



Mammalian olfactory receptors

Joerg Fleischer, Heinz Breer and Joerg Strotmann*

Institute of Physiology, University of Hohenheim, Stuttgart, Germany

Edited by:

Dieter Wicher, Max Planck Institute for Chemical Ecology, Germany

Reviewed by:

Bernd Grunewald,
Johann-Wolfgang-Goethe University,
Germany
Klemens F. Störkuhl, Ruhr Universität
Bochum, Germany

***Correspondence:**

Joerg Strotmann, Institute of
Physiology, University of Hohenheim,
Garbenstr. 30, 70599 Stuttgart,
Germany.
e-mail: strotman@uni-hohenheim.de

Perception of chemical stimuli from the environment is essential to most animals; accordingly, they are equipped with a complex olfactory system capable of receiving a nearly unlimited number of odorous substances and pheromones. This enormous task is accomplished by olfactory sensory neurons (OSNs) arranged in several chemosensory compartments in the nose. The sensitive and selective responsiveness of OSNs to odorous molecules and pheromones is based on distinct receptors in their chemosensory membrane; consequently, olfactory receptors play a key role for a reliable recognition and an accurate processing of chemosensory information. They are therefore considered as key elements for an understanding of the principles and mechanisms underlying the sense of smell. The repertoire of olfactory receptors in mammals encompasses hundreds of different receptor types which are highly diverse and expressed in distinct subcompartments of the nose. Accordingly, they are categorized into several receptor families, including odorant receptors (ORs), vomeronasal receptors (V1Rs and V2Rs), trace amine-associated receptors (TAARs), formyl peptide receptors (FPRs), and the membrane guanylyl cyclase GC-D. This large and complex receptor repertoire is the basis for the enormous chemosensory capacity of the olfactory system.

Keywords: olfaction, G protein-coupled receptor, odorant, pheromone, vomeronasal, trace amine-associated receptor, formyl peptide receptor, guanylyl cyclase

INTRODUCTION

For survival and reproduction, animals have to recognize a multitude of odorous substances related to food, predators and mating partners. Accordingly, their sense of smell has the capacity to detect and discriminate an almost unlimited number of chemical compounds. This is accomplished by an elaborated olfactory system composed of several chemosensory subsystems, including the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the septal organ (SO), and the Grueneberg ganglion (GG) (**Figure 1**; reviewed by Breer et al., 2006; Spehr et al., 2006; Ma, 2007; Munger et al., 2009). In these nasal compartments, the recognition of odorous compounds is based on highly specialized chemosensory cells, the olfactory sensory neurons (OSNs). The observation that a given odorant stimulates only a subset of OSNs (Sicard and Holley, 1984) has led to the concept that the responsiveness of individual OSNs to distinct odorants is determined by specialized receptors in their chemosensory membranes. Comprehensive research throughout the past two decades has led to the discovery of an unexpected large repertoire of olfactory receptors which is considered as the molecular basis for the enormous capacity of the olfactory system to detect and discriminate myriads of odorous compounds. Based on their structure and topographic distribution, this repertoire of olfactory receptors is categorized into several receptor families which include the odorant receptors (ORs), the vomeronasal receptors (V1Rs and V2Rs), trace amine-associated receptors (TAARs), formyl peptide receptors (FPRs), and the guanylyl cyclase GC-D (**Figure 1**). In line with the finding that odor detection depends on G protein-mediated pathways (Pace et al., 1985; Pace and Lancet, 1986; Sklar et al., 1986; Belluscio et al., 1998), most of these receptors belong to the large superfamily of G protein-coupled receptor proteins (GPCRs) which are characterized by seven transmembrane domains (**Figure 2**). Nevertheless,

olfactory receptors constitute a highly divergent group of receptors, consistent with the structural diversity of odorous compounds. In this review, structural features and functional implications of the olfactory receptor families are discussed and their common as well as their specific features are summarized.

ODORANT RECEPTORS (ORs)

STRUCTURAL FEATURES OF RECEPTOR PROTEINS

The structure of OR proteins is characterized by seven hydrophobic, putative membrane-spanning domains, the hallmark of all GPCRs. Based on their primary sequence, GPCRs are categorized into three classes: A, B or C (Jacoby et al., 2006). According to this classification, due to their domain organization, the ORs belong to GPCR class A, like e.g. rhodopsin (Jacoby et al., 2006). OR proteins have an average length of about 320 ± 25 amino acids residues; the differences in length result mainly from variable N- and C-terminal stretches. The N-terminal region which is exposed extracellularly contains a well conserved NXS/T consensus for N-linked glycosylation.

ORs are distinguishable from other GPCRs by several conserved amino acid motifs; these include an LHTPMY motif within the first intracellular loop, the most characteristic MAYDRYVAIC motif at the end of transmembrane (TM) domain 3 (TM3), a very short SY motif at the end of TM5, an FSTCSSH stretch at the beginning of TM6 and PMLNPF in TM7. Although these sequences are slightly different between species they were used to identify OR genes from many genomes. Extensive comparative analyses have identified more than 80 short motifs (Liu et al., 2003; Zhang et al., 2007), some of which are specific for distinct subfamilies or species and have been implicated in ligand binding. Seven cysteine residues are well conserved, a couple of them are thought to play a

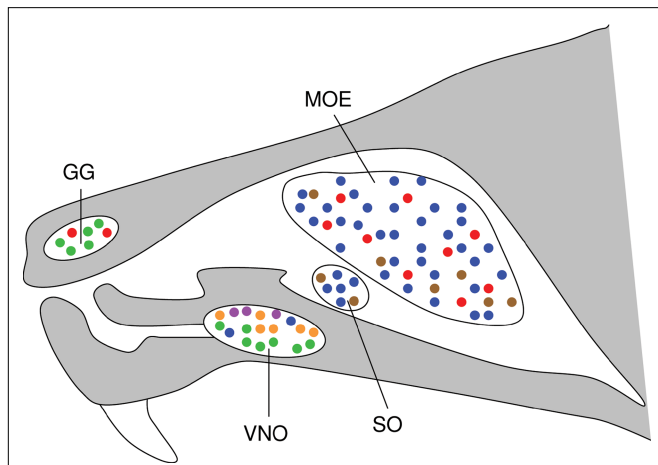


FIGURE 1 | Different olfactory compartments in the nose express distinct types of olfactory receptors. Schematic representation of the murine nose and its olfactory subsystems, including the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the septal organ (SO), and the Gruenberg ganglion (GG). The olfactory receptor types expressed in each of these organs are indicated by color: ORs in blue, V1Rs in orange, V2Rs in green, TAARs in red, FPRs in purple, GC-D in brown (modified from Fleischer et al., 2007).

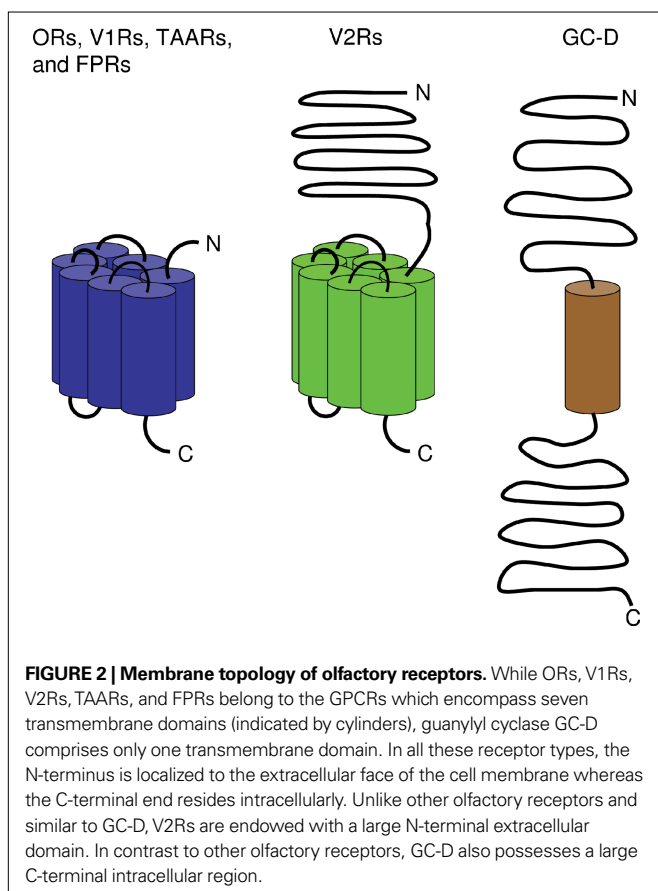


FIGURE 2 | Membrane topology of olfactory receptors. While ORs, V1Rs, V2Rs, TAARs, and FPRs belong to the GPCRs which encompass seven transmembrane domains (indicated by cylinders), guanylyl cyclase GC-D comprises only one transmembrane domain. In all these receptor types, the N-terminus is localized to the extracellular face of the cell membrane whereas the C-terminal end resides intracellularly. Unlike other olfactory receptors and similar to GC-D, V2Rs are endowed with a large N-terminal extracellular domain. In contrast to other olfactory receptors, GC-D also possesses a large C-terminal intracellular region.

role in maintaining the structural integrity of the protein. Two of these (at positions 97 and 179) are common to all GPCRs and are believed to form a disulfide link between extracellular loops 1 and 2; the other five are unique to ORs.

