with the corresponding functional groups and there would be no need for a vibrational theory of odour. Thus, in order to support this theory, any correlation between odour and molecular vibration pattern should be sought at frequencies below approximately 700 cm^{-1} (Wright, 1954).

Despite further efforts to provide a plausible odour theory based on vibration (Wright, 1977), limited success was obtained in finding correlations between odour character and low-frequency molecular vibrations. This theory also fails to explain the fact that some enantiomers produce different smells (Boelens & van Gemert, 1993) although their vibrational spectra are identical. Moreover, no biological mechanism was identified as a plausible protein-based spectroscope able to convert molecular vibrations into neuronal activation. As a result, the theory has been largely disregarded.

(3) The steric theory of olfaction

After the discovery of enzyme-substrate interactions based on molecular recognition between a protein receptor and a ligand, scientists suggested that a similar mechanism could be involved in olfaction. So, molecules with a particular shape would fit into certain complementary sites of nasal receptors, like a key into a lock, triggering a signal processed by the brain (Moncrieff, 1949). This 'steric theory' proposed by Moncrieff was later referred to as 'stereochemical theory' by Amoore (1963), who popularized the idea that molecular shape is related to odour character, a concept widely accepted today. He compared the odour description and molecular structure of a large number of compounds and found some similarity between odour and molecular shape. Accordingly, he suggested that different smells such as ethereal, floral, pepperminty, camphoraceous and musky odours were conferred by molecules sharing distinctive structural features. Camphoraceous is the term used to describe the smell of molecules like d-camphor, hexachloroethane or 1,8-cineole, that have a spherical shape (Amoore, 1963). Given this clear relationship between shape and odour, it was assumed that there was an olfactory receptor that recognized molecules with this particular shape, and consequently camphoraceous was considered as a primary odour.

Previous studies pointed out that some people are unable to smell specific substances. Cases of partial anosmia were reported in the literature for n-butylmercaptan (Patterson & Lauder, 1948), hydrogen cyanide (Kirk & Stenhouse, 1953), isobutyric acid (Amoore, 1967), amines (Amoore, 1971), musk (Whissell-Buechy & Amoore, 1973), 1,8-cineole (Pelosi & Pisanelli, 1981) and many others (Amoore, 1977). Guillot (1948) speculated that each type of specific anosmia may be caused by a defect in the corresponding primary odour detector, a hypothesis supported by the finding that some partial anosmias may be inherited (Whissell-Buechy & Amoore, 1973). Amoore (1967, 1977) adopted this idea and suggested that the identification and characterization of a specific anosmia provided evidence of a primary odour receptor specific to these substances that was inactive in those anosmic individuals. He thus proposed that the number of primary odours in the human sense of smell could be estimated using a comprehensive survey of specific anosmias, designed to identify each and every primary odour. The widely accepted stereochemical theory of olfaction was only seriously revised when experimental assays provided evidence for the existence of receptors selectively activated by odorants.

III. DISCOVERY AND CHARACTERIZATION OF OLFACTORY RECEPTORS

(1) The discovery of olfactory receptors

In 1991 Buck and Axel discovered an extremely large multigene family of transmembrane proteins that were believed to be ORs, since their expression was restricted to the rat olfactory epithelium (Buck & Axel, 1991). The numerous variants of the receptors suggested how a large number of different odorants may be discriminated. These proteins belong to the well-known family of G-protein-coupled receptors (GPCRs), that comprises a variety of receptors for a multitude of different stimuli. Their sequence contains seven segments with mostly hydrophobic amino acid residues, that are predicted to form α-helix structures buried in the lipid bilayer membrane, alternated with segments of hydrophilic residues that form loops which are stable in the water domain, interconnecting the helices (Fig. 1). The sequence in the 3rd, 4th and 5th transmembrane (TM) domains is highly variable between the different isoforms of this gene, and thus it was suggested that they would be involved in the odorant binding site (Buck & Axel, 1991; Pilpel & Lancet, 1999; Liu *et al.*, 2003). The seven TM helices pack against each other to form a bundle assembly (Fig. 1) that contains an odorant binding site on the extracellular side (corresponding to the mucus layer in the nose) and a binding site on the cytoplasmic domain that couples a heterotrimeric G-protein (guanine nucleotide binding protein), that is made up of three subunits.

Upon odorant binding, the OR undergoes some structural change that activates an olfactory-specific subtype of G-protein ($G_{\alpha olf}$), which in turn activates an adenylyl cyclase. This enzyme converts intracellular adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), triggering a cascade of events that lead to the nerve cell signal (Firestein, 2001). Membrane-bound proteins are difficult to crystallize, and the atomic-level structure was

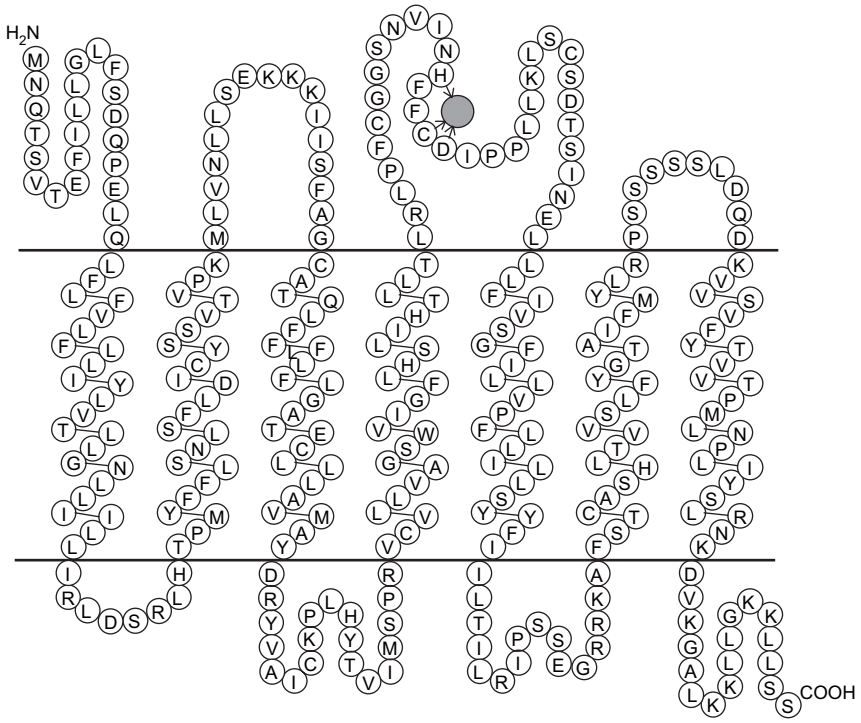

Fig. 1. Consensus amino acid sequence of class II olfactory receptors showing the predicted transmembrane (TM) regions (adapted from Zhang & Firestein, 2002; Liu *et al.*, 2003). The two black, horizontal lines approximately delimit the lipid bilayer region corresponding to the cell membrane. Positions above the upper line correspond to the extracellular side, and those below the lower line correspond to the cytoplasmic domain. Consecutive amino acid residues on a TM helix are theoretically located 100° apart (Pilpel & Lancet, 1999; Liu *et al.*, 2003) and hence each turn of the α-helix comprises 3.6 residues. The letter enclosed in each circle indicates the single-letter amino acid notation. Most residues in the TM regions are hydrophobic (leucine, L; isoleucine, I; valine, V; phenylalanine, F; alanine, A; tryptophan, W), while the most hydrophilic residues are usually located in the water domain (arginine, R; lysine, K; aspartic acid, D; glutamic acid, E). The loop between TM4 and TM5 contains the HFFCD consensus motif, that might coordinate a metal ion (filled circle) (Wang *et al.*, 2003). By contrast, other authors suggest that the cysteine (C) of this motif may form a disulphide bond with other cysteine residues (Zozulya, Echeverri & Nguyen, 2001; Liu *et al.*, 2003).

determined experimentally for bovine rhodopsin (Palczewski *et al.*, 2000) and a few other GPCRs, but not for any OR to date. Thus, details about the tertiary structure of ORs and the conformational changes upon odorant binding remain to be determined. The discovery of ORs and the organization of the olfactory system was recently awarded a Nobel Prize (http://nobelprize.org/medicine/laureates/2004/).

The first functional proof that one OR gene encodes a receptor selectively activated by an odorant was provided by Zhao *et al.* (1998), who used a recombinant adenovirus to drive the expression of a particular OR gene in the rat olfactory epithelium. Records of electrical activity in the neurons showed that increased expression of a single gene led to a greater sensitivity to a small group of odorants (Zhao *et al.*, 1998).

The mouse has more than 1000 OR genes clustered at multiple loci on many different chromosomes (Zhang & Firestein, 2002). It is now well established that each olfactory sensory neuron (OSN) expresses only one member of the OR gene family in the mouse olfactory system (Ressler, Sullivan & Buck, 1994). The mechanisms involved in this 'one neuron – one OR' rule have been investigated. Functional ORs are likely to initiate a feedback signal in order to ensure that no other OR gene will be expressed in each OSN. If a pseudogene is chosen for expression, no functional OR will be obtained and the OSN will sequentially activate additional receptor loci until a functional OR is synthesized (Serizawa *et al.*, 2003). One study found that in a few cases two OR genes can be co-expressed by a single OSN (Rawson *et al.*, 2000), which may be due to a failure in the feedback regulation process of the first OR expressed.

The axons of OSNs expressing the same OR converge to only one or a few of some 2000 modules, called glomeruli (Fig. 2), forming a highly organized topographic map on the surface of the olfactory bulb (Vosshall, 2003). The fact that a large population of OSNs converge to the same glomerulus produces an improved signal-to-noise ratio and increases the effective stimulus sensitivity. Experimental measures using optical imaging in the living olfactory bulb of rodents revealed that a given odorant activates a set of glomeruli, while different odorants activate overlapping but non-identical patterns of glomeruli (Rubin & Katz, 1999). Recent studies suggest that glomeruli activated by odorants sharing particular structural features gather together in

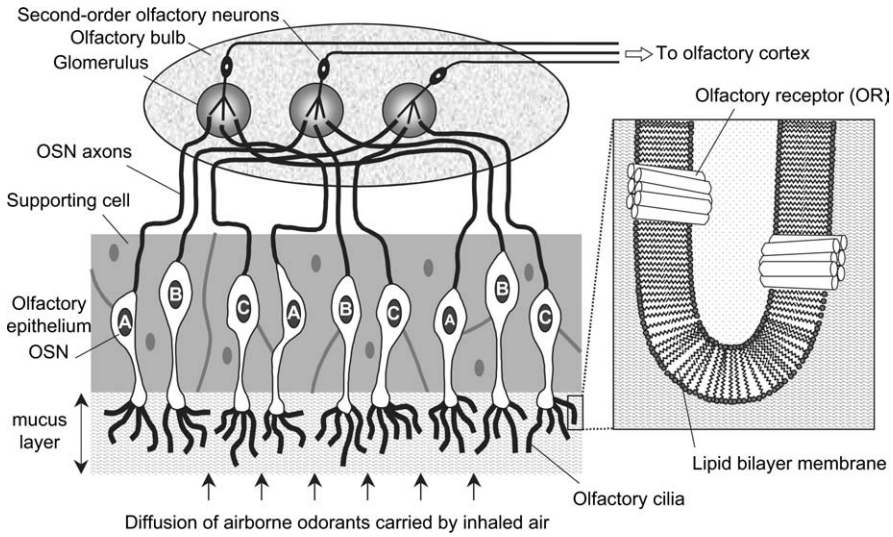

Fig. 2. Organization of the mammalian olfactory system. Volatile molecules reach the main olfactory epithelium in the nasal cavity, diffuse through the mucus layer and interact with olfactory receptors (ORs) in the fine cilia at the terminal knobs of the olfactory sensory neurons (OSNs). Three populations of OSNs (A, B, C) are depicted, each expressing only one of the approximately 1000 OR genes. Axons from all OSNs expressing the same receptor gene converge onto one or a few glomeruli in the olfactory bulb, where they synapse with the dendrites of second-order neurons, which in turn project to the olfactory cortex. The nearly 2000 glomeruli in the rat olfactory bulb are spherical knots about 50–100 μm in diameter, which contain the incoming axons of OSNs, the apical dendrites of second-order neurons, and the dendritic and axonal processes of interneurons (Firestein, 2001). Inset: ORs are seven-transmembrane proteins embedded in the membrane of OSN cilia.

proximity and form molecular-feature clusters (Mori *et al.*, 2006).

Malnic *et al.* (1999) exposed individual mouse OSNs to a range of compounds and detected which neurons were stimulated by a particular odorant. It was shown that single ORs can recognize multiple odorants, and that a single odorant elicits responses from multiple receptors, resulting in a combinatorial coding scheme for odorant recognition. The fact that no two odorants produce exactly the same odour, if assessed by trained experts (Turin & Yoshii, 2003), is consistent with the finding that odorants with a very similar chemical structure activate different combinations of receptors and hence different glomeruli. An experiment recording the response of individual mouse OSNs suggested that 40–90 different ORs can respond to a given odorant (Ma & Shepherd, 2000). Another study estimated that in the case of octanal this number could be 33–55 (Araneda *et al.*, 2004).

Searching the human genome database, Zozulya, Echeverri & Nguyen (2001) reported the identification and physical cloning of 347 full-length OR genes that they suggested represent the complete repertoire of functional human ORs. Other researchers found 322 intact OR genes (i.e. likely to encode functional ORs) (Glusman *et al.*, 2001). A similar study identified 339 intact OR genes and 297 OR pseudogenes (Malnic, Godfrey & Buck, 2004).

(2) Olfactory receptor specificity

The Olfactory Receptor Database (http://senselab.med.yale.edu) lists a set of 45 ligands identified as target odorants that activate ORs from different species of mammals, fish and invertebrates. In 2004 it was reported that the molecular receptive range (specificity) was known for at least 23 rodent and a few human ORs (Malnic *et al.*, 2004), but the number of deorphanized ORs has been growing progressively.

Many GPCRs are known to require accessory proteins for correct targeting to the cell surface membrane (Brady & Limbird, 2002). Similarly, it seems that OSNs express putative accessory proteins that are required for OR trafficking, though the details of this mechanism are still speculative (Matsunami, 2005). This is probably the reason why it has been difficult to express ORs on the surface of heterologous cells in order to assay their ligand-binding specificity. The research group of H. Matsunami has successfully overcome this limitation and has developed a high-throughput platform for screening the chemical selectivity of the large OR family (Saito *et al.*, 2004). They cloned 300 human and 250 mouse ORs using HEK293-T cells (human embryonic kidney cells). The activity of these ORs was tested with a group of 78 odorants, and it was found that 20 human and 80 mouse ORs were activated by some of the compounds (Saito *et al.*, 2006). Other researchers are also working on high-throughput functional screening methods to establish the odorant profile of large OR collections (Shirokova *et al.*, 2005), and a private enterprise is involved in a similar project (www.chemcom.be). Although few studies have thoroughly determined the specificity of one particular OR (Araneda, Kini & Firestein, 2000; Spehr *et al.*, 2003; Katada *et al.*, 2005), given the large number of compounds to be tested, this progress

in characterizing the specificity of functional ORs suggests that in the near future it will be possible to establish the first human olfactory map. This information will be a valuable tool for the rational design of new odorants and to conduct structure-odour relationship studies attempting to predict odour character, research that has produced limited success in the past (Sell, 2006).

Different experiments have tested the response of ORs to mixtures of odorants. The results have provided evidence for antagonism in the case of OR17-4 (Spehr *et al.*, 2003), rat OR-I7 (Araneda *et al.*, 2004), mOR-EG (Oka *et al.*, 2004), OR1G1 (Sanz *et al.*, 2005), and OR S86 (Shirokova *et al.*, 2005). The dual function of odorants as an agonist and an antagonist to ORs indicates a new aspect in the receptor code of odorant mixtures and provides insight into strategies to modulate perceived odour quality (Oka *et al.*, 2004). A recent study found that binary odorant mixtures can stimulate cortical neurons that are not stimulated by their individual component odorants (Zou & Buck, 2006). This complex combinatorial coding scheme is consistent with the fact that often a mixture of odorants gives rise to novel perceptual qualities that are not present in each component.