Although ORs in general are rather uniform in size and membrane topology, there are exceptions to this rule. A prominent one is represented by the so-called ‘OR37’ subfamily, which is characterized by an unusual third extracellular loop, which is six residues longer than in all other ORs (Kubick et al., 1997). Although only a few additional residues are present, they extend this loop – which is generally short – by about one-third.

Odor binding

Since the discovery of the OR genes by Buck and Axel (1991), many studies have been performed to identify the binding sites of the receptor proteins for odorous ligands. The first indications which protein domains are relevant for ligand interaction came already from the very initial sequence alignments which revealed that transmembrane domains were the most variable ones (Buck and Axel, 1991); this notion was subsequently confirmed employing larger receptor repertoires and bioinformatic approaches (Singer et al., 1996; Krautwurst et al., 1998; Zhao et al., 1998). The sequence variability of these domains thus was considered as the basis for the wide spectrum of odorous ligands that can be recognized by the receptor repertoire. Subsequent studies revealed that the most variable residues are oriented towards the inner surface of the receptor protein, whereas hydrophobic residues tended to point towards the protein/lipid interface. Using bioinformatic approaches, distinct residues have been defined which might be involved in ligand binding (Pilpel and Lancet, 1999; Lapidot et al., 2001; Katada et al., 2005; Khafizov et al., 2007); several of them could be confirmed experimentally by site-directed mutagenesis (Katada et al., 2005; Abaffy et al., 2007). All these data indicate that amino acid positions mainly in TM3, TM5 and TM6 are essential and strongly support the concept that predominantly the transmembrane domains of the OR protein form the binding pocket for odorants. The notion that a particular OR type may have a rather broad receptive range is supported by the finding that almost all analyzed ORs recognize not only a single, but multiple chemical compounds (e.g. Raming et al., 1993; Malnic et al., 1999; Araneda et al., 2000; Bozza et al., 2002; Gaillard et al., 2002; Mombaerts, 2004; Grosmaître et al., 2006; Malnic, 2007; Touhara, 2007; Saito et al., 2009).

Activation/signaling

With respect to ligand binding, ORs seem to resemble rhodopsin and related GPCRs. These GPCRs exist in one of two main conformations: an inactive and an active conformation which interacts with an intracellular heterotrimeric G protein. The transition between these conformations occurs through a movement of membrane-spanning domains. The conformational changes of a receptor that are elicited upon an interaction with a suitable odor molecule are not fully understood; however, a recent study has indicated the important role of distinct residues in an intracellular loop and the C-terminal domain (Kato et al., 2008). In this context also the DRY motif positioned at the cytoplasmic end of TM3 appears to be essential for G protein activation. Mutations within this motif caused either a constitutive activity or abolished G protein coupling (Imai et al., 2006). Based on this activation pattern, it has been proposed that upon ligand binding to the receptor, the third helix is displaced, thereby exposing the DRY motif and initiating the signal transduction pathway (Vaidehi et al., 2002; Katada et al., 2005).

Interestingly, *in vitro*, ORs can couple to various G proteins, such as $G\alpha_{olf}$, $G\alpha_s$ and $G\alpha_{15}$ (Kajiya et al., 2001) and there are indications that the interaction of a receptor with a non-typical G protein, such as $G\alpha_{15}$ instead of $G\alpha_{olf}$, can alter the ligand specificity of an OR (Shirokova et al., 2005). However, although various $G\alpha$ genes are expressed in OSNs, it is well established that $G\alpha_{olf}$ plays the major role in the chemo-electrical transduction process (Belluscio et al., 1998): odorant-activated ORs signal through $G\alpha_{olf}$ which then stimulates the adenylyl cyclase type III (ACIII), leading to a rise in cAMP concentration and opening of calcium-permeable cyclic nucleotide-gated (CNG) channels.

GENE STRUCTURE AND ORGANIZATION

OR genes have a rather unusual structure with an intronless coding region. The up- and down-stream non-coding exons are usually short, as well as the corresponding introns. Thus the transcription start site on one end and the polyadenylation signal on the other side are located in close proximity (1–10 kb) to the coding sequence. By these features, OR genes form very compact units; such an organization is supposed to favor the evolutionary dynamics of this gene family (see below). The upstream exons of several OR genes were shown to be alternatively spliced, resulting in different isoforms of OR mRNAs which, however, lead to the same protein (Asai et al., 1996; Sosinsky et al., 2000; Hoppe et al., 2003; Volz et al., 2003; Young et al., 2003).

OR genes are widely dispersed in the mammalian genomes and found on virtually all chromosomes. They generally reside at numerous locations with largely differing numbers of genes at each locus. In general, the OR clusters do not include non-OR interspersed genes. The intergenic distances vary from less than 5 kb to more than 50 kb depending on the amount of inserted repetitive sequences. Numerous clusters have meanwhile been analyzed in detail (Ben Arie et al., 1994; Glusman et al., 1996; Brand-Arpon et al., 1999; Sosinsky et al., 2000; Xie et al., 2000; Lane et al., 2001; Zhang and Firestein, 2002) indicating that each of them may contain members of several subfamilies or even families, suggesting that OR clusters have evolved through duplication of ancient precursor genes, as well as more recent duplications within gene clusters. Alternatively, genes of a given subfamily may be found in several clusters, suggesting that clusters may have been partly or completely duplicated. A high proportion of cluster sequences belongs to various families of interspersed repetitive elements. These repeats are believed to play a role in the numerous transposition/duplication events encountered in the OR repertoire during evolution.

RECEPTOR REPERTOIRES

OR genes have meanwhile been identified from numerous vertebrate species including many mammals like human, mouse, rat, dog, cow, opossum, and platypus.

Classes

Based on phylogenetic analyses, the mammalian ORs can be classified into two different groups: class I and class II. This classification is based on the original finding that the frog (*Xenopus laevis*) has two different groups of ORs: one (class I) that is similar to fish ORs and a second (class II) similar to mammalian ORs (Freitag et al., 1995). Interestingly, a comparison of the structural features

of both receptor classes from various species revealed that they differ mainly in the sequence of the second extracellular loop, and it was suggested that this loop may contribute to their ligand specificity (Freitag et al., 1998). In mammals the majority of the ORs belong to class II, but mammals do also have class I ORs (Zhang and Firestein, 2002; Tsuboi et al., 2006). Actually, more than 100 class I ORs are present e.g. in humans and mice; surprisingly, a large fraction of them are potentially functional (Niimura and Nei, 2005), suggesting that some ancient ORs were maintained and may even serve a special role in mammals.

Families and subfamilies

The complete OR gene repertoires have been characterized in several mammalian species (e.g. human, chimpanzee, mouse, rat, dog, cow, opossum, and platypus) (Glusman et al., 2001; Young and Trask, 2002; Zhang and Firestein, 2002; Godfrey et al., 2004; Malnic et al., 2004; Olender et al., 2004; Zhang et al., 2004, 2007; Quignon et al., 2005; Grus et al., 2007) demonstrating that the OR gene family is by far the largest in vertebrate genomes. ORs have been grouped in families (sequence similarity > 40%) and subfamilies (similarity > 60%). Due to the level of receptor diversification, there are large numbers of subfamilies.

Evolution

The number of OR sequences (functional and nonfunctional genes) present in the genome ranges between about 1,500 in macrosmatic species like e.g. dog or mouse and about 800 in the microsmatic primates. A rather small repertoire of functional OR genes exists in human (387) and platypus (262) (Young and Trask, 2002; Grus et al., 2007), the largest are currently known from rat (1,284) and mouse (1,194) (Zhang et al., 2007).

During mammalian evolution, many OR genes have been gained and lost (Niimura and Nei, 2007). The large turnover of OR genes in vertebrate evolution probably reflects the functional requirement for different olfactory abilities in different evolutionary lineages. The largest gene family expansion occurred in the marsupial lineage, with at least 750 novel genes. Similarly, more than 400 genes were gained in the rodent lineage. On the other hand, in the primate lineage, the number of genes that were lost is much greater than that in other lineages (Gilad et al., 2003).