A key issue in olfactory coding is how broadly the receptors are tuned. Results from physiological recordings on OSNs and functional expression of OR genes have shown that some ORs are finely tuned to a restricted set of odorants (Touhara *et al.*, 1999; Wetzel *et al.*, 1999; Spehr *et al.*, 2003), while others recognize a wider repertoire of molecules (Duchamp-Viret, Chaput & Duchamp, 1999; Malnic *et al.*, 1999; Sanz *et al.*, 2005). According to several authors (Wetzel *et al.*, 1999; Ma & Shepherd, 2000; Firestein, 2001), these apparent differences in the degree of OR specificity may indicate a range of tuning profiles from specific to broad. Although further experiments are required to investigate OR specificity, broadly tuned receptors are not so frequently encountered as finely tuned ORs.

Another issue that has yet to be resolved concerns the effect of intensity on olfactory coding. Experimental assays have revealed that the repertoire of ORs activated by a given odorant is more diverse at higher concentrations (Malnic *et al.*, 1999; Ma & Shepherd, 2000; Kajiya *et al.*, 2001). Similarly, activity recordings in the olfactory bulb have shown that additional glomeruli are recruited into the pattern of activity as the concentration of an odorant is increased, suggesting that new receptors are being activated at higher concentrations (Rubin & Katz, 1999). However, most odours remain constant in their character over a range of concentration spanning several orders of magnitude. One suggested hypothesis is that there may be a class of broadly tuned low-affinity receptors that are simply intensity detectors (Firestein, 2001). They would be activated by a large number of odorants, but only at higher concentrations, coding information not about odour character but odour intensity.

Minor structural changes in a molecule can alter the smell character completely (Sell, 2006), as is the case with some enantiomers (Boelens & van Gemert, 1993). Based on this observation, Amoore (1963) guessed that at least some ORs were very specific. However, functional characterization of OR specificity has revealed that ORs are not so specific in general as was supposed by the stereochemical theory. A more modern approach suggests that ORs probe the shape, not of the whole odorant, but of partial molecular features. These structural features responsible for a given odour were referred to as 'olfactophores' (Ham & Jurs, 1985) or 'odotopes' (Turin & Yoshii, 2003). Different olfactophore models were developed for the main odour notes of perfumery (Kraft *et al.*, 2000). Although the underlying basis of the stereochemical theory still persists, it was renamed as 'weak-shape' or 'odotope' theory (Turin & Yoshii, 2003), underscoring the idea of combinatorial activation to explain how a few hundred different receptors can result in a different odour for every different molecule.

(3) Attempts to identify key amino acid residues in olfactory receptors

Since the discovery of OR genes (Buck & Axel, 1991), several works have analysed the sequence of ORs to identify residues with a potential role in odorant recognition, using different techniques such as correlation mutation analysis (Singer *et al.*, 1995*a*) or studying the hydrophobicity moments of the α-helices (Singer *et al.*, 1996). Another reported study analysed the residue variability in TM regions of about 200 aligned OR sequences. Using a rhodopsin template, it was found that the most variable residues tended to be oriented towards the interior of the receptor barrel, and hydrophobic residues tended to point towards the lipid-protein interface. This finding allowed the identification of 17 highly variable residues thought to play a functional role in odorant recognition (Pilpel & Lancet, 1999). Almost the same residues were subsequently identified by comparing six human-mouse OR ortholog pairs (Lapidot *et al.*, 2001).

Functional residues are expected to be rather variable in equivalent positions of paralogue OR sequences (from the same species) but preserved in orthologue ORs (those from different species that evolved directly from an ancestral gene and hence are supposed to recognize similar odorants). Based on this idea, a reported study examined the OR sequences of five strains of Japanese medaka fish *Oryzias latipes* and identified 14 potential contact residues that were variable between two different OR paralogues but fully conserved within the five fish orthologues (Kondo *et al.*, 2002). A more comprehensive study classified 1332 mouse and human OR sequences according to different criteria. The most variable sequences were identified and compared with other GPCRs (Liu *et al.*, 2003). Man, Gilad & Lancet (2004) analysed 1441 mouse and human OR protein sequences, which led to the identification of 22 sequence positions supposed to be potentially involved in the odorant binding site, given that they were both highly preserved within pairs of human-mouse orthologues and considerably less preserved within paralogue pairs. It has been determined experimentally that most of these residues correspond to ligand-contact positions in other GPCRs.

Another approach to identify a potential binding pocket and highlight critical residues likely to contribute to the receptor specificity is the use of computational models to study the tertiary structure of the protein and conduct docking simulations. Computer structural models were first

applied to the rat OR5 using as a template the tertiary structure of bacteriorhodopsin to determine the relative orientation of the α-helices (Singer & Shepherd, 1994). Another study of the same receptor used a low-resolution two-dimensional map of rhodopsin (Afshar, Hubbard & Demaille, 1998). Other computational structural models were derived for mouse OR S25 (Floriano *et al.*, 2000; Floriano, Vaidehi & Goddard, 2004), specific to hexanol and heptanol (Malnic *et al.*, 1999); OR 912-93 (Gaillard *et al.*, 2004; Hummel *et al.*, 2005), activated by ketones (Gaillard *et al.*, 2002), and for the rat OR-I7 (Singer, 2000; Vaidehi *et al.*, 2002; Hall *et al.*, 2004; Lai, Singer & Crasto, 2005). The latter receptor selectively binds octanal (Zhao *et al.*, 1998), although further work has identified a wider receptive range (Araneda *et al.*, 2000). A computational study has analysed the three-dimensional structure and function of six mouse ORs (S6, S18, S19, S25, S46 and S50) (Floriano *et al.*, 2004), for which experimental odorant recognition profiles are available (Malnic *et al.*, 1999). All of these studies lacked functional assays to compensate for the ambiguity of computer simulation and to validate the accuracy of the predictions.

The mouse receptor mOR-EG is selectively activated by eugenol (Kajiya *et al.*, 2001) and inhibited by methyl isoeugenol or isosafrole (Oka *et al.*, 2004). Katada *et al.* (2005) thoroughly examined the ligand specificity of mOR-EG, and found that it recognizes 22 odorants that share certain molecular determinants. Next, a computational structural model was constructed for mOR-EG based on the atomic-level crystal structure of bovine rhodopsin (Palczewski *et al.*, 2000). A cavity search revealed a putative odorant-binding pocket made up of 26 amino acid residues. Docking analysis performed for several mOR-EG agonists suggested that 10 of these amino acids were likely candidates for being involved in odorant recognition. To determine the role of these key amino acids, they were targeted for site-directed mutagenesis and then analysed in a functional assay of odorant binding. The results indicated that nine amino acids in TM3, TM5 and TM6 were involved in odorant recognition by mOR-EG, forming a hydrophobic ligand-binding pocket. In order to validate the binding site structure, the researchers predicted changes in ligand specificity and antagonist activity if point mutations were inserted in the odorant-binding site. The predictions were successfully corroborated by functional analysis (Katada *et al.*, 2005). These results provide strong evidence for a steric and functional odotope theory.

This synergy between computational predictions and physiological assays was also applied in a recent study. The specificity of MOR42-3 was investigated for a variety of dicarboxylic acids by using site-directed mutagenesis, guided by homology modeling and ligand docking studies, to locate functionally important residues (Abaffy, Malhotra & Luetje, 2007).

(4) Structural changes in the receptor upon odorant binding

Details of the conformational changes upon odorant binding are unknown, but a mechanism similar to other GPCRs is usually assumed. Different studies have revealed that photoactivation of rhodopsin involves a rotation and tilting of TM6 with respect to TM3 (Lin & Sakmar, 1996). The β_2-adrenergic receptor was suggested upon agonist activation to undergo a conformational change similar to that seen in rhodopsin (Ghanouni *et al.*, 2001), that in this case may involve disruption of a strong ionic interaction between TM3 and TM6 (Ballesteros *et al.*, 2001). A similar mechanism was proposed for serotonin receptors (Shapiro *et al.*, 2002). The loop between TM3 and TM4 (cytoplasmic domain) contains a DRY motif (single-letter amino acid notation: aspartic acid – arginine – tyrosine) that is conserved in ORs and other GPCRs with slight variations ([DE][RH][YF]). Studies involving site-directed mutagenesis of this motif in the H(2) receptor (Alewijnse *et al.*, 2000), the α_{1b}-adrenergic receptor (Scheer *et al.*, 2000) and the AT_{1A} angiotensin receptor (Gáborik *et al.*, 2003) have revealed that it plays a pivotal role in the activation process of GPCRs. Although the details of G-protein activation are not yet clear, it seems that the relative movement of TM helices upon ligand binding exposes the DRY motif to the intracellular domain, and allows the coupling of the specific subtype of G-protein.

Based on this activation pattern, one study compared the structural model and potential binding site of several GPCRs and proposed that, upon ligand binding of rat OR-I7, the third helix might be displaced in the cytoplasmic direction. This movement would expose the DRY motif to the intracellular region and consequently initiate the signal transduction pathway (Vaidehi *et al.*, 2002). A similar mechanism was assumed in the reported structural model of mOR-EG (Katada *et al.*, 2005), though the conformational changes upon ligand binding were not studied in detail.

Interestingly, the employment of a non-typical G protein such as $G_{\alpha 15}$ instead of $G_{\alpha olf}$ can dramatically alter the odorant specificities of ORs expressed in heterologous systems (Shirokova *et al.*, 2005). Coupling of OR to $G_{\alpha 15}$ is unlikely to occur in the OSN and hence is not relevant for odorant coding. However, this kind of study might provide clues for understanding the conformational changes involved in OR activation.

IV. THE THEORY OF OLFACTORY RECEPTORS AS METALLOPROTEINS

Although the odotope theory is currently the most widely accepted, the largely divergent and characteristic odours of small molecules such as NH_3, H_2S or HCN cannot easily be explained by differences in nucleophilic or electrophilic character. The fact that alcohols (–OH) never smell like thiols (–SH) at any concentration, despite the similar shape and properties of both functional groups, suggests that the recognition mechanism must somehow be sensitive to the fine structure of the electron distribution (orbital energies, charge density, *etc.*) of the functional group (Turin & Yoshii, 2003), an aspect not properly taken into account by the odotope theory.

(1) The mechanism of odorant recognition based on Inelastic Electron Tunneling Spectroscopy

Attempting to overcome these limitations and based on previous vibrational theories, Turin (1996) proposed a transduction mechanism of primary olfactory reception suggesting that ORs act as biological spectrometers and probe the molecular vibration of a bound odorant. Therefore, ORs would be 'tuned' to the vibrational frequency of particular odorants, rather like cones in the eye are 'tuned' to certain wavelengths of light. The finding of a congenital specific anosmia to thiols or amines (Amoore, 1971) suggested the existence of specific receptors tuned to the frequency band of these functional groups. This theory was based on inelastic electron tunneling spectroscopy (IETS) (Adkins & Phillips, 1985), which relies on the interactions between electrons tunneling across a narrow gap between metallic electrodes. Metallic conductors are absent in biology, but Turin speculated that IETS was possible with proteins containing a metal ion able to interact with the odorant. This hypothesis is reasonable, given that many enzymes contain metal ions involved in electron transfer (Cass & Hill, 1980; Coleman, 1998). Recent work has further investigated the physical viability of this mechanism, and the results were consistent with observed features of smell (Brookes *et al.*, 2007). Several decades ago, Dravnieks (1962) also suggested that metals might be involved in OR activation.

A Zn(II) ion was suggested by Turin (1996) as the best candidate, given the strong link between zinc and olfaction: zinc deficiency, either dietary (Alpers, 1994), caused by treatment with histidine (Henkin *et al.*, 1975), thiocarbamides (Erikssen, Seegaard & Naess, 1975) or captopril (Zumkley *et al.*, 1985) is unique in causing a complete and rapidly reversible anosmia. Moreover, zinc is common in many metalloproteins (Coleman, 1998). Interestingly, Zn(II) is widely distributed throughout the central nervous system and is present in several regions of the brain, where it is stored in synaptic vesicles of the nerve terminals and is co-released with neurotransmitters to the synaptic gap upon neuronal activation (Frederickson, 1989; Smart, 2004). Moreover, Zn(II) binds with high affinity to and modulates the function of a number of GPCRs in neural tissues such as the tachykinin NK3 receptor (Rosenkilde *et al.*, 1998) and the β_2-adrenergic receptor (Swaminath *et al.*, 2002). Experimental assays with melanocortin MC1 and MC4 found that these GPCRs of the nervous system can be activated by Zn(II) ions, and the endogenous metal-ion site was characterized (Holst, Elling & Schwartz, 2002). These findings are consistent with the hypothesis that Zn(II) plays a key role in the activation of ORs, since they are expressed by neural cells.

The amino acid sequence CASHL (cysteine – alanine – serine – histidine – leucine) is conserved in TM6 of ORs (Fig. 1) with slight variations (Liu *et al.*, 2003), and Turin (1996) assumed that this sequence was the zinc-binding site. If this is the case, mutation of the conserved histidine residue in this sequence to phenylalanine would prevent the coordination of the metal ion, resulting in the loss of OR activity. Use of this mutation to further investigate the IETS mechanism may prove instructive. Although Turin's vibrational theory was used to predict the smells of well-documented odorants with some success (Turin, 2002), the scientific community remains skeptical, since no similar mechanism has ever been described in a biological system.

(2) The metal-ion-assisted odorant recognition mechanism

Another mechanism has been proposed, based on the involvement of a metal ion in odorant recognition. The research group of K. Suslick works with metalloporphyrins and their applications as chemical sensors (artificial olfaction and molecular recognition). They have developed a colorimetric procedure to discriminate molecules based on the ability of metalloporphyrins to form selective coordination complexes with organic compounds (Rakow & Suslick, 2000), which led to the idea that a similar mechanism could be involved in olfaction. Searching the genome sequences of human ORs, it was found that the consensus sequence HxxC[DE] (histidine – two residues usually hydrophobic – cysteine – aspartic or glutamic acid) was rather conserved in the extracellular loop between the fourth and fifth TMs (4–5 loop) (Wang, Luthey-Schulten & Suslick, 2003). Actually, this motif is present in 65% of the 347 putative human ORs identified by Zozulya *et al.* (2001). To test the metal binding properties of this sequence, a pentapeptide containing this putative binding site was synthesized and analysed with circular dichroism spectroscopy, a technique used to determine protein secondary structure. The results suggested that the pentapeptide had no particular structure (random coil), as expected for a short peptide. But upon addition of Cu(II), the spectrum changed to one characteristic of an α-helix, and the equilibrium constant revealed a high coordination affinity. Similar results were observed for Zn(II) and Ni(II) (Wang *et al.*, 2003). The metal ions Zn(II) and Cu(II) were suggested as the likely candidates to be present in ORs because they strongly coordinate amines and thiols, which are strong odorants, and similar sequences containing His, Cys, Asp or Glu are usually the metal ion-binding site in zinc and copper metalloproteins (Coleman, 1998; Cass & Hill, 1980).

The 4–5 loop is of functional importance in ligand binding for other GPCRs like the cholecystokinin-B receptor (Silvente-Poirot & Wank, 1996), chemokine receptors (Blanpain *et al.*, 1999) and aminergic receptors (Shi & Javitch, 2002). Two conserved cysteines, one at about the middle of the 4–5 loop and another at the amino-terminus of TM3, form a disulphide bond in rhodopsin. This bond pulls the 4–5 loop closer to the binding pocket, and two residues in this loop were reported to interact with retinal (Palczewski *et al.*, 2000). The same positions were suggested as functional residues in ORs (Man *et al.*, 2004). Based on the high affinity for metal binding of the 4–5 loop and considering that it plays a key role in odorant recognition in other GPCRs, the following metal-ion-assisted odorant recognition (MIAOR) mechanism was proposed for the ORs containing the HxxC[DE] sequence; this mechanism was illustrated using a computer model of the human OR o2d2 (Wang *et al.*, 2003).