EXPRESSION

OR genes are mainly expressed in OSNs of the MOE. The consensus view is that only one OR gene is expressed per OSN (monogenic). It has been shown in mice that this expression is also monoallelic, i.e. either the maternal or the paternal allele is expressed in one particular OSN (Chess et al., 1994; Mombaerts et al., 1996; Strotmann et al., 2000; Shykind, 2005). A given OR gene is expressed by a few thousand OSNs, which are usually widely scattered within a particular spatial zone of the MOE (Ressler et al., 1993; Vassar et al., 1993; Iwema et al., 2004; Miyamichi et al., 2005). Only for a few OR genes, a different pattern has been shown (Strotmann et al., 1992; Pyrski et al., 2001). A small subset of OR genes is not only expressed in the MOE, but also in other chemosensory organs, like the VNO (Levai et al., 2006) and the septal organ (Kaluza et al., 2004; Tian and Ma, 2004) or even broadly in tissues which are not involved in chemosensation (Feldmesser et al., 2006), like e.g. sperm cells

(Parmentier et al., 1992; Branscomb et al., 2000; Spehr et al., 2003; Fukuda and Touhara, 2006), autonomic ganglia (Weber et al., 2002) or cells of the cortex (Otaki et al., 2003); their functional role in these tissues is largely elusive.

VOMERONASAL RECEPTORS (VRs)

Vomeronal receptors (VRs) are classified into two major groups, V1Rs and V2Rs.

V1Rs

STRUCTURAL FEATURES OF RECEPTOR PROTEINS

The V1Rs, like the ORs, belong to class A of the GPCRs; however, they lack significant sequence homology to any other receptor from this rhodopsin-like receptor group, except for a weak relationship with the so-called T2Rs, the bitter taste receptors. In retrospect, it is therefore obvious that the V1R genes could not be uncovered by employing the homology-based approaches which had been successful for identifying the OR gene family. Instead, comparative hybridization of cDNA libraries from individual vomeronasal sensory neurons (VSNs) led to the discovery of this receptor family (Dulac and Axel, 1995). A characteristic feature of the V1Rs is their high degree of sequence diversity; only TM3 is rather well conserved and this domain is in fact under a strong negative selection pressure, i. e. selection against amino acid changes (Lane et al., 2002; Rodriguez et al., 2002). Also, a potential glycosylation site in extracellular loop 2 is rather well conserved. However, characteristic sequence motifs common to all V1R family members, as found for the ORs, are basically missing. Those that have been described are largely specific for distinct V1R families (Zhang et al., 2007). The highest sequence variability is found in TM2 and in the extracellular loops 2 and 3. The highest positive selective pressure, i.e. selection in favour of change, was surprisingly found in the first intracellular loop (Lane et al., 2002). The reason for this is currently unclear, since this domain is most likely not involved in ligand interaction.

Ligand binding and downstream signaling

Due to the similarities of the V1R membrane topology with that of the ORs, it is currently believed that the ligand binding sites – like in ORs – are located within the transmembrane regions; however, no residues that represent docking sites for ligands have been defined. Altogether, the knowledge about ligands for distinct V1Rs is still very sparse, which is mainly due to the fact that no mammalian V1R could be expressed in heterologous cells, yet. However, by means of single cell imaging and patch-clamp recordings from identified VSNs that co-express the V1R2b along with green fluorescent protein (GFP), Boschat et al. (2002) could identify 2-heptanone as a compound that activates these cells. Based on the concept that each VSN expresses only one V1R type, 2-heptanone was thus allotted as a ligand to this receptor. Interestingly, compounds which are structurally related to 2-heptanone did not activate V1R2b-expressing cells, arguing in favour of a high selectivity of this receptor. Optical imaging experiments on VNO sections independently demonstrated that distinct VSNs are activated only by very few, in the extreme by a single compound (Leinders-Zufall et al., 2000), suggesting that the respective V1Rs expressed by these cells are rather narrowly tuned. Increasing the concentrations of compounds did not activate more VSNs (Leinders-Zufall et al., 2000) – in contrast to what is generally

observed for OSNs in the MOE (Duchamp-Viret et al., 1999; Malnic et al., 1999) – further supporting this concept. Altogether, this contrasts with the relatively unspecific ligand spectrum of ORs which are generally activated by many different molecules. It thus seems conceivable that structural features of V1Rs are distinct from ORs, making their binding pocket rather rigid compared to the binding pocket of ORs which can accommodate several ligands.

In V1R-expressing VSNs several subunits of heterotrimeric G proteins have been identified including $G\alpha_{12}$, $G\alpha_o$, $G\alpha_{q/11}$, $G\beta_2$ and $G\gamma_2$ (Berghard and Buck, 1996; Jia and Halpern, 1996; Runnenburger et al., 2002; Wekesa et al., 2003). In fact, $G\alpha_{12}$, $G\alpha_o$ and $G\alpha_{q/11}$ have been found to be located in the microvilli of VSNs (Berghard and Buck, 1996; Liman et al., 1999; Menco et al., 2001). Nevertheless, it is currently not known which of these subunits is actually directly interacting with the V1Rs; thus, their precise roles in the transduction process are still elusive.

GENE STRUCTURE AND ORGANIZATION

Similar to what is known for the OR genes, the coding region of the V1R genes spans about 900 basepairs and is included in a single exon. Although additional 5' non-coding exons have been identified for several V1R genes (Lane et al., 2002), the transcriptional start site is generally positioned only a few (~5) kilobases upstream of the coding region; thus, V1R genes represent equally compact units as OR genes.

The genomic organization of the V1R repertoire has been studied most comprehensively in rodents (Rodriguez et al., 2002; Zhang et al., 2004, 2007). In the mouse, almost all V1R genes are arranged in clusters; there are only a few exceptions. The clusters rarely contain non-V1R genes, however, they appear to be densely populated with repetitive elements, mostly members of the *Line1* (L1) repeat family (Lane et al., 2002; Kambere and Lane, 2009). In one cluster residing on chromosome 6, an additional homology region of almost 1 kb length was found upstream of the transcription start site of each V1R gene; this observation led to the hypothesis that these conserved elements may be involved in controlling the expression of the respective V1R genes. The fact that they are associated exclusively with the V1R genes from this particular cluster suggested some kind of locus-specific transcriptional regulation.

RECEPTOR REPERTOIRES

The size of the V1R repertoire in most mammalian species investigated to date is significantly smaller than that of ORs; nevertheless, the 100–300 members found e.g. in rodents and marsupials (Zhang et al., 2004; Young et al., 2005; Shi and Zhang, 2007) still represent a relatively large group. Interestingly, the most 'ancient' mammal – the platypus – has the largest currently known repertoire with more than 800 V1R genes (Grus et al., 2007). Even in species with a pronounced communication by pheromones, like rodents, a large fraction of the V1R genes are pseudogenes. Extreme examples are humans and dogs which have only 5 or 8 potentially functional V1R genes (Rodriguez et al., 2000; Rodriguez and Mombaerts, 2002; Grus et al., 2005). There is substantial evidence that the VNO is not functional in adult humans, e.g. no axonal connections of VSNs to the brain were found (Meredith, 2001) and the gene encoding the TRPC2 channel, which is crucial for the VNO function,

is a pseudogene in humans (Liman and Innan, 2003; Zhang and Webb, 2003). In this context, it is not at all surprising that most V1R genes are pseudogenes in humans and the question arises what may be the function of the five potentially intact V1R genes. The finding that one of them is expressed in the MOE (Rodríguez et al., 2000) could be meaningful. A limited role of the VNO has also been proposed for the dog, and may even be pertinent for all carnivores (Grus et al., 2005). There is yet no final answer to the question why the V1R repertoires are so different in size; it has been speculated that rodents with their high numbers of V1Rs might be the exception rather than the rule.

Evolution

The V1Rs of a particular species can be grouped into distinct families which – in sharp contrast to the OR families – are phylogenetically very divergent from each other with amino acid identities of only about 15%. Within each family, however, a greater identity of up to 70% is found. As mentioned before, the size of the V1R repertoires in different species is highly divergent. A detailed study performed by Lane et al. (2002) suggested that the *L1* repeats may have promoted rearrangement events which led to the V1R expansion in the mouse. Interestingly, the activity of these *L1* elements appeared to coincide with the mouse/rat divergence and it was therefore proposed that such molecular events played a role in the speciation process by generating the species-specific V1R repertoires. In fact most V1Rs do not have orthologs in other species; in other words, the V1R repertoires are not only largely different in size, but moreover also in sequence.

EXPRESSION

The V1Rs are expressed in VSNs whose cell bodies are located in the apical layer of the VNO (Dulac and Axel, 1995). Each VSN expresses a single subtype from the repertoire, furthermore – as with the OR genes – only one allele is chosen by an individual cell (Rodríguez et al., 1999). The V1R proteins are found in the dendritic endings of VSNs (Takigami et al., 1999) such that they are in contact with the VNO lumen which is a liquid-filled, blind-ending tube (Halpern and Martinez-Marcos, 2003). A few V1R transcripts have been detected in the MOE of humans and goats (Rodríguez et al., 2000; Wakabayashi et al., 2002); however, it is currently uncertain whether there are in fact V1R proteins.