The initial or native conformation of the nonmetalated OR contains a long loop between TMs 4 and 5 (Fig. 1), and I refer to this conformation as the ELL (extracellular long loop). This 4–5 loop is stable in the extracellular domain due to the presence of an anionic residue (aspartic or glutamic acid). During a developmental stage the 4–5 loop may act as a recognition epitope to guide OSNs while they extend their axons to the correct glomeruli in the olfactory bulb, as suggested by some authors (Singer, Shepherd & Greer, 1995*b*; Skoufos, 1999). This hypothesis is consistent with the active role of ORs in organizing the connectivity of the olfactory map (Vosshall, 2003). However, new evidence suggests that ORs may control the axon-guidance process only indirectly, through modulating the intracellular concentration of cAMP in a neuron. This concentration might regulate the expression at the cell surface of axon guidance molecules (Imai, Suzuki & Sakano, 2006).

Upon metal binding the anionic charge is neutralized, the 4–5 loop becomes a new α-helical region and penetrates into the membrane, pushing the fourth TM helix (or maybe the fifth) out of the membrane towards the cytoplasmic domain, to become a long 3–4 loop. This conformation is stable in the absence of odorant binding and will be denoted ILL (intracellular long loop). The sequence HxxC[DE] now remains buried in the membrane and becomes involved in the odorant binding site. The long 3–4 loop in the ILL conformation probably hinders the DRY recognition epitope and prevents the coupling of the G-protein.

When an odorant fits into the receptor and approaches the metal ion, one of the coordinated amino acid residues is released (or maybe a coordinated water or hydroxide ion), which disrupts the local charge balance and increases the local steric interactions in the binding site, causing the original 4–5 loop to eject from the membrane, and the OR to return to the ELL form. The DRY epitope then becomes accessible in the ELL conformation due to the shorter 3–4 loop, activating the G-protein and the resulting cascade of events leading to nerve cell activity. While the G-protein is active, the metal-binding motif is exposed to the extracellular water, shifting the equilibrium of ligation and enhancing the departure of the bound odorant. When the odorant is released from the metal ion, the 4–5 loop again changes into a hydrophobic helix, the OR returns to the ILL conformation, the G-protein is deactivated, and the nerve signal ceases. The MIAOR mechanism does not necessarily assume that all molecules interact directly with a metal ion. Odours such as the camphoraceous smell that are related to a particular shape would be conferred by odorants interacting not directly with the metal ion, but with key residues of a sterically constrained binding site.

Strong evidence consistent with the MIAOR mechanism stems from the experiments of Gaillard *et al.* (2004), who cloned the orthologous genes of OR 912-93 from pig and six primate species and assayed the encoded receptors for responses to odorants. All the receptors responded to 3-heptanone except the human and orangutan ORs, which were not functional; they regained their function after restoration of the arginine residue (R) in the DRY motif required for G-protein activation. Surprisingly, the human receptor was constitutively activated in the absence of ligand stimulation, and the researchers did not provide a convincing explanation for this finding. The HFFCD motif is present in the protein sequences of all seven species except for the human one, which contains the PFFCD mutation. This sequence in the 4–5 loop would be unable to bind a metal ion, and according to the MIAOR mechanism the OR would remain permanently in the ELL conformation, that activates the G-protein cascade. This is precisely what was observed experimentally. Further studies by site-directed mutagenesis of the HxxC[DE] motif (specially the mutation Cys → Ala) in other ORs will be necessary to establish whether similar results of constitutive OR activation appear in mutated sequences preventing metal binding in the 4–5 loop. Such research is strongly recommended, since the results might suggest further targets and may provide evidence supporting or disproving the hypothesis of metal ion binding in the 4–5 loop.

The MIAOR mechanism proposes that odorant interaction occurs with the ILL conformation of the receptor, while the ELL form triggers the nervous response. By contrast, the computational structural models proposed for different ORs (OR5, OR 912-93, OR-I7, OR S6, S18, S19, S25, S46, S50 and mOR-EG), mentioned above, assume that odorants interact with ORs in the ELL conformation with no metal ion involved. All of these ORs contain the HxxC[DE] motif in the 4–5 loop, but no attention was given to the metal binding ability of this motif. Since the MIAOR mechanism was proposed, no additional computational structural models have attempted to explore the hypothesis that ORs are metalloproteins, despite evidence consistent with this theory.

The reported site-directed mutagenesis experiments with mOR-EG provide convincing evidence about the key amino acid residues involved in odorant recognition, but no metal ion was assumed to be involved. The binding site was proposed to be a hydrophobic pocket formed between TM3, TM5 and TM6 (Katada *et al.*, 2005). The mOR-EG contains the motif HFFCE in the 4–5 loop and, according to the MIAOR mechanism, upon metal binding this loop might penetrate the membrane and replace TM4. Given that TM4 was not assumed to interact directly with the ligand, these results are compatible with the MIAOR mechanism, and additional functional assays will be necessary to further investigate the role of the HFFCE motif in this receptor.

The 4–5 loop of rhodopsin contains a cysteine residue that forms a disulphide bond with another conserved cysteine of TM3, and different authors consider that this cysteine may play the same role in ORs (Zozulya *et al.*, 2001; Liu *et al.*, 2003). Maybe for this reason the MIAOR mechanism has not received much attention yet, though it seems more plausible than Turin's (1996) IETS theory. Another reason could be that there is no evidence of a similar pattern of activation in other GPCRs. The piston-like movement suggested for the conformational change seems very dramatic, and it relies on several hypotheses: the 4–5 loop is not blocked by disulphide bonds, it becomes hydrophobic upon metal binding, *etc.* Given that these conditions may not apply for all ORs containing the HxxC[DE] motif, other

alternative conformational arrangements should be considered for further investigation.

(3) Properties of olfaction consistent with the hypothesis of ORs as metalloproteins

(*a*) *Good ligands for metal coordination are likely to smell strongly*

One interesting pair of structurally related compounds with very different odours consists of methanol, which is relatively odourless, and methyl mercaptan, which has a highly powerful and disagreeable odour. Neither the slight differences in bond length, bond angle or reactivity, nor the slight difference in orientation of the methyl groups, seem capable of explaining such pronounced difference in odour quality and intensity. The major difference is the ability of mercaptans to form stable complexes with many metal ions. Thus, the difference in odour quality seems primarily connected with a distinct electronic behaviour (Klopping, 1971).

Acetonitrile (CH_3-C≡N:) presents a molecular structure similar to methyl isonitrile (CH_3-N^+≡C^-:). Both have large dipole moments with a lone electron pair on the terminal atom of the functional group that can play the role of acceptor in a hydrogen bond. Moreover, both are linear molecules with nearly the same size and shape. However, acetonitrile has a relatively weak, pleasant, ethereal odour, while methyl isonitrile has an extraordinarily vile and powerful odour. To account for the remarkable difference in odour quality, it was pointed out that isonitriles, unlike nitriles, react with salts of many heavy metals to form very stable complexes (Klopping, 1971).

Certain structural features of molecules tend to make them stronger odorants (Moncrieff, 1967). These and other examples led long ago to the belief that odorants that are good ligands for metal ion coordination are likely to smell strongly, and hence are detected at a lower concentration. Thiols, amines, nitriles and isonitriles, some of which are among the strongest odorants known, readily coordinate to zinc. Other strong odorants such as emoxyfurone, oxathiane, vanillin, diacetyl and pyrazine esters present structural features capable of bidentate binding to a metal ligand (Turin, 1996). This property of olfaction, hardly reconcilable with the odotope theory, can be interpreted under the assumption that ORs (or at least some of them) are metalloproteins, as suggested by Turin (1996) and the MIAOR mechanism. Thus, if a metal ion is involved in the receptor binding site, the odorants with a high affinity for metal ion coordination will bind most tightly to a specific subset of ORs, resulting in a strong odour perception (Wang *et al.*, 2003). This hypothesis would explain why odour intensity spans several orders of magnitude for compounds of similar size and volatility but different functional groups or molecular features. Some authors have interpreted the high sensitivity of human olfaction to detecting hydrogen sulphide (H_2S) and amines as an evolutionary adaptation to detect decaying food and toxic gases, that have been present for evolutionary significant time periods in the atmosphere (Doleman, Severin & Lewis, 1998).

Slight structural modifications of a molecule may significantly alter odour potency. Comparing the molecular structure of different strong-weak stereoisomer pairs, Ohloff (1994) suggested that when two hydrogen bond acceptors are present, the odorant is stronger when they are close to each other. This observation, known as the bifunctional rule, can be interpreted in some cases assuming that the strong isomer is a bidentate ligand for zinc, whereas the weak isomer has unfavourable geometry for zinc-binding (Turin & Yoshii, 2003). Although this rule is particularly interesting because it applies to a large number of structurally unrelated odorants, there are many exceptions. In the case of musk odorants, different researchers (see Section V.1) have identified particular key features that discriminate musk *versus* odourless compounds with an analogous structure, but did not investigate metal coordination. Thus, differences in odour intensity cannot always be ascribed to the accessibility of one or more metal coordinating groups, and steric restrictions may also be crucial, probably because certain molecular shapes are more favourable than others for binding the target receptors.

(*b*) *Different functional groups and even different atoms may produce similar odours*

It was long ago observed that chemical compounds with different functional groups can smell alike. One of the best known cases is the bitter-almond odour character, shared by molecules of a widely different structure (Zakarya, Yahiaoui & Fkihtetouani, 1993). Another example is that of nitriles and aldehydes: benzaldehyde and benzonitrile have similar odours, as do agrunitril and citronellal, cinnamaldehyde and cinnamalva (Turin, 1996). The nitrile group can usually replace an aldehyde with only a minor change in odour character, making it duller and somewhat oily (Bedoukian, 1986). Some molecules with an acetylenic –C≡C– triple bond share a mustard-like smell typical of isothiocyanates (–NS), which is clearly recognizable for example in acetylene and in methyloctynoate (Turin & Yoshii, 2003). These findings are difficult to reconcile with the odotope theory, unless we assume that they bind different receptors sharing a common perceptual pathway. The fact that about one in ten persons cannot smell the bitter-almond odour of hydrogen cyanide but perceive this odour in benzaldehyde and nitrobenzene (Kirk & Stenhouse, 1953) seems consistent with this hypothesis. According to the MIAOR mechanism, compounds with different functional groups that produce a similar smell probably share a similar ability to form a coordinate bond with a metal ion, though additional chemical properties and steric constraints may also be involved. Turin's (1996) hypothesis would be that they share some similar pattern in vibrational spectra.

Sulphuraceous odours are produced by molecules containing sulphur such as hydrogen sulphide, thioethers and thiols. Garlic and onion produce a characteristic odour referred to as alliaceous, that is conferred by thioethers (Bersuker *et al.*, 1989). Thus, sulphuraceous and alliaceous are related odours. However, other atoms can produce a similar smell. Boranes (molecules with the moiety –BH)

were reported to produce a sulphuraceous smell, though they do not contain sulphur (Stock & Massenez, 1913). Decaborane ($B_{10}H_{14}$) is a stable compound that smells strongly of boiled onion (an alliaceous odour), and other less stable boranes also have this sulphuraceous odour character (Turin & Yoshii, 2003). A similar case occurs with arsenic: heated arsenic, hydrogen arsenide (AsH_3), and cacodyle [$As_2(CH_3)_4$] were reported to smell alliaceous (Harper, Bate-Smith & Land, 1968). Oxidizing agents like chlorine, bromine, or iodine have also been described as having alliaceous odours (Harper *et al.*, 1968), though they give off a characteristic clear, clean smell when diluted (Jennings-White, 1984). According to Turin's theory, this similarity in smell should correspond to a certain common pattern in vibrational spectra.

(*c*) *Molecules with an almost identical structure may smell different*

Ferrocene and nickelocene are composed of a metal ion of Fe(II) and Ni(II), respectively, almost completely encased by two cyclopentadienyl rings (Fig. 3). The resulting coordination complex is hydrophobic and volatile and the structures are nearly identical. However, ferrocene has a camphoraceous odour reminiscent of chloroalkanes, while nickelocene smells oily-chemical (a typical cycloalkene odour) (Turin, 1996). Although the slight variations in molecular shape might account for differences in the repertoire of activated ORs, their different smell can also be interpreted as resulting from a different affinity to form a coordinate bond with a metal ion, given that each encased metal atom (Fe^{2+} or Ni^{2+}) produces a hybridised resonant orbital of unique characteristics.

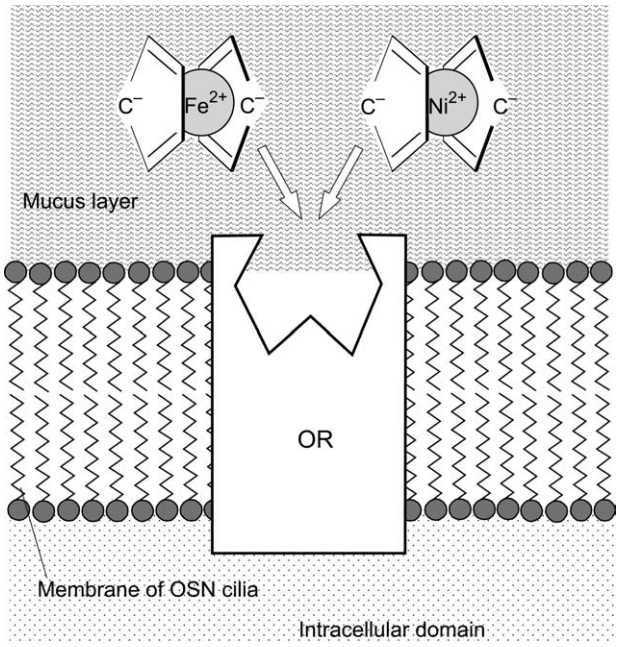

Fig. 3. Ferrocene and nickelocene: same molecular shape but different smell. Two cyclopentadienyl ions coordinating a Fe(II) cation become a ferrocene molecule (*upper left*). Same structure for nickelocene, replacing Fe(II) by Ni(II) (*upper right*). The structures are nearly identical, since the atomic radii of the metals are very similar (1.72 Å for Fe and 1.62 Å for Ni), which suggests *a priori* that they are likely to activate the same set of olfactory receptors (ORs). However, this is not the case, since they smell different. This observation is consistent with the hypothesis that at least some ORs are metalloproteins able to discriminate the fine electron orbitals of resonant structures. OSN, olfactory sensory neuron.

Another finding consistent with Turin's (1996) vibrational theory but difficult to explain by the odotope theory is that some isotopes produce a different smell compared to their parent compounds, despite their identical structure; they only differ in the number of neutrons of some atoms. Differences between the odour of deuterated and regular benzaldehyde have been described (Haffenden, Yaylayan & Fortin, 2001). Dimethyl sulphide was reported to smell repulsive, green and cabbage-like, but its fully deuterated analogue dimethylsulphide-d_6 smells cleaner, more truffle-like, without the gassy cabbage-like note of the parent compound (Turin & Yoshii, 2003). Similarly, acetophenone and acetophenone-d_8 produce a similar but not identical odour profile that can be distinguished by trained experts. Acetophenone-d_8 has stronger fruity and bitter-almond notes, while acetophenone smells more like toluene (Turin, 1996). These differences were not found in a later report (Keller & Vosshall, 2004), maybe because panelists were not experts in odour description. Computational studies could help to determine if reported variations in smell correspond to a different affinity for metal coordination.

V. PERIRECEPTOR MECHANISMS INVOLVED IN ODORANT TRANSPORT AND BIOTRANSFORMATION

Terrestrial animals smell airborne hydrophobic molecules that must traverse the aqueous mucus layer covering the nasal epithelium before reaching the receptors on the olfactory cilia. The mechanisms involved in odorant transport and biotransformation in the receptor environment are referred to as perireceptor events (Breer, 2003), and are considered an important part of the chemical sensing process.

(1) Theories suggesting odorant partition in the membrane

Different theories have attempted to interpret why small changes in the chemical structure of a molecule can greatly alter odour potency. Given that stronger odorants are generally detected at lower concentrations, odour detection thresholds are commonly used to characterize odour strength of volatile organic compounds (VOCs). This threshold is defined as the lowest concentration of a VOC that can be perceived or discerned from a blank stimulus or background noise (Doty, 1992).