V2Rs

The fact that V1R genes are expressed exclusively in the apical $G\alpha_{12}$ -positive layer of the VNO suggested that the $G\alpha_0$ -positive VNS in the basal layers may express other GPCR subtypes. Indeed, an additional multigene GPCR family was discovered which is expressed in $G\alpha_0$ -positive VSNs (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997); accordingly, they were named V2Rs. In these cells, the V2R proteins are localized to the dendritic terminals (Martini et al., 2001). One particular V2R subtype – V2r83 – is also expressed outside the VNO in neurons of the GG (Fleischer et al., 2006).

STRUCTURAL FEATURES OF RECEPTOR PROTEINS

Unlike ORs and V1Rs, the V2Rs belong to the class C of GPCRs. A characteristic feature of class C receptors, which also include

the taste receptors for sweet/umami, the metabotropic glutamate receptors, and the Ca^{2+} -sensing receptor is their large (~70 kDa) N-terminal extracellular domain (Pin et al., 2003); this domain is joined to the heptahelical transmembrane part of the receptor protein via a cysteine-rich linker region. Typically, class C receptors dimerize via hydrophobic stretches which are present within the long N-terminal domain. It has therefore been proposed that also the V2Rs dimerize (Martini et al., 2001); a direct proof for this concept is still missing. Most of the V2R genes are expressed in a mutually exclusive manner in small subpopulations of VSNs. In these cells, they appear to be co-expressed with a receptor belonging to the so-called V2R2 family of V2Rs – a distinct family of V2Rs (also designated as family C of V2Rs) – whose members are present in an exceptionally high number of VNO neurons (Martini et al., 2001; Yang et al., 2005; Silvotti et al., 2007), indicating that VSNs in the basal layer express two distinct V2Rs.

Some V2Rs seem to require additional interaction partners. It was found that individual V2R-expressing VSNs also express particular members of non-classical major histocompatibility complex (MHC) class Ib genes (Ishii et al., 2003; Loconto et al., 2003). It has been demonstrated that these MHC molecules, together with the $\beta 2$ -microglobulin, are necessary for escorting distinct V2Rs to the plasma membrane and it was proposed that they might form a multimolecular complex at the membrane (Loconto et al., 2003). More recently, it was reported, however, that defined V2Rs are correctly targeted to the plasma membrane also in the absence of MHC1b proteins (Ishii and Mombaerts, 2008) and furthermore, that MHC1b genes are present only in rodents (Shi and Zhang, 2007). These findings suggest that the concept of V2Rs forming complexes with immune system-related proteins may not be generally applicable.

Ligand binding

V2Rs possess a long extracellular N-terminus (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997), suggesting a special mode of ligand recognition. Indeed, it has been shown for GPCRs of class C that this domain forms a Venus flytrap-like structure to which the ligand can bind (Bridges and Lindsley, 2008). Whether V2Rs employ the same mechanism is unclear. Specific ligands for distinct V2Rs have not even been identified, yet. In view of other class C GPCRs, V2R ligands are probably well soluble in water, rather than very hydrophobic molecules. In this context, it is intriguing that many other class C receptors bind amino acids, even the Ca^{2+} -sensing receptor (Conigrave et al., 2000). Consistent with this knowledge an *in vitro* study has provided evidence that in the rat VNO, protein pheromones activated the $G\alpha_0$ subunit (Krieger et al., 1999). Due to these considerations, the major urinary proteins (MUPs) have been viewed as promising candidates for V2R ligands (Dulac and Torello, 2003; Cheetham et al., 2007; Sherborne et al., 2007); however, the MUPs belong to the group of lipocalins which are rather carriers of small hydrophobic molecules; so this concept is still under debate. A recent study revealed, however, that purified MUPs alone are in fact sufficient to activate dissociated $G\alpha_0$ -positive VSNs (Chamero et al., 2007). It is noteworthy that in the V2R2s – but not in the other V2Rs – the residues to which amino acids bind and which are thus present in almost all other class C GPCRs, are conserved (Silvotti et al., 2005).

Other potential V2R ligands identified so far are peptides. Two distinct groups of peptides were shown to activate V2R-expressing VSNs: on one hand members from the exocrine gland-secreting peptide (ESP) family (Kimoto et al., 2005) and on the other hand, the MHC class I peptides (Leinders-Zufall et al., 2004), small peptides that are presented by MHC proteins at the cell surface. This finding may be relevant for the fact that mice can discriminate the body odors of conspecifics which are genetically different only in the MHC haplotype (Yamaguchi et al., 1981).

GENE STRUCTURE AND ORGANIZATION

V2R genes are also organized in clusters which are distributed on several chromosomes. The organization of individual V2R genes, however, is much more complex. The coding sequence of V2Rs is comprised of several exons, a unique feature among the olfactory GPCRs; this greatly increases the length (~20 kb) of individual genes and complicates the extraction of V2R coding sequences from genomic databases (Yang et al., 2005). Therefore, our current knowledge about the repertoires and evolution of V2R genes in mammals are still rather limited. The V2R repertoire in rodents comprises more than 200 members; it is slightly smaller in marsupials and in platypus (Shi and Zhang, 2007; Young and Trask, 2007). Again, similar to what has been found for the V1R repertoire, a very large part of the respective V2R genes are pseudogenes. In each species, the genes can be grouped into distinct families. Interestingly, in the mouse, one family is extremely large and comprises almost all (80%) of the V2R genes, whereas another one is very small with only four members. Surprisingly, in some mammalian species, like dog and cow, the V2R repertoire is completely degenerated (Young and Trask, 2007). In those species which have lost all of their functional V2R genes, usually one member from the V2R2 family is still present and contains only very few mutations, indicating a very recent pseudogenization event (Young and Trask, 2007).

TRACE AMINE-ASSOCIATED RECEPTORS (TAARs)

Searching for novel receptors, Borowsky et al. (2001) accidentally identified a group of GPCRs which are characterized by distinct sequence motifs (Lindemann and Hoener, 2005; Lindemann et al., 2005; Hussain et al., 2009; see below). Due to their activation by trace amines (Borowsky et al., 2001; Bunzow et al., 2001), such as β -phenylethylamine, p-tyramine, tryptamine, and octopamine, they were initially designated as trace amine receptors (TAs or TARs). Since it is more than doubtful that all members of this receptor family are sensitive to trace amines (Borowsky et al., 2001; Lindemann et al., 2005), they are now designated as trace amine-associated receptors (TAARs) (Lindemann and Hoener, 2005; Lindemann et al., 2005; Lewin, 2006). The coding sequence of TAAR genes – like those for ORs and V1Rs – encompasses about 1 kb and represents a single exon (Lindemann et al., 2005). TAARs reveal structural hallmarks characteristic of the rhodopsin/ β -adrenergic receptor superfamily, including short N- and C-terminal domains. Nevertheless, in line with their clustered genomic localization and a characteristic fingerprint motif in TM7, TAARs represent a well-defined, coherent receptor family (Lindemann et al., 2005). Compared to ORs, the number of distinct TAAR subtypes is rather low (15 TAARs in mice and 6 TAARs in humans; Lindemann et al., 2005).

TAARs are strongly expressed in the murine MOE and each TAAR subtype (except TAAR1) is expressed by a small subset of OSNs in a mutually exclusive manner, i.e., each cell expresses one TAAR type only. OSNs expressing a given TAAR subtype are distributed in the MOE in a manner reminiscent of the zonal expression pattern of ORs (Liberles and Buck, 2006). In addition to the MOE, some TAARs are also present in a distinct population of neurons in the GG (Fleischer et al., 2007). TAARs are activated by certain amine ligands (Borowsky et al., 2001; Bunzow et al., 2001; Liberles and Buck, 2006). Some of these amines are present in mouse urine in gender- or stress-dependent concentrations, leading to speculations that TAARs might be involved in the detection of some 'urine-borne' pheromones (Liberles and Buck, 2006). The signaling elements downstream of TAARs are unknown. In the murine MOE, TAARs are co-expressed with the $G\alpha_s$ -related G protein $G\alpha_{olf}$ (Liberles and Buck, 2006); in the GG, however, TAARs are co-expressed with $G\alpha_{i2}$ (Fleischer et al., 2007).

FORMYL PEPTIDE RECEPTORS (FPRs)

Two decades ago, a novel group of GPCRs called formyl peptide receptors (FPRs) was discovered (Boulay et al., 1990). FPR-encoding genes are clustered on a single chromosome (human chromosome 19 and mouse chromosome 17; reviewed by Migeotte et al., 2006). Their coding sequences are intronless and their open reading frames encode proteins of about 350 amino acid residues (Gao et al., 1998; Wang and Ye, 2002) with highly conserved transmembrane domains and more variable extracellular domains; the latter are supposed to be involved in ligand binding (Migeotte et al., 2006). FPRs were reported to be expressed in diverse tissues (reviewed by Migeotte et al., 2006; Panaro et al., 2006). Most recently, it has been shown that out of the seven murine FPR subtypes, some are predominantly expressed in the VNO. In fact, each of these FPR subtypes is expressed in about 1% of the VNO sensory neurons; apparently, these cells do not co-express vomeronasal receptors (Riviere et al., 2009).

In cells of the immune system, FPRs were found to be activated by their name-giving ligands, formylated peptides, which are released by bacteria; moreover, FPRs also bind to some other peptides and proteins associated with disease or inflammation (reviewed by Migeotte et al., 2006; Panaro et al., 2006; Le et al., 2007). For the FPR subtypes expressed in the VNO, it was observed that they are also activated by formylated peptides and other disease-related compounds which also induced responses in subsets of VNO sensory neurons, indicating that these cells might allow detection of infected conspecifics or contaminated food (Riviere et al., 2009).

MEMBRANE GUANYLYL CYCLASE GC-D

Among the various membrane guanylyl cyclases, subtype GC-D was found to be expressed in a subset of OSNs in the MOE which are therefore designated as GC-D neurons (Fülle et al., 1995; Juilfs et al., 1997). These cells lack signaling elements characteristic of the canonical cAMP pathway in OSNs of the MOE. Instead, they are endowed with the cGMP-dependent phosphodiesterase PDE2A and a cGMP-sensitive cyclic nucleotide-gated ion channel (Juilfs et al., 1997; Meyer et al., 2000; Hu et al., 2007). In addition to GC-D neurons in the MOE, GC-D is also expressed in some neurons of the septal organ (Walz et al., 2007). Similar to other OSNs, GC-D

neurons project their axons to the olfactory bulb where they converge on distinct glomeruli; these glomeruli encircle the caudal olfactory bulb and are therefore called 'necklace glomeruli' (Juilfs et al. 1997; Hu et al., 2007; Leinders-Zufall et al., 2007; Walz et al., 2007). In GC-D neurons, GC-D is mainly localized to apical cilia which are considered as the principal site of odor detection; this finding suggests an olfactory role of GC-D (Juilfs et al., 1997). In search of the chemosensory role of GC-D, it was found that the urinary peptides uroguanylin and guanylin activate GC-D neurons in a GC-D-dependent manner (Leinders-Zufall et al., 2007). The notion that GC-D is a receptor for such peptides was lately supported by studies on cells heterologously expressing GC-D (Duda and Sharma, 2008). Other findings indicate that GC-D may also

be involved in the detection of carbon dioxide (CO₂), since GC-D neurons – in contrast to other OSNs – respond to low concentrations of CO₂ (Hu et al., 2007; Sun et al., 2009). It is supposed that CO₂ is converted into bicarbonate in GC-D neurons via carbonic anhydrase and that bicarbonate then activates GC-D (Hu et al., 2007; Guo et al., 2009; Sun et al., 2009). In contrast to rodents, CO₂ is odorless to humans. In this context, it is interesting to note that in humans and several other primate species, the GC-D gene is a pseudogene (Young et al., 2007).

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

- Abaffy, T., Malhotra, A., and Luetje, C. W. (2007). The molecular basis for ligand specificity in a mouse olfactory receptor: a network of functionally important residues. *J. Biol. Chem.* 282, 1216–1224.
- Araneda, R. C., Kini, A. D., and Firestein, S. (2000). The molecular receptive range of an odorant receptor. *Nat. Neurosci.* 3, 1248–1255.
- Asai, H., Kasai, H., Matsuda, Y., Yamazaki, N., Nagawa, F., Sakano, H., and Tsuboi, A. (1996). Genomic structure and transcription of a murine odorant receptor gene: differential initiation of transcription in the olfactory and testicular cells. *Biochem. Biophys. Res. Commun.* 221, 240–247.
- Belluscio, L., Gold, G. H., Nemes, A., and Axel, R. (1998). Mice deficient in G(olf) are anosmic. *Neuron* 20, 69–81.
- Ben Arie, N., Lancet, D., Taylor, C., Khen, M., Walker, N., Ledbetter, D. H., Carrozzo, R., Patel, K., Sheer, D., Lehrach, H., and North, M. A. (1994). Olfactory receptor gene cluster on human chromosome 17: possible duplication of an ancestral receptor repertoire. *Hum. Mol. Genet.* 3, 229–235.
- Berghard, A., and Buck, L. B. (1996). Sensory transduction in vomeronasal neurons: evidence for G alpha o, G alpha i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. *J. Neurosci.* 16, 909–918.
- Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek, K. L., Durkin, M. M., Lakhiani, P. P., Bonini, J. A., Pathirana, S., Boyle, N., Pu, X., Kouranova, E., Lichtblau, H., Ochoa, F. Y., Branchek, T. A., and Gerald, C. (2001). Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proc. Natl. Acad. Sci. U.S.A.* 98, 8966–8971.
- Boschat, C., Pelofi, C., Randin, O., Roppolo, D., Luscher, C., Broillet, M. C., and Rodriguez, I. (2002). Pheromone detection mediated by a V1r vomeronasal receptor. *Nat. Neurosci.* 5, 1261–1262.
- Boulay, F., Tardif, M., Brouchon, L., and Vignais, P. (1990). The human N-formylpeptide receptor. Characterization of two cDNA isolates and evidence for a new subfamily of G-protein-coupled receptors. *Biochemistry* 29, 11123–11133.
- Bozza, T., Feinstein, P., Zheng, C., and Mombaerts, P. (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *J. Neurosci.* 22, 3033–3043.
- Brand-Arpon, V., Rouquier, S., Massa, H., de Jong, P. J., Ferraz, C., Ioannou, P. A., Demaille, J. G., Trask, B. J., and Giorgi, D. (1999). A genomic region encompassing a cluster of olfactory receptor genes and a myosin light chain kinase (MYLK) gene is duplicated on human chromosome regions 3q13-q21 and 3p13. *Genomics* 56, 98–110.
- Branscomb, A., Seger, J., and White, R. L. (2000). Evolution of odorant receptors expressed in mammalian testes. *Genetics* 156, 785–797.
- Breer, H., Fleischer, J., and Strotmann, J. (2006). The sense of smell: multiple olfactory subsystems. *Cell. Mol. Life Sci.* 63, 1465–1475.
- Bridges T. M., and Lindsley, C. W. (2008). G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. *ACS Chem. Biol.* 3, 530–541.
- Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65, 175–187.
- Bunzow, J. R., Sonders, M. S., Arttamangkul, S., Harrison, L. M., Zhang, G., Quigley, D. I., Darland, T., Suchland, K. L., Pasumamula, S., Kennedy, J. L., Olson, S. B., Magenis, R. E., Amara, S. G., and Grandy, D. K. (2001). Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol. Pharmacol.* 60, 1181–1188.
- Chamero, P., Marton, T. F., Logan, D. W., Flanagan, K., Cruz, J. R., Saghatelian, A., Cravatt, B. F., and Stowers, L. (2007). Identification of protein pheromones that promote aggressive behaviour. *Nature* 450, 899–902.
- Cheetham, S. A., Thom, M. D., Jury, F., Ollier, W. E., Beynon, R. J., and Hurst, J. L. (2007). The genetic basis of individual-recognition signals in the mouse. *Curr. Biol.* 17, 1771–1777.
- Chess, A., Simon, I., Cedar, H., and Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. *Cell* 78, 823–834.
- Conigrave, A. D., Quinn, S. J., and Brown, E. M. (2000). L-amino acid sensing by the extracellular Ca²⁺-sensing receptor. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4814–4819.
- Duchamp-Viret, P., Chaput, M. A., and Duchamp, A. (1999). Odor response properties of rat olfactory receptor neurons. *Science* 284, 2171–2174.
- Duda, T., and Sharma, R. K. (2008). ONE-GC membrane guanylate cyclase, a trimodal odorant signal transducer. *Biochem. Biophys. Res. Commun.* 367, 440–445.
- Dulac, C., and Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83, 195–206.
- Dulac, C., and Torello, A. T. (2003). Molecular detection of pheromone signals in mammals: from genes to behaviour. *Nat. Rev. Neurosci.* 4, 551–562.
- Feldmesser, E., Olender, T., Khen, M., Yanai, I., Ophir, R., and Lancet, D. (2006). Widespread ectopic expression of olfactory receptor genes. *BMC Genomics* 7, 121.
- Fleischer, J., Schwarzenbacher, K., Besser, S., Hass, N., and Breer, H. (2006). Olfactory receptors and signaling elements in the Gruenberg ganglion. *J. Neurochem.* 98, 543–554.
- Fleischer, J., Schwarzenbacher, K., and Breer, H. (2007). Expression of trace amine-associated receptors in the Gruenberg ganglion. *Chem. Senses* 32, 623–631.
- Freitag, J., Krieger, J., Strotmann, J., and Breer, H. (1995). Two classes of olfactory receptors in *Xenopus laevis*. *Neuron* 15, 1383–1392.
- Freitag, J., Ludwig, G., Andreini, I., Rossler, P., and Breer, H. (1998). Olfactory receptors in aquatic and terrestrial vertebrates. *J. Comp. Physiol. A* 183, 635–650.
- Fukuda, N., and Touhara, K. (2006). Developmental expression patterns of testicular olfactory receptor genes during mouse spermatogenesis. *Genes Cells* 11, 71–81.
- Fülle, H. J., Vassar, R., Foster, D. C., Yang, R. B., Axel, R., and Garbers, D. L. (1995). A receptor guanylyl cyclase expressed specifically in olfactory sensory neuron. *Proc. Natl. Acad. Sci. U.S.A.* 92, 3571–3575.
- Gaillard, I., Rouquier, S., Pin, J. P., Mollard, P., Richard, S., Barnabe, C., Demaille, J., and Giorgi, D. (2002). A single olfactory receptor specifically binds a set of odorant molecules. *Eur. J. Neurosci.* 15, 409–418.
- Gao, J. L., Chen, H., Filie, J. D., Kozak, C. A., and Murphy, P. M. (1998). Differential expansion of the N-formylpeptide receptor gene cluster in human and mouse. *Genomics* 51, 270–276.
- Gilad, Y., Man, O., Paabo, S., and Lancet, D. (2003). Human specific loss of olfactory receptor genes. *Proc. Natl. Acad. Sci. U.S.A.* 100, 3324–3327.
- Glusman, G., Clifton, S., Roe, B., and Lancet, D. (1996). Sequence analysis in the olfactory receptor gene cluster on human chromosome 17: recombinatorial events affecting receptor diversity. *Genomics* 37, 147–160.
- Glusman, G., Yanai, I., Rubin, I., and Lancet, D. (2001). The complete human olfactory subgenome. *Genome Res.* 11, 685–702.
- Godfrey, P. A., Malnic, B., and Buck, L. B. (2004). The mouse olfactory receptor