In the course of investigations on the kinetics of lysis of red blood cells by haemolytic agents, it was observed that

the addition of small amounts of various odorants accelerated the haemolysis process. Measurements of the haemolytic accelerating power of 16 odorants proved tightly correlated with olfactory thresholds. Based on this observation, it was proposed that olfactory stimulation was induced upon adsorption of odorant molecules in the olfactory cell membrane and cell penetration by a puncturing mechanism (Davies & Taylor, 1954). A further study calculated the olfactory threshold of several classes of compounds based on two parameters: the partition coefficient of the substance between air and a water-oil interface, and the cross-sectional area of the odorous molecule (Davies & Taylor, 1959). Another early study found a significant correlation between the olfactory threshold of 16 compounds and the odorant concentration required to produce a certain increase in the surface tension of lipid layers. This result was assumed to be caused by the penetration of odorants into the lipid layer (Koyama & Kurihara, 1972). The hypothesis of intracellular odorant penetration is no longer accepted, but these results suggest that odorants may be adsorbed into the lipid layer of OSN membranes.

In an attempt to predict odour thresholds, another study used different descriptors calculated for compounds of very different structure types, functional groups and heteroatoms. The descriptor log P (the log[1-octanol/water partition coefficient]) was strongly correlated with odour intensity within homologous groups. Log P is commonly used in drug design and reflects the molecular hydrophobicity and the tendency to partition in the lipid bilayer of cell membranes. It was thus postulated that the critical factor determining odour intensity was the ability of an odorant to partition through mucus and membrane layers in order to reach the olfactory receptor sites (Greenberg, 1979).

Similar results were obtained in subsequent studies using musk odorants. The molecular structure descriptors that best discriminate musk *versus* non-musk compounds were studied for monocyclic nitrobenzenes (Ham & Hurs, 1985). Further research examined 10 pairs of structurally similar odourless and odoriferous musk benzenoids. Searching among 34 molecular descriptors, it was found that three of them properly discriminated between odoriferous musks and odourless analogues: two structural parameters and the octanol/water partition coefficient (Yoshii *et al.*, 1991). Based on the molecular structure of two strong benzenoid musks, a further study proposed a three-dimensional (3D) structure model that successfully classified 40 benzenoids (30 musks and 10 odourless) (Yoshii *et al.*, 1992). Klopman & Ptchelintsev (1992) developed a multiple linear regression equation with 87 musk odorants and 65 of their odourless structural analogues. The proposed equation calculated musk intensity using as predictive variables log P and 23 structural descriptors accounting for the presence in the molecule of particular features. The model successfully predicted the musky odour or lack of it in 20 chemicals not included in the training set.

Hau & Connell (1998) proposed an empirical model to predict odour detection thresholds (ODTs) of acetates, alcohols, ketones and amines, using the air-water partition coefficient (K_w) and the octanol-water partition coefficient (log P):

$$\log[\mathrm{ODT} \cdot K_w] = -a \cdot \log P + b.$$

The coefficients a and b differed for each homologous series. According to this equation it was suggested that ODT is related to a partition process of VOCs between the air phase and a biophase where the olfactory signal is triggered. A three-stage model was proposed: VOCs dissolve in the aqueous mucus layer, are transported to the biophase that contains the ORs and finally bind the receptors. According to this mechanism, K_w refers to the equilibrium between the gas phase and the aqueous layer, while log P refers to the equilibrium between the aqueous phase and the biophase (Hau & Connell, 1998).

Abraham *et al.* (2002) obtained a multiple linear regression model to predict the ODT of 64 compounds from several homologous series using different types of molecular descriptors. The regression coefficients of this equation proved to be of the same sign and similar order of magnitude as those obtained when the same equation was used to predict gas-solvent partitions. Consequently, it was suggested that the main mechanism contributing to the overall threshold effect was a simple passive transport process of VOCs from the gas phase to the biophase containing the receptors. This transport would be driven by equilibrium constants depending on the VOC physicochemical properties. An additional effect was found for aldehydes and carboxylic acids, that was interpreted as some actual chemical reaction of these compounds (Abraham *et al.*, 2002).

The detailed structure of rhodopsin was determined experimentally (Palczewski *et al.*, 2000), and this receptor is commonly used as a template for structural models of ORs. However, the entry pathway for the hydrophobic ligand 11-*cis*-retinal is unknown. The primary ligand is stable in the lipid bilayer domain, and a recent simulation study shows that it enters a site located in the TM region between helices 5 and 6 (Hubert *et al.*, 2005). A similar mechanism was proposed in the case of ORs activated by acetates, alcohols and ketones, speculating that these compounds probably bind to a receptor site located in the hydrophobic interior of the lipid bilayer membrane of the OSN cilia (Hau & Connell, 1998). A different pattern was found for amines, and it was suggested that they probably bind to a receptor site located in a different position, closer to the mucus layer (Hau & Connell, 1998). Given the reported evidence that hydrophobic odorants may partition in the membrane of OSN cilia, the hypothesis that the binding site of ORs faces the extracellular aqueous domain should be revised, though it is widely accepted.

A similar molecular mechanism that involves ligand diffusion into the membrane was described for the way that calcium channels and β-adrenergic blockers bind to their specific receptors in cardiac membranes (Herbette, Rhodes & Mason, 1991). The hypothesis proposes partitioning of the molecule into the lipid bilayer, where it may become oriented and then diffuse laterally to a specific binding site in the cell membrane receptor. The rat olfactory mucosa

contains a high polyunsaturated fatty acid content (Russell, Evans & Dodd, 1989), which suggests that the membrane of OSN cilia is not tightly packed, which would favour the mobility of hydrophobic odorants dissolved in the membrane.

(2) Odorant-binding proteins

(*a*) *The chromatographic theory of olfaction*

Experimental studies found a correlation between chromatographic properties of different compounds and physiological measures on the frog olfactory mucosa, which led to the idea that olfaction may share certain features with chromatography (Mozell & Jagodowicz, 1973; Mozell *et al.*, 1987). A reported theoretical model has simulated odour response assuming a chromatographic step to be involved in olfaction (Nachbar & Morton, 1981). Based on this theory, a regression equation was proposed to describe the ODT of 60 di-substituted pyrazines using two variables: one retention chromatographic index and one structural parameter (Mihara & Masuda, 1988). These chromatographic properties of olfaction observed a few decades ago have not received much attention by the scientific community. However, I speculate that they might partly be explained by the presence in the aqueous nasal mucus of soluble proteins that bind hydrophobic molecules and may enhance their transport throughout the aqueous layer.

(*b*) *The sharp molecular cut-off in odour detection*

Not all molecules can be smelled. They require certain volatility to reach the olfactory epithelium in the nose and a molecular mass less than 300 Da. According to Ohloff (1994), a labdane with a molecular mass of 296 Da is the largest known odorant. Vapour pressure (volatility) decreases rapidly with molecular size, but molecules larger than 300 Da in molecular weight are odourless though they may develop appreciable partial pressures, suggesting that they are too large to fit into any receptor (Turin & Yoshii, 2003). This cut-off effect in olfaction due to size is evident in the case of musk odorants. Near the maximum size, most subjects are anosmic to one or more of the large musks like pentadecanolide or Tonalide (Turin, 1996). One of the most striking cases of this sharp cut-off is that the substitution of a carbon in a benzenoid musk with a slightly larger silicon atom causes it to become odourless (Wrobel & Wannagat, 1982). Most molecules with a musk odour have a reduced flexibility, which may be connected with the sharp cut-off for musks. Moncrieff (1967) proposed that a flexible molecule will have a better chance of accommodating itself on a receptor site, whereas if a molecule has a fixed shape it can fit only one type of site. This suggests that flexible molecules such as aliphatic compounds should not have such a sharp cut-off as musks, but experimental assays will be necessary to verify this hypothesis. If a small change to a large molecule renders it completely odourless, as opposed to less odorous or nondescript in character, it is likely to be because it no longer fits any receptor. However, another hypothesis is a size-limit constraint for being carried by transport proteins. Thus, any musk-type compounds unable to bind any of these proteins will experience serious restriction on reaching the ORs, given their hydrophobic character, and hence are likely to be odourless.

(*c*) *Functional characterization of odorant-binding proteins*

In order to reach the ORs embedded in the membrane of OSN cilia, airborne odorants have to be transferred across the aqueous nasal mucus (10–30 μm thick) that covers the olfactory epithelium (Fig. 2). In the case of hydrophobic odorants, it is supposed that this transport is enhanced by odorant-binding proteins (OBPs), but details about their function are still unclear. Vertebrate OBPs are small soluble proteins secreted at high levels by the olfactory epithelium. They belong to the lipocalin family, which serve as carriers in other body fluids. Structure analysis revealed that the polypeptide chain is folded into eight antiparallel β-sheets forming a continuously hydrogen-bonded β-barrel structure, resulting in an internal non-polar cavity that reversibly binds small hydrophobic molecules (Bianchet *et al.*, 1996). OBPs have not been identified in fish, which suggests that these carrier proteins represent a functional adaptation of the olfactory system to an aerial environment (Millery *et al.*, 2005).

Pevsner *et al.* (1985) purified a soluble OBP in the bovine nasal epithelium and found that the binding affinity of this protein to a homologous series of pyrazines was correlated with the human ODT of these compounds. A similar general correlation was reported for the binding of cycloalkanethiazoles to bovine OBP (Topazzini *et al.*, 1985). By comparing the structure of different pyrazine derivatives it was found that the steric and electrostatic features of the derivatives were correlated with human ODT values, and a quantitative 3D model was proposed that allowed the prediction of ODTs for other pyrazines (Yoshii & Hirono, 1996). A similar study experimentally obtained the ODT of 80 alkylpyrazines and reported significant differences according to the positions of radicals. The minimized structure of pyrazines with low ODT was compared with those with high ODT, which allowed the identification of sterically forbidden regions in the theoretical receptor that binds these compounds (Wagner *et al.*, 1999). These results suggest that the ODT of pyrazines depends on the interaction with OBPs. Attempting to determine if similar results apply for other compounds, another study characterized the binding properties of over 80 odorant molecules of different chemical classes to bovine OBP, and no significant correlation was found with ODT values. It was found that OBP does not display a uniquely high affinity for any single chemical class. Higher affinity binding for OBP is associated to a limited degree with more lipophilic ligands. However, lipophilicity alone is not a major determinant of affinity for OBP, as a number of relatively hydrophilic odorants display high affinity for OBP and a number of highly lipophilic molecules display low affinity for OBP (Pevsner *et al.*, 1990).

Different OBP subtypes occur simultaneously in the same animal species. Eight OBPs were identified from the nasal tissue of the porcupine *Hystrix cristata* (Felicioli *et al.*, 1993),

and probably a similar number applies for most mammals. Interestingly, in *Drosophila melanogaster* the actual number of OBPs was estimated to be at least 15 (Pikielny *et al.*, 1994). This similar number is probably related to a similar role performed by OBPs in vertebrates and insects, despite their belonging to structurally different classes. In the case of rats, three subtypes were identified. A reported investigation of rat OBP-binding properties showed that the three OBPs are specifically tuned towards distinct chemical classes of odorants. Rat OBP-1 preferentially binds heterocyclic compounds such as pyrazine derivatives. OBP-2 appears to be more specific for long-chain aliphatic aldehydes and carboxylic acids (Löbel *et al.*, 1998). Similarly, it was recently reported that the human variant hOBP-2A binds numerous odorants of different chemical classes with a higher affinity for aldehydes and fatty acids. Site-directed mutagenesis experiments revealed that the specificity toward aldehydes is determined by a lysine residue of the binding pocket, stabilizing odorant docking (Tcatchoff *et al.*, 2006). OBP-3 was described as interacting strongly with odorants composed of saturated or unsaturated ring structure (Löbel *et al.*, 2001). A further study analysed the ligand profile of three rat OBPs using a large number of odorous compounds from different chemical classes. It was found that most of the compounds bind only one OBP subtype, and that each OBP displays a characteristic ligand binding profile, interacting with a different subset of exogenous organic compounds (Löbel *et al.*, 2002). These results suggest that OBPs operate as a selective filter in odour preselection rather than as unspecific carriers for all hydrophobic compounds. Actually, a small side chain change in the pyrazine and thiazole derivatives caused overlapping binding affinities to rat-OBP1 and rat-OBP3 (Löbel *et al.*, 2002). Further research is still required to determine how odorant transport mediated by OBPs impacts odour character.

(*d*) *Hypotheses about the role of odorant-binding proteins*

Hydrophilic odorants will easily dissolve in the mucus aqueous layer of the olfactory epithelium. No transport mediated by OBPs is therefore necessary, and they probably interact with receptor binding sites facing the extracellular water domain (Fig. 4). Actually, different studies have expressed ORs in heterologous systems, and the results demonstrated that OR activation by odorant is not dependent upon the presence of OBPs (Malnic *et al.*, 1999; Wetzel *et al.*, 1999). On the contrary, the odorants with enough volatility to reach the olfactory epithelium but which are highly hydrophobic will have lower diffusivity across the mucus layer, and their transport needs to be enhanced by OBPs. Two scenarios appear in this case (Fig. 4). One possibility is that odorants may be transported to the lipid bilayer membrane and then diffuse laterally to reach specific entry sites in the receptors. Another hypothesis is that odorant-loaded OBPs interact with the extracellular loops of the receptors, so that the transported molecule can easily reach the binding site facing the water domain. Consistent with this hypothesis, a reported study expressed two human ORs using cell lines and found that in the absence of any ligand, porcine OBP interacted with high affinity only with one of the receptors (Matarazzo *et al.*, 2002). These results suggest that, under physiological conditions, unliganded OBP can selectively bind an OR. But the formation of an OBP-OR-odorant complex to initiate an olfactory response still remains to be established. The loops between TM2 – TM3 and TM4 – TM5 were suggested as candidates for the OBP-OR interaction, given that these loops contain motifs conserved in ORs but not in other GPCRs (Skoufos, 1999). For those partially hydrophobic and hydrophilic odorants both types of transport and OR activation patterns might possibly be involved. These mechanisms will depend upon odorant concentration. Thus, the role of OBPs as carriers will be specially relevant at low odorant concentrations, while as the concentration increases free odorant could also reach a specific OR directly. It was suggested that OBPs may play the opposite role at high ligand concentrations by preventing binding site saturation (Matarazzo *et al.*, 2002). Further studies will be required to elucidate the details of this transport process.

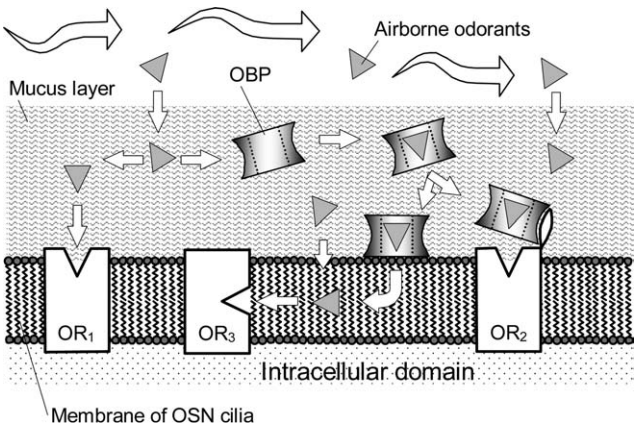

Fig. 4. Diagram of hypothetical mechanisms transporting odorants to the binding site of olfactory receptors (ORs). When a hydrophobic odorant adsorbs to the perireceptor fluid (mucus layer) it is bound by an odorant binding protein (OBP) to form a complex. This complex diffuses through the mucus layer and presents the ligand to the membrane-bound OR. Transport mediated by OBPs may not apply to hydrophilic odorants, which dissolve easily in the mucus layer. OR_1: for receptors activated by hydrophilic odorants, the binding site is assumed to face the extracellular domain. OR_2: odorant-loaded OBPs interact with the extracellular loops of the receptor, so that the ligand carried can easily reach the binding site facing the water domain. OR_3: the ligand is transported to the membrane (by diffusion through the mucus or by means of OBPs) and then diffuses laterally to reach a specific entry site in the receptor.

(3) The role of biotransformation enzymes

Mammals scan the chemical environment through consecutive sniffing. Odorants received by a given sniff have to be rapidly inactivated and extinguished, which is a prerequisite

for the olfactory system to receive iterative incoming signals. For this purpose, the olfactory epithelium contains different types of enzymes involved in reactions of degradation and biotransformation that inactivate and clear up the dissolved VOCs once they are perceived (Breer, 2003).

Assays in isolated tissue preparations and *in vivo* have found that the olfactory epithelium is very active in drug metabolism, in many cases more active than the liver (Dahl *et al.*, 1982). In order to study the enzymatic activity of the olfactory epithelium, a reported experiment screened 32 potential substrates for cytochrome P-450-dependent monooxygenases with rat nasal microsomes and found that 18 compounds were metabolized to produce formaldehyde (Dahl & Hadley, 1983). Nef *et al.* (1989) first cloned and reported a cytochrome P-450 (CYP) uniquely expressed in the olfactory epithelium. This protein belongs to the family of cytochrome P-450II enzymes that catalyse metabolic reactions of numerous hydrophobic odorants. Thus, it was assumed that CYP enzymes specifically expressed in the olfactory epithelium catalyse the conversion of odorants, either as a means of generating a more active stimulus or to turn off the olfactory signal so that new stimuli can be perceived (Nef *et al.*, 1989). A human P-450 enzyme (CYP2A13) that is predominantly expressed in nasal tissue was characterized, revealing a high efficiency to transform specific molecules (Su *et al.*, 2000). A recent study has found several biotransformation enzymes expressed preferentially in the nasal mucosa (ALDH6, CYP1B1, CYP2F1, CYP4B1), while others were at least as abundant in the nasal mucosa as in the liver (Zhang *et al.*, 2005).

Cytochrome P-450 enzymes are usually membrane-bound proteins. Some of them probably contain a binding site facing the extracellular domain and are responsible for the inactivation of hydrophilic odorants, while others would degrade hydrophobic compounds dissolved in the membrane bilayer. Experimental assays suggest that CYP enzymes expressed in the nasal epithelium are more sensitive to being inhibited than those in the liver (Jenner & Dodd, 1988). Thus, nasal metabolism may influence the levels of odorants in the olfactory receptor environment, activate odourless molecules to odorants, convert odorants to non-odorants, produce compounds with a different odour character or potency with respect to the parent compounds, and even metabolites that may inhibit intermediate degradation enzymes.

A similar complex pattern of agonist/antagonist effects was proposed long ago by Kistiakowsky (1950), who speculated that odorant molecules might be transformed by a multi-step enzymatic reaction, each step being catalysed by a separate enzyme. The molecules able reversibly to inhibit one or more of these enzymes would cause a shift in the relative concentration of the intermediate compounds, triggering a signal in a distinctive nerve. Based on this assumption, smell intensity would be connected with the extent of inhibition, and the compounds producing a nonreversible inhibition would result in a persistent odour (Kistiakowsky, 1950).

Interestingly, CYP enzymes can be inhibited, but the effect of CYP antagonists on odour intensity, character or persistency has not been studied in detail. These enzymes contain a heme prosthetic group that tetrahedrally coordinates an iron atom. This ferric ion is directly involved in the active site of the enzyme, and reacts with the substrate (Sono *et al.*, 1996). I speculate that the VOCs able to form a coordinate bond with ferric ions, like thiols or amines, might act as competitive inhibitors of these enzymes, resulting in a decrease in their activity. Thus, the stronger smell of these VOCs could be due to a high affinity to activating target ORs (assuming a metal ion to be involved in the binding site) and a simultaneous (maybe synergic) inhibitory effect of P-450 cytochromes.

VI. EVOLUTION AND FUNCTIONAL DIVERSITY OF OLFACTORY RECEPTORS

(1) Classification of G-protein-coupled receptors

The family of GPCRs comprises a variety of receptors for a multitude of different stimuli, that include light, neurotransmitters, odorants, biogenic amines, lipids, proteins, hormones, nucleotides and many others. All known members of this superfamily have seven transmembrane α-helices separated by extracellular and intracellular loops, with an extracellular amino-terminus and an intracellular carboxy-terminus. The presence of GPCRs in the genomes of bacteria, yeast, plants, nematodes, and other invertebrate groups argues in favour of a relatively early evolutionary origin of this receptor family. On the basis of shared sequence motifs, mammalian GPCRs are grouped into four classes: A, B, C, and F/S (Horn *et al.*, 1998). Rhodopsin is the prototype of class A, and hence receptors of this class are also called rhodopsin-like GPCRs. Another classification is based on their function. One group are receptors for sensory signals of external origin that are sensed as odours, pheromones, or tastes, and are referred to as chemosensory GPCRs (csGPCRs). The other group responds to endogenous signals that are involved in numerous physiological processes, and are referred to as endogenous GPCRs (endoGPCRs).

Searching the human genome, a total of 367 endoGPCRs were found: 284 belonged to class A, 50 to class B, 17 to class C, and 11 to class F/S (Vassilatis *et al.*, 2003). A phylogenetic analysis and sequence comparison indicated that the human endoGPCR superfamily can be divided into 95 families. Multiple receptor families with related functions or that recognize ligands of a particular chemical class were grouped in the same large branch. This organization is of predictive value for about 143 endoGPCRs with unknown ligands (Vassilatis *et al.*, 2003).

Given that there are approximately 350 functional ORs and 30 other chemosensory receptors in humans, the total number of human GPCRs was proposed to be around 750. In the case of mice, 392 endoGPCRs were found, 343 of which were common to humans (Vassilatis *et al.*, 2003). The persistence of the vast majority of endoGPCRs over the 50–60 million years of evolutionary time separating the two species is significant, and suggests that the functions of most endoGPCRs are conserved in humans and mice. In sharp

contrast, mice present a higher number of OR genes than humans and a lower number of pseudogenes (Niimura & Nei, 2005). Another remarkable distinction is that csGPCR genes are primarily expressed in the sensory organs, while multiple endoGPCR genes are widely expressed in different tissues and cell types, with a high preference for the brain (Vassilatis *et al.*, 2003).

(2) Classification of olfactory receptors

Olfactory receptors are the largest family of csGPCRs. OR genes are often highly divergent, and there are dramatic differences in the size of this gene family among species. OR genes from the fly, mosquito, and moth have been studied; the sequences are diverse, share no identity with vertebrate OR genes, and the repertoire appears to be under 100. In lower vertebrates, such as fish, the repertoire of OR genes ranges between 50 and 100 (Breer, 2003).

OR genes have been identified by molecular cloning techniques in species representing different levels of vertebrate evolution. Amphibians live in aquatic as well as terrestrial environments, and are capable of detecting both water-soluble and volatile odorants. Amphibians in general possess a gene repertoire encoding two distinct classes of olfactory receptors: class I are related to receptors of fish, and class II are similar to receptors of mammals (Freitag *et al.*, 1998). These results support the notion that class I receptors may be specialized in detecting water-soluble odorants, and class II receptors in recognizing volatile odorants. Interestingly, a comparison of the structural features of both receptor classes from various species revealed that they differ mainly in the sequence of the extracellular 4–5 loop, and it was suggested that this loop may contribute to ligand specificity (Freitag *et al.*, 1998).

A reported phylogenetic analysis conducted with published sequences from 24 vertebrate species classified OR genes into 32 distinct families. A clear separation appeared between 'fish-like' ORs (class I) and genes from tetrapods (class II). OR genes described to date in fish all belong to class I, with a few exceptions. On the other hand, while in tetrapods a majority of the OR genes belong to class II, many non-fish species (mammals, birds, and amphibians) were found to have class I ORs as well. These findings suggest that some ancient fish ORs were maintained and even expanded in mammals (Glusman *et al.*, 2000).

In an attempt to explore the functional properties of the two OR classes from *Xenopus laevis* (an amphibian), ten different ORs were expressed in *X. laevis* oocytes (four class I ORs and six class II ORs). It was found that ORs of each class did indeed detect different categories of odorants. Class I receptors responded to complex mixtures of water-soluble substances as well as to amino acids. By contrast, class II receptors specifically responded to complex mixtures of small hydrophobic compounds (Mezler, Fleischer & Breer, 2001). Interestingly, the six class II ORs in this study contain the HFFCD motif in the extracellular loop between TM4 and TM5, that is supposed to be involved in odorant recognition according to the MIAOR mechanism. By contrast, this motif is absent in the four ORs of class I, which might account for the differences in specificity.

The divergence of the OR genome of fish and arthropods is probably the result of adaptive processes, allowing the fish to deal with a limited number of water-soluble molecules and mammals to detect a large variety of hydrophobic, volatile compounds. It was postulated that class I ORs in mammals might have evolved to recognize volatile compounds, although they are still more sensitive to relatively hydrophilic compounds, whereas class II ORs might favour more hydrophobic compounds (Zhang & Firestein, 2002).

(3) Classification of human olfactory receptors

The classification of human intact OR genes into related groups of sequences is an essential prerequisite for rational structure-function studies of this vast receptor family. A phylogenetic analysis found that OR genes are clearly separated into class I and class II. Class I comprised 57 OR genes, and class II was formed by 331 genes (Niimura & Nei, 2003). On the basis of phylogenetic clustering, chromosomal localization, and amino acid sequence similarity, 347 human intact ORs were classified into 119 subfamilies (Zozulya *et al.*, 2001).

GPCRs with a similar sequence tend to display a similar function (Vassilatis *et al.*, 2003), and the same is expected for ORs. It was suggested that ORs with 60% or more sequence identity are likely to recognize structurally related odorants (Malnic *et al.*, 2004). According to this criterion, 172 OR subfamilies were found, most of which are encoded by genes at a single chromosomal locus. Each subfamily may be dedicated to the detection of a particular class of odorant structures. Members of the same subfamily would recognize partially overlapping sets of odorants, thereby allowing for the fine discrimination of odorants with highly related structures (Malnic *et al.*, 2004). The extensive variability of ORs is consistent with the ability of the OR repertoire to interact with odorous chemicals with diverse structures.

A proposed nomenclature classifies human ORs into families and subfamilies. Receptor sequences with 40% or more amino acid identity are considered members of the same family, whereas those sharing 60% or more identity constitute a subfamily. According to this criterion, ORs were classified into 17 families (four corresponding to class I and 13 to class II). Four of these families account for 58% of the ORs classified (Glusman *et al.*, 2001). In a similar piece of research, a phylogenetic analysis showed that class II OR genes can further be classified into 19 phylogenetic clades. Moreover, it was found that functional human OR genes belonging to the same clade tend to be located close to one another on a chromosome (Niimura & Nei, 2003).

Different studies suggest that the number of odour classes or dimensions in odour space is about 20–30. A statistical analysis of an odour profile database classified 146 odour character descriptors into 17 groups (Jeltema & Southwick, 1986). A similar study used a different odour database with 126 odour descriptors, that were classified into 19 clusters (Abe *et al.*, 1990). Interestingly, the number of dimensions in

odour space is similar to the number of OR families or phylogenetic clades proposed by different authors (Glusman *et al.*, 2001; Niimura & Nei, 2003). This observation suggests that one OR family might code for one dimension in odour space.

(4) Evolutionary aspects of human olfactory receptors

Mammalian ORs are encoded by a large gene superfamily formed by 900–1400 genes that are likely to comprise some 1–3% of the entire genome. Mice have 2.7 times as many functional OR genes as humans, but a phylogenetic tree constructed with sequences of human and mouse OR families revealed that the overall structures of the two OR repertoires were similar, and they covered more or less the same 'receptor space'. This result suggests that the human olfactory system has retained the ability to recognize a broad, if perhaps less discriminating, spectrum of chemicals by keeping fewer ORs in each family (Zhang & Firestein, 2002; Godfrey, Malnic & Buck, 2004). Another comparative study of the genome of both species also postulated that human ORs cover the same diversity of sequence motifs as mouse ORs (Liu *et al.*, 2003).

A phylogenic analysis suggested that the most recent common ancestor of human and mouse had approximately 750 functional OR genes and that mice acquired approximately 350 new OR genes after the human-mouse divergence 50–60 million years ago by repeated gene duplication, whereas approximately 430 OR genes in the common ancestor have become pseudogenes in the human lineage (Niimura & Nei, 2005). Mouse-specific gene expansion indicates a greater selective pressure to maintain a larger repertoire of functional ORs, probably as an adaptation to cope with the wide variety of environments in which mice live. The smaller human OR repertoire is consistent with the observation that humans have a poor sense of smell compared to other mammals.

ORs are disposed in clusters on most human chromosomes. Chromosome 11 alone contains 42% of all human ORs, including nine of the 13 class II families and all class I receptors, located in a single cluster. Moreover, it also has the two largest OR clusters in the entire genome (Glusman *et al.*, 2001). Based on the genomic location of OR clusters, the following genome expansion history was proposed: first, the generation of a 'tetrapod-specific' class II OR cluster on chromosome 11 by local duplications, then a single-step duplication of this cluster to chromosome 1, and finally an avalanche of duplication events out of chromosome 1 to most other chromosomes (Glusman *et al.*, 2001). Genes encoding viral coat proteins are often encountered in OR clusters. The density of such genes was found to be twice as high in OR clusters as in the rest of the genome, which suggests a possible viral-based mechanism of gene duplication and relocation (Zhang & Firestein, 2002).

Approximately 50% of human OR genes carry one or more coding region disruptions and are therefore considered pseudogenes (Malnic *et al.*, 2004). A reported study estimated the percentage of pseudogenes in the OR genome of 18 primate species other than humans to be approximately 31% in 11 species, and a significantly lower value of approximately 17% for the remaining seven species. A similar value, close to 20%, has been reported in the mouse (Zhang & Firestein, 2002) and dog. Strikingly, humans and the 11 primate species with a higher rate of pseudogenes possess full trichromatic vision, which is not the case for the other seven species (Gilad *et al.*, 2004). The higher loss of functional OR genes in primates with trichromatic vision occurred probably as a result of relaxed selective pressure to maintain or expand olfactory capabilities as species became less dependent on olfaction for survival, compensated by an increased reliance on vision and hearing.

A recent study has suggested that about 135 intact human ORs are likely to encode nonfunctional proteins, and hence the potential number of human OR pseudogenes could be up to 70% (Menashe, Aloni & Lancet, 2006). The high number of pseudogenes in the human OR genome suggests that it is degenerating at a relatively rapid rate. The most recent of these disruptions correspond to OR genes showing both functional and inactive alleles in the human population (Fig. 5), that are referred to as segregating pseudogenes (SPG). Attempting to study the variability of OR genes among individuals, 51 candidate OR genes were genotyped in 189 ethnically diverse humans. The results showed an unprecedented prevalence of SPGs, identifying one of the most pronounced cases of functional population diversity in the human genome (Menashe *et al.*, 2003). In a later study, 38 SPGs were found among the 384 intact human OR genes (Menashe *et al.*, 2006). Individualized SPG combinations generate an olfactory 'barcode', whereby every human nose is genetically different. Similar results were found in another study that compared the sequences of 16 OR genes in a sample of 95 dogs of 20 different breeds (Tacher *et al.*, 2005).

Humans exhibit high variability of both general olfactory thresholds and sensitivities towards specific odorants (i.e. partial anosmia). These phenotypes can partly be attributed

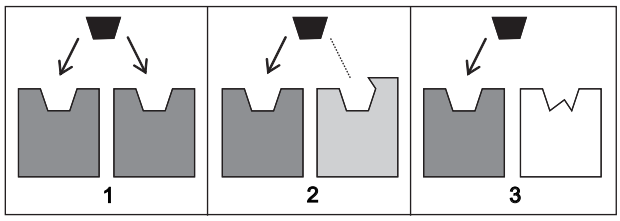

Fig. 5. Genotypic olfactory variation in different individuals. Each olfactory sensory neuron expresses only one allele of a given olfactory receptor (OR) gene (Chess *et al.*, 1994). The olfactory receptor is homozygously functional in individual 1 (the maternal and paternal alleles are functional and can be activated by a specific odorant molecule). In individual 2, the two alleles express sequence variants with slightly different odorant-binding affinities. Individual 3 shows heterozygosity for this OR (a mutation in one of the alleles renders it inactive). The lighter-grey receptor presents a lower affinity for the odorant, and the white receptor is inactive.

to genetic variation due to SPGs in the OR genome. Interestingly, some partial anosmias are genetically determined (Whissell-Buechy & Amoore, 1973). This issue is of particular interest for the fragrance industry, since it suggests that a given perfume will not smell the same to everybody.