- gene family. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2156–2161.
- Grosmaire, X., Vassalli, A., Mombaerts, P., Shepherd, G. M., and Ma, M. (2006). Odorant responses of olfactory sensory neurons expressing the odorant receptor MOR23: a patch clamp analysis in gene-targeted mice. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1970–1975.
- Grus, W. E., Shi, P., and Zhang, J. (2007). Largest vertebrate vomeronasal type I receptor gene repertoire in the semiaquatic platypus. *Mol. Biol. Evol.* 24, 2153–2157.
- Grus, W. E., Shi, P., Zhang, Y. P., and Zhang, J. (2005). Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5767–5772.
- Guo, D., Zhang, J. J., and Huang, X. Y. (2009). Stimulation of guanylyl cyclase-D by bicarbonate. *Biochemistry* 48, 4417–4422.
- Halpern, M., and Martinez-Marcos, A. (2003). Structure and function of the vomeronasal system: an update. *Prog. Neurobiol.* 70, 245–318.
- Herrada, G., and Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell* 90, 763–773.
- Hoppe, R., Frank, H., Breer, H., and Strotmann, J. (2003). The clustered olfactory receptor gene family 262: genomic organization, promoter elements, and interacting transcription factors. *Genome Res.* 13, 2674–2685.
- Hu, J., Zhong, C., Ding, C., Chi, Q., Walz, A., Mombaerts, P., Matsunami, H., and Luo, M. (2007). Detection of near-atmospheric concentrations of CO₂ by an olfactory subsystem in the mouse. *Science* 317, 953–957.
- Hussain, A., Saraiva, L. R., and Korsching, S. I. (2009). Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4313–4318.
- Imai, T., Suzuki, M., and Sakano, H. (2006). Odorant receptor-derived cAMP signals direct axonal targeting. *Science* 314, 657–661.
- Ishii, T., Hirota, J., and Mombaerts, P. (2003). Combinatorial coexpression of neural and immune multigene families in mouse vomeronasal sensory neurons. *Curr. Biol.* 13, 394–400.
- Ishii, T., and Mombaerts, P. (2008). Expression of nonclassical class I major histocompatibility genes defines a tripartite organization of the mouse vomeronasal system. *J. Neurosci.* 28, 2332–2341.
- Iwema, C. L., Fang, H., Kurtz, D. B., Youngentob, S. L., and Schwob, J. E. (2004). Odorant receptor expression patterns are restored in lesion-recovered rat olfactory epithelium. *J. Neurosci.* 24, 356–369.
- Jacoby, E., Bouhelal, R., Gerspacher, M., and Seuwen, K. (2006). The 7 TM G-protein-coupled receptor target family. *Chem. Med. Chem.* 1, 761–782.
- Jia, C., and Halpern, M. (1996). Subclasses of vomeronasal receptor neurons: differential expression of G proteins (Gi alpha 2 and G(o alpha)) and segregated projections to the accessory olfactory bulb. *Brain Res.* 719, 117–128.
- Juilfs, D. M., Fülle, H. J., Zhao, A. Z., Houslay, M. D., Garbers, D. L., and Beavo, J. A. (1997). A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3388–3395.
- Kajiyama, K., Inaki, K., Tanaka, M., Haga, T., Kataoka, H., and Touhara, K. (2001). Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants. *J. Neurosci.* 21, 6018–6025.
- Kaluza, J. F., Gussing, F., Bohm, S., Breer, H., and Strotmann, J. (2004). Olfactory receptors in the mouse septal organ. *J. Neurosci. Res.* 76, 442–452.
- Kambere, M. B., and Lane, R. P. (2009). Exceptional LINE density at V1R loci: the Lyon repeat hypothesis revisited on autosomes. *J. Mol. Evol.* 68, 145–159.
- Katada, S., Hirokawa, T., Oka, Y., Suwa, M., and Touhara, K. (2005). Structural basis for a broad but selective ligand spectrum of a mouse olfactory receptor: mapping the odorant-binding site. *J. Neurosci.* 25, 1806–1815.
- Kato, A., Katada, S., and Touhara, K. (2008). Amino acids involved in conformational dynamics and G protein coupling of an odorant receptor: targeting gain-of-function mutation. *J. Neurochem.* 107, 1261–1270.
- Khafizov, K., Anselmi, C., Menini, A., and Carloni, P. (2007). Ligand specificity of odorant receptors. *J. Mol. Model.* 13, 401–409.
- Kimoto, H., Haga, S., Sato, K., and Touhara, K. (2005). Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. *Nature* 437, 898–901.
- Krautwurst, D., Yau, K. W., and Reed, R. R. (1998). Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* 95, 917–926.
- Krieger, J., Schmitt, A., Lobel, D., Gudermann, T., Schultz, G., Breer, H., and Boehhoff, I. (1999). Selective activation of G protein subtypes in the vomeronasal organ upon stimulation with urine-derived compounds. *J. Biol. Chem.* 274, 4655–4662.
- Kubick, S., Strotmann, J., Andreini, I., and Breer, H. (1997). Subfamily of olfactory receptors characterized by unique structural features and expression patterns. *J. Neurochem.* 69, 465–475.
- Lane, R. P., Cutforth, T., Axel, R., Hood, L., and Trask, B. J. (2002). Sequence analysis of mouse vomeronasal receptor gene clusters reveals common promoter motifs and a history of recent expansion. *Proc. Natl. Acad. Sci. U.S.A.* 99, 291–296.
- Lane, R. P., Cutforth, T., Young, J., Athanasiou, M., Friedman, C., Rowen, L., Evans, G., Axel, R., Hood, L., and Trask, B. J. (2001). Genomic analysis of orthologous mouse and human olfactory receptor loci. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7390–7395.
- Lapidot, M., Pilpel, Y., Gilad, Y., Falcovitz, A., Sharon, D., Haaf, T., and Lancet, D. (2001). Mouse-human orthology relationships in an olfactory receptor gene cluster. *Genomics* 71, 296–306.
- Le, Y., Wang, J. M., Liu, X., Kong, Y., Hou, X., Ruan, L., and Mou, H. (2007). Biologically active peptides interacting with the G protein-coupled formyl-peptide receptor. *Protein Pept. Lett.* 14, 846–853.
- Leinders-Zufall, T., Brennan, P., Widmayer, P., Chandramani, P. S., Maul-Pavicic, A., Jager, M., Li, X. H., Breer, H., Zufall, F., and Boehm, T. (2004). MHC class I peptides as chemosensory signals in the vomeronasal organ. *Science* 306, 1033–1037.
- Leinders-Zufall, T., Cockerham, R. E., Michalakis, S., Biel, M., Garbers, D. L., Reed, R. R., Zufall, F., and Munger, S. D. (2007). Contribution of the receptor guanylyl cyclase GC-D to chemosensory function in the olfactory epithelium. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14507–14512.
- Leinders-Zufall, T., Lane, A. P., Puche, A. C., Ma, W., Novotny, M. V., Shipley, M. T., and Zufall, F. (2000). Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* 405, 792–796.
- Levai, O., Feistel, T., Breer, H., and Strotmann, J. (2006). Cells in the vomeronasal organ express odorant receptors but project to the accessory olfactory bulb. *J. Comp. Neurol.* 498, 476–490.
- Lewin, A. H. (2006). Receptors of mammalian trace amines. *AAPS. J.* 8, E138–E145.
- Liberles, S. D., and Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium. *Nature* 442, 645–650.
- Liman, E. R., Corey, D. P., and Dulac, C. (1999). TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5791–5796.
- Liman, E. R., and Innan, H. (2003). Relaxed selective pressure on an essential component of pheromone transduction in primate evolution. *Proc. Natl. Acad. Sci. U.S.A.* 100, 3328–3332.
- Lindemann, L., Ebeling, M., Kratochwil, N. A., Bunzow, J. R., Grandy, D. K., and Hoener, M. C. (2005). Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics* 85, 372–385.
- Lindemann, L., and Hoener, M. C. (2005). A renaissance in trace amines inspired by a novel GPCR family. *Trends Pharmacol. Sci.* 26, 274–281.
- Liu, A. H., Zhang, X., Stolovitzky, G. A., Califano, A., and Firestein, S. J. (2003). Motif-based construction of a functional map for mammalian olfactory receptors. *Genomics* 81, 443–456.
- Loconto, J., Papes, F., Chang, E., Stowers, L., Jones, E. P., Takada, T., Kumanovics, A., Fischer, L. K., and Dulac, C. (2003). Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. *Cell* 112, 607–618.
- Ma, M. (2007). Encoding olfactory signals via multiple chemosensory systems. *Crit. Rev. Biochem. Mol. Biol.* 42, 463–480.
- Malnic, B. (2007). Searching for the ligands of odorant receptors. *Mol. Neurobiol.* 35, 175–181.
- Malnic, B., Godfrey, P. A., and Buck, L. B. (2004). The human olfactory receptor gene family. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2584–2589.
- Malnic, B., Hirono, J., Sato, T., and Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell* 96, 713–723.
- Martini, S., Silvotti, L., Shirazi, A., Ryba, N. J., and Tirindelli, R. (2001). Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ. *J. Neurosci.* 21, 843–848.
- Matsunami, H., and Buck, L. B. (1997). A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* 90, 775–784.
- Menco, B. P., Carr, V. M., Ezeh, P. I., Liman, E. R., and Yankova, M. P. (2001). Ultrastructural localization of G-proteins and the channel protein TRP2 to microvilli of rat vomeronasal receptor cells. *J. Comp. Neurol.* 438, 468–489.
- Meredith, M. (2001). Human vomeronasal organ function: a critical review of best and worst cases. *Chem. Senses* 26, 433–445.
- Meyer, M. R., Angele, A., Kremmer, E., Kaupp, U. B., and Muller, F. (2000). A

- cGMP-signaling pathway in a subset of olfactory sensory neurons. *Proc. Natl. Acad. Sci. U.S.A.* 97, 10595–10600.
- Migotte, I., Communi, D., and Parmentier, M. (2006). Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev.* 17, 501–519.
- Miyamichi, K., Serizawa, S., Kimura, H. M., and Sakano, H. (2005). Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelium determine the dorsal/ventral positioning of glomeruli in the olfactory bulb. *J. Neurosci.* 25, 3586–3592.
- Mombaerts, P. (2004). Genes and ligands for odorant, vomeronasal and taste receptors. *Nat. Rev. Neurosci.* 5, 263–278.
- Mombaerts, P., Wang, F., Dulac, C., Chao, S. K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Visualizing an olfactory sensory map. *Cell* 87, 675–686.
- Munger, S. D., Leinders-Zufall, T., and Zufall, F. (2009). Subsystem organization of the mammalian sense of smell. *Annu. Rev. Physiol.* 71, 115–140.
- Niimura, Y., and Nei, M. (2005). Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods. *Proc. Natl. Acad. Sci. U.S.A.* 102, 6039–6044.
- Niimura, Y., and Nei, M. (2007). Extensive gains and losses of olfactory receptor genes in Mammalian evolution. *PLoS ONE* 2, e708. doi: 10.1371/journal.pone.0000085.
- Olender, T., Fuchs, T., Linhart, C., Shamir, R., Adams, M., Kalush, F., Khen, M., and Lancet, D. (2004). The canine olfactory subgenome. *Genomics* 83, 361–372.
- Otaki, J. M., Yamamoto, H., and Firestein, S. (2003). Odorant receptor expression in the mouse cerebral cortex. *J. Neurobiol.* 58, 315–327.
- Pace, U., Hanski, E., Salomon, Y., and Lancet, D. (1985). Odorant-sensitive adenylyl cyclase may mediate olfactory reception. *Nature* 316, 255–258.
- Pace, U., and Lancet, D. (1986). Olfactory GTP-binding protein: signaltransducing polypeptide of vertebrate chemosensory neurons. *Proc. Natl. Acad. Sci. U.S.A.* 83, 4947–4951.
- Panaro, M. A., Acquafredda, A., Sisto, M., Lisi, S., Maffione, A. B., and Mitolo V. (2006). Biological role of the N-formyl peptide receptors. *Immunopharmacol. Immunotoxicol.* 28, 103–127.
- Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., Ledent, C., Mollereau, C., Gerard, C., Perret, J., Grootegoed, A., and Vassart, G. (1992). Expression of members of the putative olfactory receptor gene family in mammalian germ cells. *Nature* 355, 453–455.
- Pilpel, Y., and Lancet, D. (1999). The variable and conserved interfaces of modeled olfactory receptor proteins. *Protein Sci.* 8, 969–977.
- Pin, J. P., Galvez, T., and Prezeau, L. (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol. Ther.* 98, 325–354.
- Pyrski, M., Xu, Z., Walters, E., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., and Margolis, F. L. (2001). The OMP-lacZ transgene mimics the unusual expression pattern of OR-Z6, a new odorant receptor gene on mouse chromosome 6: implication for locus-dependent gene expression. *J. Neurosci.* 21, 4637–4648.
- Quignon, P., Giraud, M., Rimbault, M., Lavigne, P., Tacher, S., Morin, E., Retout, E., Valin, A. S., Lindblad-Toh, K., Nicolas, J., and Galibert, F. (2005). The dog and rat olfactory receptor repertoires. *Genome Biol.* 6, R83.
- Raming, K., Krieger, J., Strotmann, J., Boekhoff, I., Kubick, S., Baumstark, C., and Breer, H. (1993). Cloning and expression of odorant receptors. *Nature* 361, 353–356.
- Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1993). A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* 73, 597–609.
- Riviere, S., Challet, L., Fluegge, D., Spehr, M., and Rodriguez, I. (2009). Formyl peptide receptor-like proteins are a novel family of vomeronasal chemosensors. *Nature* 459, 574–577.
- Rodriguez, I., Del Punta, K., Rothman, A., Ishii, T., and Mombaerts, P. (2002). Multiple new and isolated families within the mouse superfamily of V1r vomeronasal receptors. *Nat. Neurosci.* 5, 134–140.
- Rodriguez, I., Feinstein, P., and Mombaerts, P. (1999). Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. *Cell* 97, 199–208.
- Rodriguez, I., Greer, C. A., Mok, M. Y., and Mombaerts, P. (2000). A putative pheromone receptor gene expressed in human olfactory mucosa. *Nat. Genet.* 26, 18–19.
- Rodriguez, I., and Mombaerts, P. (2002). Novel human vomeronasal receptor-like genes reveal species-specific families. *Curr. Biol.* 12, R409–R411.
- Runnenburger, K., Breer, H., and Boekhoff, I. (2002). Selective G protein beta gamma subunit compositions mediate phospholipase C activation in the vomeronasal organ. *Eur. J. Cell Biol.* 81, 539–547.
- Ryba, N. J., and Tirindelli, R. (1997). A new multigene family of putative pheromone receptors. *Neuron* 19, 371–379.
- Saito, H., Chi, Q., Zhuang, H., Matsunami, H., and Mainland, J. D. (2009). Odor coding by a Mammalian receptor repertoire. *Sci. Signal.* 2, ra9.
- Sherborne, A. L., Thom, M. D., Paterson, S., Jury, F., Ollier, W. E., Stockley, P., Beynon, R. J., and Hurst, J. L. (2007). The genetic basis of inbreeding avoidance in house mice. *Curr. Biol.* 17, 2061–2066.
- Shi, P., and Zhang, J. (2007). Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. *Genome Res.* 17, 166–174.
- Shirokova, E., Schmiedeberg, K., Bedner, P., Niessen, H., Willecke, K., Raguse, J. D., Meyerhof, W., and Krautwurst, D. (2005). Identification of specific ligands for orphan olfactory receptors. G protein-dependent agonism and antagonism of odorants. *J. Biol. Chem.* 280, 11807–11815.
- Shykind, B. M. (2005). Regulation of odorant receptors: one allele at a time. *Hum. Mol. Genet.* 14(Spec No. 1), R33–R39.
- Sicard, G., and Holley, A. (1984). Receptor cell responses to odorants: similarities and differences among odorants. *Brain Res.* 292, 283–296.
- Silvotti, L., Giannini, G., and Tirindelli, R. (2005). The vomeronasal receptor V2R2 does not require escort molecules for expression in heterologous systems. *Chem. Senses* 30, 1–8.
- Silvotti, L., Moiani, A., Gatti, R., and Tirindelli, R. (2007). Combinatorial co-expression of pheromone receptors, V2Rs. *J. Neurochem.* 103, 1753–1763.
- Singer, M. S., Weisinger-Lewin, Y., Lancet, D., and Shepherd, G. M. (1996). Positive selection moments identify potential functional residues in human olfactory receptors. *Receptors. Channels* 4, 141–147.
- Sklar, P. B., Anholt, R. R., and Snyder, S. H. (1986). The odorant-sensitive adenylyl cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. *J. Biol. Chem.* 261, 15538–15543.
- Sosinsky, A., Glusman, G., and Lancet, D. (2000). The genomic structure of human olfactory receptor genes. *Genomics* 70, 49–61.
- Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J. A., Wetzel, C. H., Zimmer, R. K., and Hatt, H. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* 299, 2054–2058.
- Spehr, M., Spehr, J., Ukhanov, K., Kelliher, K. R., Leinders-Zufall, T., and Zufall, F. (2006). Parallel processing of social signals by the mammalian main and accessory olfactory systems. *Cell. Mol. Life Sci.* 63, 1476–1484.
- Strotmann, J., Conzelmann, S., Beck, A., Feinstein, P., Breer, H., and Mombaerts, P. (2000). Local permutations in the glomerular array of the mouse olfactory bulb. *J. Neurosci.* 20, 6927–6938.
- Strotmann, J., Wanner, I., Krieger, J., Raming, K., and Breer, H. (1992). Expression of odorant receptors in spatially restricted subsets of chemosensory neurones. *Neuroreport* 3, 1053–1056.
- Sun, L., Wang, H., Hu, J., Han, J., Matsunami, H., and Luo, M. (2009). Guanylyl cyclase-D in the olfactory CO2 neurons is activated by bicarbonate. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2041–2046.
- Takigami, S., Osada, T., Yoshida-Matsuoka, J., Matsuoka, M., Mori, Y., and Ichikawa, M. (1999). The expressed localization of rat putative pheromone receptors. *Neurosci. Lett.* 272, 115–118.
- Tian, H., and Ma, M. (2004). Molecular organization of the olfactory septal organ. *J. Neurosci.* 24, 8383–8390.
- Touhara, K. (2007). Deorphanizing vertebrate olfactory receptors: recent advances in odorant-response assays. *Neurochem. Int.* 51, 132–139.
- Tsuboi, A., Miyazaki, T., Imai, T., and Sakano, H. (2006). Olfactory sensory neurons expressing class I odorant receptors converge their axons on an antero-dorsal domain of the olfactory bulb in the mouse. *Eur. J. Neurosci.* 23, 1436–1444.
- Vaidehi, N., Floriano, W. B., Trabanino, R., Hall, S. E., Freddolino, P., Choi, E. J., Zamanakos, G., and Goddard, W. A., III (2002). Prediction of structure and function of G protein-coupled receptors. *Proc. Natl. Acad. Sci. U.S.A.* 99, 12622–12627.
- Vassar, R., Ngai, J., and Axel, R. (1993). Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* 74, 309–318.
- Volz, A., Ehlers, A., Younger, R., Forbes, S., Trowsdale, J., Schnorr, D., Beck, S., and Ziegler, A. (2003). Complex transcription and splicing of odorant receptor genes. *J. Biol. Chem.* 278, 19691–19701.
- Wakabayashi, Y., Mori, Y., Ichikawa, M., Yazaki, K., and Hagino-Yamagishi, K. (2002). A putative pheromone receptor gene is expressed in two distinct olfactory organs in goats. *Chem. Senses* 27, 207–213.
- Walz, A., Feinstein, P., Khan, M., and Mombaerts, P. (2007). Axonal wiring of guanylate cyclase-D-expressing olfactory neurons is dependent on