(5) The sensory evaluation of genotype

To fight infection, vertebrates have evolved a sophisticated chemical recognition system that can discriminate between 'self' and 'non-self'. The major histocompatibility complex (MHC) is a family of approximately 50 genes characterized in many species by an extreme diversity. These genes code for proteins that bind and shuttle to the cell surface intracellular peptides of 8–10 amino acids long that are generated as intermediates during intracellular proteolytic degradation. In the context of immune surveillance, these MHC-peptide complexes are assessed by T lymphocytes through their highly diverse antigen receptors. The range of peptides displayed by the MHC molecules of an individual mirrors the structural diversity of its MHC alleles (Boehm & Zufall, 2006). The human MHC is called HLA (human leukocyte antigen).

In addition to their well-known immunological role in antigen presentation, MHC is involved in the generation of volatile and non-volatile chemosignals that can be used to distinguish between conspecifics. Secretions such as urine contain a mixture of volatile chemosignals that convey information on MHC haplotype. This genetically programmed unique body odour distinguishes one individual from another, and is referred to as 'odortype'. Experimental evidence reveals that the concentration in urine of a subset of compounds differs in mice according to MHC types (Willse *et al.*, 2005). Mice differing even in one MHC gene have a unique odour. The mechanisms underlying the generation of odortype are still poorly understood. Odortype is used as a means of animal communication and is involved in individual recognition, choice of mate, parent-infant interactions, physiological responses to other mice, and perhaps other aspects of the mouse's social and reproductive behaviour (Penn & Potts, 1999).

Different studies indicate that volatile odortypes alone are sufficient to carry information on MHC haplotype (Restrepo *et al.*, 2006). Interestingly, MHC-peptide complexes are shed from the cell surface and their fragments appear in serum, saliva, sweat and urine (Singh, Brown & Roser, 1987), acting as social cues for animal communication. Mouse urine contains fragments of MHC class I proteins, and a diverse mixture of MHC peptides (Singh *et al.*, 1987). Thus, in addition to volatile odortypes, MHC peptides in urine themselves act as an additional non-volatile chemosignal of MHC identity, providing an individual with unique olfactory signatures that are likely to be involved in conspecific communication and the regulation of mammalian social behaviour (Boehm & Zufall, 2006). It was recently discovered that MHC peptides can stimulate some mouse OSNs at remarkably low concentrations (Boehm & Zufall, 2006; Spehr *et al.*, 2006), but peptide-specific receptors have not been characterized yet. Given that MHC peptides are non-volatile compounds, how do they reach the olfactory mucosa? Based on experimental evidence it was postulated that MHC peptides present in mouse urine or other bodily secretions might gain access to the olfactory epithelium during behavioural situations involving direct physical contact between conspecifics (Spehr *et al.*, 2006).

In many mammals there is an accessory olfactory system located in the vomeronasal organ (VNO), separate from the main olfactory epithelium. The VNO has been identified with the action of pheromones, molecules produced and emitted by other members of the same species that are implicated in mating and other animal behaviours. Vomeronasal sensory neurons express over 200 receptors in rodents, that belong to two families of GPCRs unrelated to ORs. These neurons are highly specific for particular compounds, that can be detected at remarkably low concentrations (Firestein, 2001). Given that the VNO is specialized for the detection of highly specific compounds involved in conspecific communication, it was rather unexpected to find that certain receptors of the main olfactory epithelium are also implicated in a similar role.

It has been suggested that animals use body odour as a guide to identify possible mates as MHC-similar or MHC-dissimilar from their own genotype. Preference for a MHC-dissimilar partner enhances MHC heterozygosity of an individual's offspring, which may have improved immunocompetence and greater resistance to pathogens (Penn & Potts, 1999). Another possible adaptive advantage is clear: MHC-disassortative mating preferences is a mechanism for avoiding inbreeding (Penn & Potts, 1999). The combination of both the odortype (volatile) and the MHC peptide (non-volatile) as a chemosignal of MHC haplotype would provide the animal with a more robust signal of MHC haplotype and hence individual identity, which represents a distinct adaptive advantage (Restrepo *et al.*, 2006).

In the case of humans, experimental evidence suggests that each individual is also characterized by a unique body odour, that can be identified by trained dogs. But, in our species, vision is more important than olfaction to distinguish one individual from another, and obviously olfaction plays a minor role as a means of social communication. Odour preferences in humans are likely to be influenced by different factors such as psychological context, personal history, and hormonal status. Nonetheless, different studies have implicated HLA genes in the preference for body odours (for review, see Penn, 2002). This evidence was found for the odour of sweat, but not for urine (Santos *et al.*, 2005). The results suggest that HLA genes influence body odour production and/or perception. Extrapolative considerations on human mating preferences still fail to provide solid empirical evidence, and additional studies are needed to establish the role of odours in influencing basic human behaviours (Santos *et al.*, 2005). Interestingly, one study found a significant and repeatable association between individual preferences for perfume ingredients and HLA type (Milinski & Wedekind, 2000). Understanding the factors involved in odour preference is of considerable interest for the perfume industry.

(6) A second class of receptors in the olfactory epithelium

A recent study has reported the discovery of a second family of chemosensory receptors in the mouse olfactory epithelium (Liberles & Buck, 2006). Some of these receptors are activated by amines that are found at trace levels (i.e. very low concentrations) in mammalian tissues of the nervous system, and hence these compounds are referred to as trace amines (TAs). TAs include *p*-tyramine, β-phenylethylamine, tryptamine, and octopamine. Their molecular structure is similar to classical biogenic amines such as norepinephrine, dopamine, and serotonin, whose well-characterized effects as neurotransmitters are mediated by interactions with subfamilies of GPCRs. The role of TAs remained rather elusive until the discovery of a multigene family of GPCRs specific to TAs (Borowsky *et al.*, 2001). A later study completed the identification of all members of this novel GPCR family by screening the human, chimpanzee, rat, and mouse genome sequences, which led to the identification of 53 genes overall. These GPCRs were referred to as trace-amine-associated receptors (TAARs) (Lindemann *et al.*, 2005).

TAs have long been suspected to be involved in psychiatric disorders such as depression and schizophrenia. However, a different role of TAARs has emerged since the discovery that all mouse TAAR genes, except TAAR1, are expressed primarily in the olfactory epithelium, whereas none was detected in the brain (Liberles & Buck, 2006). Individual mouse TAARs are expressed in unique subsets of OSNs dispersed in the olfactory epithelium, and co-expression with other TAARs or ORs seems unlikely. This expression pattern is reminiscent of that displayed by the canonical ORs, which suggests that a major function of TAARs is the detection of olfactory cues by the nose (Liberles & Buck, 2006).

In rodents and primates the entire family of TAAR genes maps to a narrow region of a single chromosome. The degree of homology between members of the TAAR family within a species is extremely high, but the degree of amino acid identity among orthologues is moderate to low (Lindemann *et al.*, 2005). A phylogenetic analysis of the TAAR genes across species revealed that TAARs can be classified into three receptor subfamilies, that are clearly distinguished from all other GPCRs. Each subfamily was assumed to present a distinct pharmacological profile. This analysis also suggested that TAARs originated from a common ancestor and underwent a total of eight gene duplication events that led to a cluster of nine genes before the primate and rodent lineage split. These findings suggest that TAARs evolved relatively recently. In the case of rodents, TAAR7 and TAAR8 underwent multiple gene duplications that did not occur in primates. The diversification of these genes may indicate that, in rodents, TAARs were actively involved in adaptation processes during evolution, but not so much in the case of primates. This hypothesis is consistent with the fact that the number of TAAR pseudogenes in rodents (two in rat, one in mouse) is lower than those found in primates (six in chimpanzee, three in human) (Lindemann *et al.*, 2005).

Zebrafish *Danio rerio* reportedly has 57 TAAR genes with intact open reading frames (Gloriam *et al.*, 2005). Many of them are likely to be expressed in the fish olfactory epithelium (Liberles & Buck, 2006). Amine compounds are highly soluble in water, which makes them suitable candidates as chemosensory stimuli for fish. This might be the reason why a lower number of intact TAAR genes was found in rodents (17 in rat, 15 in mouse) as well as in primates (three in chimpanzee, six in human) (Lindemann *et al.*, 2005).

Since the discovery of TAARs, it was observed that only a subset of these receptors was specifically activated by TAs (Borowsky *et al.*, 2001). The remaining TAARs were also assumed to recognize amine compounds, though no functional response was found, which suggested the name TA 'associated' receptors (Lindemann *et al.*, 2005). A similar result was found after screening the ability of more than 300 odorous compounds, including almost 100 amines, to activate tissue culture cells engineered to express mouse and human TAARs. Activating compounds were found for five of the receptors tested, that were specific for different amine structures (Liberles & Buck, 2006). Notably, three ligands identified for mouse TAARs are natural components of mouse urine, a major source of social cues in rodents. Mouse TAAR4 recognizes a volatile amine that is elevated in urine in response to stress. Mouse TAAR3 is activated by isoamylamine, that in male urine is reported to act as a male-derived pheromone that accelerates the onset of puberty in female mice. Mouse TAAR5 responded robustly to extremely diluted mouse urine from sexually mature males, but not from females or prepubescent males. The ligand responsible for this activation was supposed to be trimethylamine. Thus, using TAAR5, mice could in theory determine the gender and sexual status of other mice. Taken together, these results suggest that, in mice, TAARs may mediate behavioural and physiological responses to amine-based social cues present in urine (Liberles & Buck, 2006). The discovery of TAARs and peptide-sensitive OSNs cautions against assuming that all aspects of odour perception are mediated by the same class of ORs.

VII. CONCLUSIONS

(1) Olfaction is a chemical sense that allows the perception and discrimination of airborne volatile chemicals. It plays a key role for survival, allowing the identification of food, predators or toxic compounds. Moreover, it is involved in animal communication. Hence, clarifying the underlying mechanisms of olfaction will provide a better understanding of animal behaviour.

(2) Odours are detected by a large repertoire of receptors expressed in the olfactory epithelium of the mammalian nose. Since the discovery of ORs in 1991, great progress has been achieved in understanding the physiological basis of olfaction. Odorants are recognized by means of a combinatorial coding scheme that explains how a few hundred different receptors can discriminate among thousands of

different molecules. This review suggests that the combinatorial theory of odour encoding should also include the perireceptor events involved in odorant transport and biotransformation: a given odorant can activate or inhibit different combinations of receptors, OBPs or biotransformation enzymes, while each one of the large number of proteins involved in odorant transport, recognition and transformation can be activated or inhibited by different molecules. This complex scenario reveals the great difficulty involved in modeling odour character and intensity, but provides a scientific basis for rational odorant design.

(3) ORs belong to the family of GPCRs that comprises a variety of receptors for a multitude of different stimuli. The sequence of all intact OR genes is currently known for several species. Different studies have analysed and compared these OR genomes, providing interesting hypotheses about evolutionary aspects of olfaction. It was recently suggested that the human OR genome is degenerating at a relatively rapid rate, probably as a result of selective pressure to maintain olfactory capabilities compensated by an increased reliance on vision and hearing. Consistent with this hypothesis, recent studies have found a functional population diversity in the human OR genome, which partially accounts for the variability in odour perception of each individual.

(4) The large repertoire of ORs in the olfactory epithelium is usually regarded as a non-specific chemical sense that evolved to recognize volatile chemicals. Another function recently reported is a direct implication in animal communication, by recognizing very specific molecules that act as social recognition chemosignals. These olfactory cues such as amines or peptides are involved in individual recognition, choice of mate and parent-infant interactions. The mechanism by which animals can identify conspecifics through smell is rather complex, and involves genes of the immune system. These findings provide a molecular mechanism by which an individual can sense the composition and compatibility of vital immune system molecules of a conspecific, with direct consequences for social behaviour. Functional genome analysis by the mammalian nose is assumed to avoid inbreeding, as well as to enhance offspring immunocompetence and provide greater resistance to pathogens. In humans, MHC genes are also involved in odour preference.

(5) Despite the progress in understanding the physiological fundamentals of olfaction, the molecular basis of odorant recognition and the conformational changes that ORs undergo upon ligand binding are still poorly understood. In some cases odour character and intensity is connected with the presence of a particular functional group in the molecule, and different authors have suggested that the olfactory system is sensitive to the fine structure of the electron distribution of the functional group. The hypothesis that a metal ion might be involved in odorant recognition is conjectural, but it provides a natural interpretation for this fact and other properties of olfaction that are not properly understood yet. According to this hypothesis, Turin (1996) proposed a mechanism based on inelastic electron tunneling spectroscopy which has been largely neglected because no similar mechanism has ever been found in biological systems. The metal-ion-assisted odorant recognition mechanism also assumes that olfactory receptors are metalloproteins. However, to date this mechanism has not received much attention. Despite the recent progress in understanding the fundamentals of olfaction, no clear evidence has appeared to validate or disprove the MIAOR mechanism. Further research is strongly recommended about the likely role of metal ions in olfaction.

VIII. ACKNOWLEDGEMENTS

This work was partly supported by a postdoctoral grant jointly sponsored by the Fulbright program and the Spanish Ministry of Education and Science – State Secretariat of Universities and Research. Sponsorship from Procter & Gamble Co. (Corporate Modeling and Simulations) is also acknowledged. I am grateful to D. Stanton and B. Murch for valuable discussion and support.

IX. REFERENCES

Abaffy, T., Malhotra, A. & Luetje, C. W. (2007). The molecular basis for ligand specificity in a mouse olfactory receptor: a network of functionally important residues. *Journal of Biological Chemistry* **282**, 1216–1224.

Abe, H., Kanaya, S., Komukai, T., Takahashi, Y. & Sasaki, S. (1990). Systematization of semantic descriptions of odors. *Analytica Chimica Acta* **239**, 73–85.

Abraham, M. H., Gola, J. M. R., Cometto-Muñiz, J. E. & Cain, W. S. (2002). A model for odour thresholds. *Chemical Senses* **27**, 95–104.

Adkins, C. J. & Phillips, W. A. (1985). Inelastic electron tunnelling spectroscopy. *Journal of Physics C: Solid State Physics* **18**, 1313–1346.

Afshar, M., Hubbard, R. E. & Demaille, J. (1998). Towards structural models of molecular recognition in olfactory receptors. *Biochimie* **80**, 129–135.

Alewijnse, A. E., Timmerman, H., Jacobs, E. H., Smit, M. J., Roovers, E., Cotecchia, S. & Leurs, R. (2000). The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. *Molecular Pharmacology* **57**, 890–898.

Alpers, D. H. (1994). Zinc and deficiencies of taste and smell. *JAMA* **272**, 1233–1234.

Amoore, J. E. (1963). Stereochemical theory of olfaction. *Nature* **198**, 271–272.

Amoore, J. E. (1967). Specific anosmia: a clue to the olfactory code. *Nature* **214**, 1095–1098.

Amoore, J. E. (1971). Olfactory genetics and anosmia. In *Handbook of Sensory Physiology*, (ed. L. M. Beidler), pp. 245–256. Springer Verlag, Berlin

Amoore, J. E. (1977). Specific anosmia and the concept of primary odors. *Chemical Senses* **2**, 267–281.

Araneda, R. C., Kini, A. D. & Firestein, S. (2000). The molecular receptive range of an odorant receptor. *Nature Neuroscience* **3**, 1248–1255.

Araneda, R. C., Peterlin, Z., Zhang, X., Chesler, A. & Firestein, S. (2004). A pharmacological profile of the aldehyde

receptor repertoire in rat olfactory epithelium. *Journal of Physiology 555.3*, 743–756.

Ballesteros, J. A., Jensen, A. D., Liapakis, G., Rasmussen, S. G., Shi, L., Gether, U. & Javitch, J. A. (2001). Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *Journal of Biological Chemistry* **276**, 29171–29177.

Bedoukian, P. Z. (1986). *Perfumery and Flavoring Synthetics*. Allured Publishing Co., Wheaton, IL.

Beets, M. G. J. (1957). Structure and odour. In *Molecular Structure and Organoleptic Quality*, S.C.I. Monograph No. 1, pp. 54–90. Society of Chemical Industry, London

Bersuker, I. B., Dimoglo, A. S., Gorbachov, M. Y., Greni, A. I., Vysotskaya, L. E. & Mikhailova, T. V. (1989). Study of the electronic and structural properties of the chemical compounds in garlic aroma. *Food / Nahrung* **33**, 405–411.

Bianchet, M. A., Bains, G., Pelosi, P., Pevsner, J., Snyder, S. H., Monaco, H. L. & Amzel, L. M. (1996). The three-dimensional structure of bovine odorant binding protein and its mechanism of odor recognition. *Nature Structural Biology* **3**, 934–939.

Blanpain, C., Lee, B., Vakili, J., Doranz, B. J., Govaerts, C., Migeotte, I., Sharron, M., Dupriez, V., Vassart, G., Doms, R. W. & Parmentier, M. (1999). Extracellular cysteines of CCR5 are required for chemokine binding, but dispensable for HIV-I coreceptor activity. *Journal of Biological Chemistry* **274**, 18902–18908.

Boehm, T. & Zufall, F. (2006). MHC peptides and the sensory evaluation of genotype. *Trends in Neurosciences* **29**, 100–107.

Boelens, M. H. & van Gemert, L. J. (1993). Sensory properties of optical isomers. *Perfumer & Flavorist* **18**(6), 1–15.

Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek, K. L., Durkin, M. M., Lakhlani, P. P., Bonini, J. A., Pathirana, S., Boyle, N., Pu, X., Kouranova, E., Lichtblau, H., Ochoa, F. Y., Branchek, T. A. & Gerald, C. (2001). Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proceedings of the National Academy of Sciences of USA* **98**, 8966–8971.

Brady, A. E. & Limbird, L. E. (2002). G protein-coupled receptor interacting proteins: emerging roles in localization and signal transduction. *Cell Signaling* **14**, 297–309.

Breer, H. (2003). Olfactory receptors: molecular basis for recognition and discrimination of odors. *Analytical & Bioanalytical Chemistry* **377**, 427–433.

Brookes, J. C., Hartoutsiou, F., Horsfield, A. P. & Stoneham, A. M. (2007). Could humans recognize odor by phonon assisted tunneling? *Physical Review Letters* **98**, 038101.

Brower, K. R. & Schafer, R. (1975). The recognition of chemical types by odor and the effect of steric hindrance at the functional group. *Journal of Chemical Education* **52**, 538–540.

Buck, L. & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65**, 175–187.

Burr, C. (2002). *The Emperor of Scent*. Random House, New York

Cass, A. E. & Hill, H. A. (1980). Copper proteins and copper enzymes. *CIBA Foundation Symposium* **79**, 71–91.

Chess, A., Simon, I., Cedar, H. & Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. *Cell* **78**, 823–834.

Coleman, J. E. (1998). Zinc enzymes. *Current Opinion in Chemical Biology* **2**, 222–234.

Dahl, A. R., Hadley, W. M., Hahn, F. F., Benson, J. M. & McMlellan, R. O. (1982). Cytochrome P-450-dependent monooxygenases in olfactory epithelium of dogs: possible role in tumorigenicity. *Science* **216**, 57–59.

Dahl, A. R. & Hadley, W. M. (1983). Formaldehyde production promoted by rat nasal cytochrome P-450-dependent monooxygenases with nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine as substrates. *Toxicology & Applied Pharmacology* **67**, 200–205.

Davies, J. T. & Taylor, F. H. (1954). A model system for the olfactory membrane. *Nature* **174**, 693–694.

Davies, J. T. & Taylor, F. H. (1959). The role of adsorption and molecular morphology in olfaction: the calculation of olfactory thresholds. *Biological Bulletin* **117**, 222–238.

Doleman, B. J., Severin, E. J. & Lewis, N. S. (1998). Trends in odor intensity for human and electronic noses: relative roles of odorant vapor pressure vs. molecularly specific odorant binding. *Proceedings of the National Academy of Sciences of USA* **95**, 5442–5447.

Doty, R. L. (1992). Psychophysical measurement of odor perception in humans. In *The Human Sense of Smell*, (eds. D. G. Laing, R. L. Doty & W. Breipohl), pp. 95–134. Springer-Verlag, Berlin

Dravnieks, A. (1962). Possible mechanism of olfaction. *Nature* **194**, 245–247.

Duchamp-Viret, P., Chaput, M. A. & Duchamp, A. (1999). Odor response properties of rat olfactory receptor neurons. *Science* **284**, 2171–2174.

Dyson, G. M. (1938). The scientific basis of odour. *Chemistry & Industry* **57**, 647–651.

Erikssen, J., Seegaard, E. & Naess, K. (1975). Side-effects of thiocarbamides. *Lancet* **1**, 231–232.

Felicioli, A., Ganni, M., Garibotti, M. & Pelosi, P. (1993). Multiple types and forms of odorant-binding proteins in the old-world porcupine *Hystrix cristtata*. *Computational Biochemistry Physiology* **105B**, 775–784.

Firestein, S. (2001). How the olfactory system makes sense of scents. *Nature* **413**, 211–218.

Floriano, W. B., Vaidehi, N., Goddard III, W. A., Singer, M. S. & Shepherd, G. M. (2000). Molecular mechanisms underlying differential odor responses of a mouse olfactory receptor. *Proceedings of the National Academy of Sciences of USA* **97**, 10712–10716.

Floriano, W. B., Vaidehi, N. & Goddard III, W. A. (2004). Making sense of olfaction through predictions of the 3-D structure and function of olfactory receptors. *Chemical Senses* **29**, 269–290.

Frederickson, C. J. (1989). Neurobiology of zinc and zinc-containing neurons. *International Review of Neurobiology* **31**, 145–238.

Freitag, J., Ludwig, G., Andreini, I., Rossler, P. & Breer, H. (1998). Olfactory receptors in aquatic and terrestrial vertebrates. *Journal of Comparative Physiology A* **183**, 635–650.

Gáborik, Z., Jagadeesh, G., Zhang, M., Spät, A., Catt, K. J. & Hunyady, L. (2003). The role of a conserved region of the second intracellular loop in AT_1 angiotensin receptor activation and signaling. *Endocrinology* **144**, 2220–2228.

Gaillard, I., Rouquier, S., Chavanieu, A., Mollard, P. & Giorgi, D. (2004). Amino-acid changes acquired during evolution by olfactory receptor 912-93 modify the specificity of odorant recognition. *Human Molecular Genetics* **13**, 771–780.

Gaillard, I., Rouquier, S., Pin, J. P., Mollard, P., Richard, S., Barnabe, C., Demaille, J. & Giorgi, D. (2002). A single olfactory receptor specifically binds a set of odorant molecules. *European Journal of Neuroscience* **15**, 409–418.

Ghanouni, P., Steenhuis, J. J., Farrens, D. L. & Kobilka, B. K. (2001). Agonist-induced conformational changes in the G-protein-coupling domain of the β_2 adrenergic receptor. *Proceedings of the National Academy of Sciences of USA* **98**, 5977–6002.

Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D. & Pääbo, S. (2004). Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PloS Biology* **2**, 120–125.

Gloriam, D. E. I., Bjarnadóttir, T. K., Yan, Y. L., Postlethwait, J. H., Schiöth, H. B. & Fredriksson, R. (2005). The repertoire of trace amine G-protein-coupled receptors: large expansion in zebrafish. *Molecular Phylogenetics and Evolution* **35**, 470–482.

Glusman, G., Bahar, A., Sharon, D., Pilpel, Y., White, J. & Lancet, D. (2000). The olfactory receptor gene superfamily: data mining, classification, and nomenclature. *Mammalian genome* **11**, 1016–1023.

Glusman, G., Yanai, I., Rubin, I. & Lancet, D. (2001). The complete human olfactory subgenome. *Genome Research* **11**, 685–702.

Godfrey, P. A., Malnic, B. & Buck, L. B. (2004). The mouse olfactory receptor gene family. *Proceedings of the National Academy of Sciences of USA* **101**, 2156–2161.

Goeke, A. (2002). Sulfur-containing odorants in fragrance chemistry. *Sulfur Reports* **23**, 243–278.

Greenberg, M. J. (1979). Dependence of odor intensity on the hydrophobic properties of molecules. A quantitative structure odor intensity relationship. *Journal of Agricultural & Food Chemistry* **27**, 347–352.

Guillot, M. (1948). Anosmies partielles et odeurs fondamentales. *Comptes Rendus de l'Académie des Sciences, Paris* **226**, 1307–1308.

Haffenden, L. J. W., Yaylayan, V. A. & Fortin, J. (2001). Investigation of vibrational theory of olfaction with variously labelled benzaldehydes. *Food Chemistry* **73**, 67–72.

Hall, S. E., Floriano, W. B., Vaidehi, N. & Goddard III, W. A. (2004). Predicted 3-D structures for mouse I7 and rat I7 olfactory receptors and comparison of predicted odor recognition profiles with experiment. *Chemical Senses* **29**, 595–616.

Ham, C. L. & Jurs, P. C. (1985). Structure-activity studies of musk odorants using pattern recognition: monocyclic nitrobenzenes. *Chemical Senses* **10**, 491–505.

Harper, R., Bate-Smith, E. C. & Land, D. G. (1968). *Odour Description and Odour Classification: a Multidisciplinary Examination*. Elsevier, New York

Hau, K. M. & Connell, D. W. (1998). Quantitative Structure-Activity Relationships (QSAR) for odor thresholds of volatile organic compounds (VOCs). *Indoor Air* **8**, 23–33.

Henkin, R. I., Patten, B. M., Re, P. K. & Bronzert, D. A. (1975). A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. *Archives of Neurology* **32**, 745–751.

Herbette, L. B., Rhodes, D. G. & Mason, R. P. (1991). New approaches to drug design and delivery based on drug-membrane interactions. *Drug Design & Delivery* **7**, 75–118.

Holst, B., Elling, C. E. & Schwartz, T. W. (2002). Metal ion-mediated agonism and agonist enhancement in melanocortin MC1 and MC4 receptors. *Journal of Biological Chemistry* **277**, 47662–47670.

Horn, F., Weare, J., Beukers, M. W., Hörsch, S., Bairoch, A., Chen, W., Edvardsen, O., Campagne, F. & Vriend, G. (1998). GPCRDB: an information system for G protein-coupled receptors. *Nucleic Acids Research* **26**, 275–279.

Hubert, T., Gunnison, K. M., Kazmi, M. A., Chang, B. S. W. & Sakmar, T. P. (2005). Identification of the primary entry site in visual rhodopsins: an intramembranous pathway from mutagenesis and MD simulations. *Biophysical Journal* **88**, 507A–508A (abstracts issues supplement)

Hummel, P., Vaidehi, N., Floriano, W. B., Hall, S. E. & Goddard III, W. A. (2005). Test of the binding threshold hypothesis for olfactory receptors: explanation of the differential binding of ketones to the mouse and human orthologs of olfactory receptor 912-93. *Protein Science* **14**, 703–710.

Imai, T., Suzuki, M. & Sakano, H. (2006). Odorant receptor-derived cAMP signals direct axonal targeting. *Science* **314**, 657–661.

Jeltema, M. A. & Southwick, E. W. (1986). Evaluations and application of odor profiling. *Journal of Sensory Studies* **1**, 123–136.

Jenner, J. & Dodd, G. H. (1988). The interaction of metyrapone and alpha-naphthoflavone with rat olfactory cytochrome P-450. *Biochemical Pharmacology* **37**, 558–559.

Jennings-White, C. (1984). Human primary odors. *Perfumer & Flavorist* **9** (6), 46–58.

Kajiya, K., Inaki, K., Tanaka, M., Haga, T., Kataoka, H. & Touhara, K. (2001). Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. *Journal of Neuroscience* **21**, 6018–6025.

Katada, S., Hirokawa, T., Oka, Y., Suwa, M. & Touhara, K. (2005). Structural basis for a broad but selective ligand spectrum of a mouse olfactory receptor: mapping the odorant-binding site. *Journal of Neuroscience* **25**, 1806–1815.

Keller, A. & Vosshall, L. B. (2004). A psychophysical test of the vibration theory of olfaction. *Nature Neuroscience* **7**, 337–338.

Kirk, R. L. & Stenhouse, N. S. (1953). Ability to smell solutions of potassium cyanide. *Nature* **171**, 698–699.

Kistiakowsky, G. B. (1950). On the theory of odors. *Science* **112**, 154–155.

Klopman, G. & Ptchelintsev, D. (1992). Application of the computer automated structure evaluation methodology to a QSAR study of chemoreception. Aromatic musky odorants. *Journal of Agricultural & Food Chemistry* **40**, 2244–2251.

Klopping, H. L. (1971). Olfactory theories and the odors of small molecules. *Journal of Agricultural & Food Chemistry* **19**, 999–1004.

Kondo, R., Kaneko, S., Sun, H., Sakaizumi, M. & Chigusa, S. I. (2002). Diversification of olfactory receptor genes in the Japanese medaka fish, *Oryzias latipes*. *Gene* **282**, 113–120.

Koyama, N. & Kurihara, K. (1972). Effect of odorants on lipid monolayers from bovine olfactory epithelium. *Nature* **236**, 402–404.

Kraft, P., Bajgrowicz, J. A., Denis, C. & Fráter, G. (2000). Odds and trends: recent developments in the chemistry of odorants. *Angewandte Chemie International Edition* **39**, 2980–3010.

Lai, P. C., Singer, M. S. & Crasto, C. J. (2005). Structural activation pathways from dynamic olfactory receptor-odorant interactions. *Chemical Senses* **30**, 781–792.

Lapidot, M., Pilpel, Y., Gilad, Y., Falcovitz, A., Sharon, D., Haaf, T. & Lancet, D. (2001). Mouse-human orthology relationships in an olfactory receptor gene cluster. *Genomics* **71**, 296–306.

Liberles, S. D. & Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium. *Nature* **442**, 645–650.

Lin, S. W. & Sakmar, T. P. (1996). Specific tryptophan UV-absorbance changes are probes of the transition of rhodopsin to its active state. *Biochemistry* **35**, 11149–11159.

Lindemann, L., Ebeling, M., Kratochwil, N. A., Bunzow, J. R., Grandy, D. K. & Hoener, M. C. (2005). Trace amine-associated receptors from structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics* **85**, 372–385.

Liu, A. H., Zhang, X., Stolovitzky, G. A., Califano, A. & Firestein, S. J. (2003). Motif-based construction of a functional map for mammalian olfactory receptors. *Genomics* **81**, 443–456.

Löbel, D., Marchese, S., Krieger, J., Pelosi, P. & Breer, H. (1998). Subtypes of odorant-binding proteins: heterologous expression and ligand binding. *European Journal of Biochemistry* **254**, 318–324.

Löbel, D., Strotmann, J., Jacob, M. & Breer, H. (2001). Identification of a third rat odorant-binding protein. *Chemical Senses* **26**, 673–680.

Löbel, D., Jacob, M., Völkner, M. & Breer, H. (2002). Odorants of different chemical classes interact with distinct odorant binding protein subtypes. *Chemical Senses* **27**, 39–44.

Ma, M. & Shepherd, G. M. (2000). Functional mosaic organization of mouse olfactory receptor neurons. *Proceedings of the National Academy of Sciences of USA* **97**, 12869–12874.

Malnic, B., Godfrey, P. A. & Buck, L. B. (2004). The human olfactory receptor gene family. *Proceedings of the National Academy of Sciences of USA* **101**, 2584–2589.

Malnic, B., Hirono, J., Sato, T. & Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell* **96**, 713–723.

Man, O., Gilad, Y. & Lancet, D. (2004). Prediction of the odorant binding site of olfactory receptor proteins by human-mouse comparisons. *Protein Science* **13**, 240–254.

Matarazzo, V., Zsürger, N., Guillemot, J. C., Clot-Faybesse, O., Botto, J. M., Farra, C. D., Crowe, M., Demaille, J., Vincent, J. P., Mazella, J. & Ronin, C. (2002). Porcine odorant-binding protein selectively binds to a human olfactory receptor. *Chemical Senses* **27**, 691–701.

Matsunami, H. (2005). Functional expression of mammalian odorant receptors. *Chemical Senses* **30** (suppl 1), i95–i96.

Menashe, I., Man, O., Lancet, D., Gilad, Y. (2003). Different noses for different people. *Nature Genetics* **34**, 143–144.

Menashe, I., Aloni, R. & Lancet, D. (2006). A probabilistic classifier for olfactory receptor pseudogenes. *BMC Bioinformatics* **7**, 393.

Mezler, M., Fleischer, J. & Breer, H. (2001). Characteristic features and ligand specificity of the two olfactory receptor classes from *Xenopus laevis*. *Journal of Experimental Biology* **204**, 2987–2997.

Mihara, S. & Masuda, H. (1988). Structure-odor relationships for disubstitued pyrazines. *Journal of Agricultural & Food Chemistry* **36**, 1242–1247.

Milinski, M. & Wedekind, C. (2000). Evidence for MHC-correlated perfume preferences in humans. *Behavioral Ecology* **12**, 140–149.

Millery, J., Briand, L., Bézirard, V., Blon, F., Fenech, C., Richard-Parpaillon, L., Quennedey, B., Pernollet, J. C. & Gascuel, J. (2005). Specific expression of olfactory binding protein in the aerial olfactory cavity of adult and developing *Xenopus*. *European Journal of Neuroscience* **22**, 1389–1399.

Moncrieff, R. W. (1949). A new theory of odour. *Perfumery & Essential Oil Record* **40**, 279–285.

Moncrieff, R. W. (1967). *The Chemical Senses*. Cleveland, CRC Press

Mori, K., Takahashi, Y. K., Igarashi, K. M. & Yamaguchi, M. (2006). Maps of odorant molecular features in the mammalian olfactory bulb. *Physiological Reviews* **86**, 409–433.

Mozell, M. & Jagodowicz, M. (1973). Chromatographic separation of odorants by the nose: retention times measured across in vivo olfactory mucosa. *Science* **181**, 1247–1249.

Mozell, M. M., Sheehe, P. R., Hornung, D. E., Kent, P. F., Youngentob, S. L. & Murphy, S. J. (1987). "Imposed" and "Inherent" mucosal activity patterns. *Journal of General Physiology* **90**, 625–650.

Nachbar, R. B. & Morton, T. H. (1981). A gas chromatographic (GLPC) model for the sense of smell. Variation of olfactory sensitivity with conditions of stimulation. *Journal of Theoretical Biology* **89**, 387–407.

Nef, P., Heldman, J., Lazard, D., Margalit, T., Jaye, M., Hanukoglu, I. & Lancet, D. (1989). Olfactory-specific cytochrome P-450. *Journal of Biological Chemistry* **264**, 6780–6785.

Niimura, Y. & Nei, M. (2003). Evolution of olfactory receptor genes in the human genome. *Proceedings of the National Academy of Sciences of USA* **100**, 12235–12240.

Niimura, Y. & Nei, M. (2005). Evolutionary changes of the number of olfactory receptor genes in the human and mouse lineages. *Gene* **346**, 23–28.

Ohloff, G. (1994). *Scent and Fragrances: The Fascination of Odors and Their Chemical Perspectives*. Springer, Berlin

Oka, Y., Omura, M., Kataoka, H. & Touhara, K. (2004). Olfactory receptor antagonism between odorants. *EMBO Journal* **23**, 120–126.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M. & Miyano, M. (2000). Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **289**, 739–745.

Patterson, P. M. & Lauder, B. A. (1948). The incidence and probable inheritance of smell blindness to normal butyl mercaptan. *Journal of Heredity* **39**, 295–297.

Pelosi, P. & Pisanelli, A. M. (1981). Specific anosmia to 1,8-cineole: the camphor primary odor. *Chemical Senses* **6**, 87–93.

Penn, D. J. (2002). The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology* **801**, 1–21.

Penn, D. J. & Potts, W. K. (1999). The evolution of mating preferences and major histocompatibility complex genes. *American Naturalist* **153**, 145–164.

Pevsner, J., Trifiletti, R. R., Strittmatter, S. M. & Snyder, S. H. (1985). Isolation and characterization of an olfactory receptor protein for odorant pyrazines. *Proceedings of the National Academy of Sciences of USA* **82**, 3050–3054.

Pevsner, J., Hou, V., Snowman, A. M. & Snyder, S. H. (1990). Odorant-binding protein: characterization of ligand binding. *Journal of Biological Chemistry* **265**, 6118–6125.

Pikielny, C. W., Hasan, G., Rouyer, F. & Rosbash, M. (1994). Members of a family of *Drosophila* putative odorant-binding proteins are expressed in different subsets of olfactory hairs. *Neuron* **12**, 35–49.

Pilpel, Y. & Lancet, D. (1999). The variable and conserved interfaces of modeled olfactory receptor proteins. *Protein Science* **8**, 969–977.

Rakow, N. A. & Suslick, K. S. (2000). A colorimetric sensor array for odour visualization. *Nature* **406**, 710–714.

Rawson, N. E., Eberwine, J., Dotson, R., Jackson, J., Ulrich, P. & Restrepo, D. (2000). Expression of mRNAs encoding for two different olfactory receptors in a subset of olfactory receptor neurons. *Journal of Neurochemistry* **75**, 185–195.

Ressler, K. J., Sullivan, S. L. & Buck, L. B. (1994). A molecular dissection of spatial patterning in the olfactory system. *Current Opinion in Neurobiology* **4**, 588–596.

Restrepo, D., Lin, W., Salcedo, E., Yamazaki, K. & Beauchamp, G. (2006). Odortypes and MHC peptides: complementary chemosignals of MHC haplotype? *Trends in Neurosciences* **29**, 604–609.

Rosenkilde, M. M., Lucibello, M., Holst, B. & Schwartz, T. W. (1998). Natural agonist enhancing bis-His zinc-site in transmembrane segment V of the tachykinin NK3 receptor. *FEBS Letters* **439**, 35–40.

Rubin, B. D. & Katz, L. C. (1999). Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* **23**, 499–511.

Russell, Y., Evans, P. & Dodd, G. H. (1989). Characterization of the total lipid and fatty acid composition of rat olfactory mucosa. *Journal of Lipid Research* **30**, 877–884.

Saito, H., Kubota, M., Roberts, R. W., Chi, Q. & Matsunami, H. (2004). RTP family members induce functional expression of mammalian odorant receptors. *Cell* **119**, 679–691.

Saito, H., Chi, Q., Zhuang, H. & Matsunami, H. (2006). The functional properties of mammalian odorant receptors. *Chemical Senses* **31**, 479.A65

Santos, P. S. C., Schinemann, J. A., Gabardo, J. & Bicalho, M. G. (2005). New evidence that the MHC influences odor perception in humans: a study with 58 Southern Brazilian students. *Hormones and Behavior* **47**, 384–388.

Sanz, G., Schlegel, C., Pernollet, J. C. & Briand, L. (2005). Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. *Chemical Senses* **30**, 69–80.

Scheer, A., Costa, T., Fanelli, F., De Benedetti, P. G., Mhaouty-Kodja, S., Abuin, L., Nenniger-Tosato, M. & Cotecchia, S. (2000). Mutational analysis of the highly conserved arginine within the Glu/Asp-Arg-Tyr motif of the α_{1b}-adrenergic receptor: effects on receptor isomerization and activation. *Molecular Pharmacology* **57**, 219–231.

Sell, C. S. (2006). On the unpredictability of odor. *Angewandte Chemie International Edition* **45**, 6254–6261.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y. & Sakano, H. (2003). Negative feedback regulation ensures the one receptor – one olfactory neuron rule in mouse. *Science* **302**, 2088–2094.

Shapiro, D. A., Kristiansen, K., Weiner, D. M., Kroeze, W. K. & Roth, B. L. (2002). Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. *Journal of Biological Chemistry* **277**, 11441–11449.

Shi, L. & Javitch, J. A. (2002). The binding site of aminergic G protein-coupled receptors: the transmembrane segmens and second extracellular loop. *Annual Review of Pharmacology & Toxicology* **42**, 437–467.

Shirokova, E., Schmiedeberg, K., Bedner, P., Niessen, H., Willecke, K., Raguse, J. D., Meyerhof, W. & Krautwurst, D. (2005). Identification of specific ligands for orphan olfactory receptors: G protein-dependent agonism and antagonism of odorants. *Journal of Biological Chemistry* **280**, 11807–11815.

Silvente-Poirot, S. & Wank, S. A. (1996). A segment of five amino acids in the second extracellular loop of the cholecystokinin-B receptor is essential for selectivity of the peptide agonist gastrin. *Journal of Biological Chemistry* **271**, 14698–14706.

Singer, M. S. (2000). Analysis of the molecular basis for octanal interactions in the expressed rat I7 olfactory receptor. *Chemical Senses* **25**, 155–165.

Singer, M. S., & Shepherd, G. M. (1994). Molecular modeling of ligand-receptor interactions in the OR5 olfactory receptor. *NeuroReport* **5**, 1297–1300.

Singer, M. S., Oliveira, L., Vriend, G. & Shepherd, G. M. (1995*a*). Potential ligand-binding residues in rat olfactory receptors identified by correlated mutation analysis. *Receptors & Channels* **3**, 89–95.

Singer, M. S., Shepherd, G. M. & Greer, C. A. (1995*b*). Olfactory receptors guide axons. *Nature* **377**, 19–20.

Singer, M. S., Weisinger-Lewin, Y., Lancet, D. & Shepherd, G. M. (1996). Positive selection moments identify potential functional residues in human olfactory receptors. *Receptors & Channels* **4**, 141–147.

Singh, P. B., Brown, R. E. & Roser, B. (1987). MHC antigens in urine as olfactory recognition cues. *Nature* **327**, 161–164.

Skoufos, E. (1999). Conserved sequence motifs of olfactory receptor-like proteins may participate in upstream and downstream signal transduction. *Receptors & Channels* **6**, 401–413.

Smart, T. G. (2004). Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist* **10**, 432–442.

Sono, M., Roach, M. P., Coulter, E. D. & Dawson, J. H. (1996). Heme-containing oxygenases. *Chemical Reviews* **96**, 2841–2887.

Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J. A., Wetzel, C. H., Zimmer, R. K. & Hatt, H. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* **299**, 2054–2058.

Spehr, M., Kelliher, K. R., Li, X. H., Boehm, T., Leinders-Zufall, T. & Zufall, F. (2006). Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. *Journal of Neuroscience* **26**, 1961–1970.

Stock, A. & Massenez, C. (1913). Boron hydrides. *Berichte* **45**, 3539–3568.

Su, T., Bao, Z., Zhang, Q. -Y., Smith, T. J., Hong, J. -Y. & Ding, X. (2000). Human cytochrome P450 CYP2A13: predominant expression in the respiratory tract and is high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Research* **60**, 5074–5079.

Swaminath, G., Steenhuis, J., Kobilka, B. & Lee, T. W. (2002). Allosteric modulation of b_2-adrenergic receptor by Zn^{2+}. *Molecular Pharmacology* **61**, 65–72.

Tacher, S., Quignon, P., Rimbault, M., Dreano, S., Andre, C. & Galibert, F. (2005). Olfactory receptor sequence polymorphism within and between breeds of dogs. *Journal of Heredity* **96**, 812–816.

Tcatchoff, L., Nespoulous, C., Pernollet, J. C. & Briand, L. (2006). A single lysyl residue defines the binding specificity of a human odorant-binding protein for aldehydes. *FEBS Letters* **580**, 2102–2108.

Topazzini, A., Pelosi, P., Pasqualetto, P. L. & Baldaccini, N. E. (1985). Specificity of a pyrazine binding protein from cow olfactory mucosa. *Chemical Senses* **10**, 45–49.

Touhara, K., Sengoku, S., Inaki, K., Tsuboi, A., Hirono, J., Sato, T., Sakano, H. & Haga, T. (1999). Functional identification and reconstitution of an odorant receptor in single olfactory neurons. *Proceedings of the National Academy of Sciences of USA* **96**, 4040–4045.

Turin, L. (1996). A spectroscopic mechanism for primary olfactory reception. *Chemical Senses* **21**, 773–791.

Turin, L. (2002). A method for the calculation of odor character from molecular structure. *Journal of Theoretical Biology* **216**, 367–385.

Turin, L. & Yoshii, F. (2003). Structure-odor relations: a modern perspective. In *Handbook of Olfaction and Gustation*, (ed. R. L. Doty), pp. 275–294. Marcel Dekker, New York

Vaidehi, N., Floriano, W. B., Trabanino, R., Hall, S. E., Freddolino, P., Choi, E. J., Zamanakos, G. & Goddard III, W. A. (2002). Prediction of structure and function of G protein-coupled receptors. *Proceedings of the National Academy of Sciences of USA* **99**, 12622–12627.

Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., Brown, A., Rodriguez, S. S., Weller, J. R., Wright, A. C., Bergmann, J. E. & Gaitanaris, G. A. (2003). The G protein-coupled receptor repertoires of human and mouse. *Proceedings of the National Academy of Sciences of USA* **100**, 4903–4908.

Vosshall, L. B. (2003). Putting smell on the map. *Trends in Neurosciences* **26**, 169–170.

Wagner, R., Czerny, M., Bielohradsky, J. & Grosch, W. (1999). Structure-odour-activity of alkylpyrazines. *European Food Research and Technology* **208**, 308–316.

Wang, J., Luthey-Schulten, Z. A. & Suslick, K. A. (2003). Is the olfactory receptor a metalloprotein? *Proceedings of the National Academy of Sciences of USA* **100**, 3035–3039.

Wetzel, C. H., Oles, M., Wellerdieck, C., Kuczkowiak, M., Gisselman, G. & Hatt, H. (1999). Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus Laevis* Oocytes. *Journal of Neuroscience* **19**, 7426–7433.

Whissell-Buechy, D. & Amoore, J. E. (1973). Odour-blindness to musk: simple recessive inheritance. *Nature* **242**, 271–273.

Willse, A., Belcher, A., Preti, G., Wahl, J. H., Thresher, M., Yang, P., Yamazaki, K. & Beauchamp, G. K. (2005). Identification of major histocompatibility complex-regulated body odorants by statistical analysis of a comparative gas chromatography/mass spectrometry experiment. *Analytical Chemistry* **77**, 2348–2361.

Wright, R. H. (1954). Odour and chemical constitution. *Nature* **173**, 831.

Wright, R. H. (1977). Odor and molecular vibration: neural coding of olfactory information. *Journal of Theoretical Biology* **64**, 473–502.

Wrobel, D. & Wannagat, U. (1982). Sila substituted perfumes; 4: Sila derivatives of some musk scents. *Journal of Organometallic Chemistry* **225**, 203–210.

Yoshii, F., Hirono, S., Liu, Q. & Moriguchi, I. (1991). Quantitative structure-activity relationships of structurally similar odorless and odoriferous benzenoid musks. *Chemical Senses* **16**, 319–328.

Yoshii, F., Hirono, S., Liu, Q. & Moriguchi, I. (1992). Three-dimensional structure model for benzenoid musks expressed by computer graphics. *Chemical Senses* **17**, 573–582.

Yoshii, F. & Hirono, S. (1996). Construction of a quantitative three-dimensional model for odor quality using Comparative Molecular Field Analysis (CoMFA). *Chemical Senses* **21**, 201–210.

Zakarya, D., Yahiaoui, M., & Fkihtetouani, S. (1993). Structure-odor relations for bitter almond odorants. *Journal of Physical Organic Chemistry* **6**, 627–633.

Zhang, X. & Firestein, S. (2002). The olfactory receptor gene superfamily of the mouse. *Nature Neuroscience* **5**, 124–133.

Zhang, X., Zhang, Q. -Y., Liu, D., Su, T., Weng, Y., Ling, G., Chen, Y., Gu, J., Schilling, B. & Ding, X. (2005). Expression of Cytochrome P450 and other biotransformation genes in fetal and adult human nasal mucosa. *Drug Metabolism & Dispositon* **33**, 1423–1428.

Zhao, H., Ivic, L., Otaki, J. M., Hashimoto, M., Mikoshiba, K. & Firestein, S. (1998). Functional expression of a mammalian odorant receptor. *Science* **279**, 237–242.

Zou, Z. & Buck, L. B. (2006). Combinatorial effects of odorant mixes in olfactory cortex. *Science* **311**, 1477–1481.

Zozulya, S., Echeverri, F. & Nguyen, T. (2001). The human olfactory receptor repertoire. *Genome Biology* **2**, 18.1–18.12.

Zumkley, H., Bertram, H. P., Vetter, H., Zidek, W. & Losse, H. (1985). Zinc metabolism during captopril treatment. *Hormone & Metabolic Research* **17**, 256–258.