- neuropilin 2 and semaphorin 3F. *Development* 134, 4063–4072.
- Wang, Z. G., and Ye, R. D. (2002). Characterization of two new members of the formyl peptide receptor gene family from 129S6 mice. *Gene* 299, 57–63.
- Weber, M., Pehl, U., Breer, H., and Strotmann, J. (2002). Olfactory receptor expressed in ganglia of the autonomic nervous system. *J. Neurosci. Res.* 68, 176–184.
- Wekesa, K. S., Miller, S., and Napier, A. (2003). Involvement of G(q/11) in signal transduction in the mammalian vomeronasal organ. *J. Exp. Biol.* 206, 827–832.
- Xie, S. Y., Feinstein, P., and Mombaerts, P. (2000). Characterization of a cluster comprising approximately 100 odorant receptor genes in mouse. *Mamm. Genome* 11, 1070–1078.
- Yamaguchi, M., Yamazaki, K., Beauchamp, G. K., Bard, J., Thomas, L., and Boyse, E. A. (1981). Distinctive urinary odors governed by the major histocompatibility locus of the mouse. *Proc. Natl. Acad. Sci. U.S.A.* 78, 5817–5820.
- Yang, H., Shi, P., Zhang, Y. P., and Zhang, J. (2005). Composition and evolution of the V2r vomeronasal receptor gene repertoire in mice and rats. *Genomics* 86, 306–315.
- Young, J. M., Kambere, M., Trask, B. J., and Lane, R. P. (2005). Divergent V1R repertoires in five species: amplification in rodents, decimation in primates, and a surprisingly small repertoire in dogs. *Genome Res.* 15, 231–240.
- Young, J. M., Shykind, B. M., Lane, R. P., Tonnes-Priddy, L., Ross, J. A., Walker, M., Williams, E. M., and Trask, B. J. (2003). Odorant receptor expressed sequence tags demonstrate olfactory expression of over 400 genes, extensive alternate splicing and unequal expression levels. *Genome Biol.* 4, R71.
- Young, J. M., and Trask, B. J. (2002). The sense of smell: genomics of vertebrate odorant receptors. *Hum. Mol. Genet.* 11, 1153–1160.
- Young, J. M., and Trask, B. J. (2007). V2R gene families degenerated in primates, dog and cow, but expanded in opossum. *Trends Genet.* 23, 212–215.
- Young, J. M., Waters, H., Dong, C., Fülle, H. J., and Liman, E. R. (2007). Degeneration of the olfactory guanylyl cyclase D gene during primate evolution. *PLoS ONE* 2, e884. doi: 10.1371/journal.pone.0000884.
- Zhang, J., and Webb, D. M. (2003). Evolutionary deterioration of the vomeronasal pheromone transduction pathway in catarrhine primates. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8337–8341.
- Zhang, X., and Firestein, S. (2002). The olfactory receptor gene superfamily of the mouse. *Nat. Neurosci.* 5, 124–133.
- Zhang, X., Rodriguez, I., Mombaerts, P., and Firestein, S. (2004). Odorant and vomeronasal receptor genes in two mouse genome assemblies. *Genomics* 83, 802–811.
- Zhang, X., Zhang, X., and Firestein, S. (2007). Comparative genomics of odorant and pheromone receptor genes in rodents. *Genomics* 89, 441–450.
- Zhao, H., Ivic, L., Otaki, J. M., Hashimoto, M., Mikoshiba, K., and Firestein, S. (1998). Functional expression of a mammalian odorant receptor. *Science* 279, 237–242.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 June 2009; paper pending published: 14 July 2009; accepted: 07 August 2009; published online: 27 August 2009.

Citation: Fleischer J, Breer H and Strotmann J (2009) Mammalian olfactory receptors. *Front. Cell. Neurosci.* 3:9. doi: 10.3389/neuro.03.009.2009

Copyright © 2009 Fleischer, Breer and Strotmann. This is an open-access article subject to an exclusive license agreement between the authors and the Frontiers Research Foundation, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited.