# HHS Public Access

Author manuscript

*J Neurochem*. Author manuscript; available in PMC 2015 June 04.

Published in final edited form as:
*J Neurochem*. 2009 June ; 109(6): 1570–1583. doi:10.1111/j.1471-4159.2009.06085.x.

# Olfactory receptors: GPCRs and beyond

**Marc Spehr**[1] and **Steven D. Munger**[2,3]

[1]Department of Cellular Physiology, Ruhr-University Bochum, Germany

[2]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201 USA

## Abstract

Sensing the chemical environment is critical for all organisms. Diverse animals from insects to mammals utilize highly organized olfactory system to detect, encode, and process chemostimuli that may carry important information critical for health, survival, social interactions and reproduction. Therefore, for animals to properly interpret and react to their environment it is imperative that the olfactory system recognizes chemical stimuli with appropriate selectivity and sensitivity. Because olfactory receptor proteins play such an essential role in the specific recognition of diverse stimuli, understanding how they interact with and transduce their cognate ligands is a high priority. In the nearly two decades since the discovery that the mammalian odorant receptor (OR) gene family constitutes the largest group of G protein-coupled receptor (GPCR) genes, much attention has been focused on the roles of GPCRs in vertebrate and invertebrate olfaction. However, is has become clear that the "family" of olfactory receptors is highly diverse, with roles for enzymes and ligand-gated ion channels as well as GPCRs in the primary detection of olfactory stimuli.

## Introduction

The discovery by Buck and Axel nearly two decades ago of a family of odorant receptors (ORs) in rodents (Buck & Axel 1991) was a watershed event for understanding olfactory function. Knowledge of these proteins, which are members of the superfamily of G protein-coupled receptors (GPCRs), not only provided invaluable tools for elucidating the function and organization of the main olfactory system, but they served as a template for identifying other chemosensory receptors in mammals, other vertebrates and invertebrates (Mombaerts 2004a, Imai & Sakano 2008, Keller & Vosshall 2008, Touhara & Vosshall 2009). However, a number of recent reports have greatly expanded the types of receptors implicated in the detection of chemosensory cues by olfactory systems, revealing a structural and functional diversity that was not anticipated even a few years ago.

Animals must detect and respond to a large number of structurally diverse chemical cues in their environment. These chemosignals can convey important information critical for health, survival and reproduction: the type and quality of foods or the existence of toxins; the presence and characteristics of predators, prey, competitors, or potential mates; or social

[3]To whom correspondence should be addressed. smung001@umaryland.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

signals that impact stereotyped behaviors or hormonal responses. Animals employ distinct molecular, cellular and neural mechanisms to detect, discriminate and react to these varied stimuli (Munger *et al.* 2009, Chandrashekar *et al.* 2006, Jordt *et al.* 2003). For example, it has become clear that the olfactory system contains numerous subsystems that can be distinguished by the chemostimuli to which they respond, the chemosensory receptors and other signaling proteins that they express, and their connections to the brain (Munger et al. 2009, Ma 2007, Breer *et al.* 2006) (Figure 1). With this newfound appreciation of the diversity of olfactory subsystems comes the recognition of surprising differences in receptor structure and function that confer an important degree of stimulus selectivity for each subsystem. In this review, we will consider the rapidly expanding repertoire of chemosensory receptor families employed by vertebrate and insect olfactory systems. In particular, we will highlight recent findings in rodents and *Drosophila* indicating that not all olfactory receptors are members of the GPCR superfamily. The reader interested in more comprehensive reviews of the main or accessory olfactory systems, olfactory transduction mechanisms, the roles of olfactory receptors in olfactory development, or insights into the contributions of chemosensory receptors outside the olfactory system can consult any of a number of informative reviews [e.g., (Munger et al. 2009, Tirindelli *et al.* 1998, Firestein 2001, Zufall & Munger 2001, Mombaerts 2004a, Brennan & Zufall 2006, Zufall & Leinders-Zufall 2007, Buck 2000, Gold 1999, Dulac & Torello 2003, Dulac & Wagner 2006, Ache & Young 2005, Imai & Sakano 2008, Keller & Vosshall 2008, Spehr *et al.* 2006, Bargmann 2006)].

## The mammalian olfactory system is composed of several subsystems

The mammalian olfactory system contains a number of distinct subsystems that can be differentiated based on a number of molecular, functional and anatomical criteria (Munger et al. 2009, Ma 2007, Breer et al. 2006). However, the most fundamental division is between the main and accessory olfactory systems (Munger et al. 2009, Ma 2007, Breer et al. 2006). Olfactory sensory neurons (OSNs) of the main olfactory system are located in the main olfactory epithelium (MOE) and send their axons to synapse with second order neurons in the main olfactory bulb (Munger et al. 2009, Firestein 2001). In contrast, sensory neurons in the accessory system are found within the vomeronasal organ (VNO, which lies above the hard palate and at the base of the nasal septum); axons of these vomeronasal sensory neurons (VSNs) project to the accessory olfactory bulb (Munger et al. 2009, Brennan & Zufall 2006). Anatomical and genetic evidence indicates that a functional accessory olfactory system is not maintained in higher primates, including humans (Brennan & Zufall 2006).

These two divisions of the mammalian olfactory system are not homogeneous (Munger *et al.* 2009). The MOE contains a number of sensory neuron subpopulations that respond to distinct types of chemosensory stimuli, express different receptors and/or utilize different transduction cascades. Similarly, the vomeronasal organ contains at least two different groups of chemosensory neurons that vary in stimulus selectivity and mechanisms of chemosensory transduction. In the next section we will discuss the diversity of mammalian olfactory receptors in the context of these different subsystems.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## GPCRs and the detection of olfactory stimuli in mammals

### ORs: the canonical odorant receptors

Prior to the discovery of mammalian ORs, several studies supported a G protein-coupled, cAMP-mediated transduction mechanism for odor detection in the main olfactory system (Pace *et al.* 1985, Pace & Lancet 1986, Sklar *et al.* 1986, Anholt *et al.* 1987, Nakamura & Gold 1987). These findings strongly suggested that mammalian odorant receptors would be members of the GPCR superfamily. In addition, the large repertoire of odorous molecules recognized by the main olfactory system implicated a multigene family of odorant receptors. Based on these criteria, Buck and Axel devised a successful cloning strategy to isolate novel cDNAs exhibiting GPCR homology from the MOE of rats (Buck & Axel 1991). The identified transcripts encoded proteins with key hallmarks of GPCRs, including seven putative membrane-spanning helices, three putative intracellular and three putative extracellular loops connecting these helices, and a conserved DRY amino acid motif (a hallmark of GPCRs) within the second intracellular loop (Buck & Axel 1991, Mombaerts 2004a). The encoded proteins also contain hypervariable regions that likely contribute to selective binding of different odorants (Buck & Axel 1991, Katada *et al.* 2005, Bozza *et al.* 2002, Zhao *et al.* 1998, Krautwurst *et al.* 1998, Abaffy *et al.* 2006, Shirokova *et al.* 2005).

Genome sequencing has confirmed Buck and Axel's initial findings that the ORs are part of a large, multigene family: it is estimated that humans express ~350 functional ORs, while rodents express over 1000 (Buck & Axel 1991, Glusman *et al.* 2001, Zozulya *et al.* 2001, Young *et al.* 2002, Zhang & Firestein 2002, Niimura & Nei 2003)! However, ORs are not restricted to mammals; they are expressed in the OSNs of both terrestrial and aquatic vertebrates (Mombaerts 2004a), including the primitive lamprey (Freitag *et al.* 1999). OR genes can be divided into two primary phylogenetic classes: mammals contain both Class I and Class II OR genes, while the genomes of jawed fishes contain only the more ancient Class I OR genes (Mombaerts 2004a).

Consistent with a role in odor recognition, most OR genes are expressed in OSNs (Buck & Axel 1991, Mombaerts 2004a, Zhang *et al.* 2004, Zhang *et al.* 2007, Feldmesser *et al.* 2006). Individual OSNs each seem to express only a single OR protein, and thus individual OR genes are expressed in only a subset of OSNs (Mombaerts 2004b). While the choice of which OR gene to express appears to be largely stochastic, monogenic (indeed, monoallelic) OR gene expression is regulated in OSNs through a negative feedback mechanism mediated by the OR proteins themselves (Serizawa *et al.* 2003, Lewcock & Reed 2004). Though those OSNs expressing the same OR intermingle with OSNs expressing other ORs, the OSN cohort expressing any one OR is restricted to one of several anatomical zones within the MOE (Mombaerts 2004a). The functional relevance of these OR-expression zones remains unknown.

A number of genes are classified as ORs based on sequence similarity but are expressed outside the MOE, including in the vomeronasal epithelium (Levai *et al.* 2006) and in non-olfactory tissues (Zhang et al. 2007, Feldmesser et al. 2006, Zhang et al. 2004, Tian & Ma 2004, Fleischer *et al.* 2006). While ORs expressed in chemosensory organs like the VNO and the septal organ of Masera (Tian & Ma 2004), part of an olfactory subsystem that shares

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

many functional and molecular biological features with the MOE, likely serve a chemosensory role, other "ectopically-expressed" ORs may have additional chemosensory functions in the body (Spehr *et al.* 2004) or may not function to detect odors at all (De la Cruz *et al.* 2008).

A chemosensory role for many ORs has been confirmed through both *in vitro* and *in vivo* functional studies. ORs confer selective odor responsiveness on either OSNs [e.g., (Zhao et al. 1998, Bozza et al. 2002, Grosmaitre *et al.* 2006, Touhara *et al.* 1999)] or heterologous cells [e.g., (Krautwurst et al. 1998, Abaffy et al. 2006, Kajiya *et al.* 2001, Wetzel *et al.* 1999, Saito *et al.* 2009)]; thus, the OR dictates the stimulus tuning of the OSN. All of the ORs deorphaned to date respond to volatile odorants of diverse chemical classes, and most ORs are broadly tuned to multiple stimuli (Zhao et al. 1998, Krautwurst et al. 1998, Mombaerts 2004a, Abaffy et al. 2006). In addition, multiple ORs can respond to the same odorant, though usually with different efficacies (Malnic *et al.* 1999, Mombaerts 2004a). This is consistent with the observation that most OSNs recognize multiple odorants (Firestein 2001). Thus, odors are encoded using a combinatorial strategy employing multiple ORs of overlapping stimulus selectivity (Malnic *et al.* 1999, Buck 2000). Interestingly, some odors are OR antagonists (Shirokova et al. 2005, Spehr *et al.* 2003, Oka *et al.* 2004) and could therefore increase the complexity of odor coding.

## Trace amine-associated receptors (TAARs)

The MOE is responsive to diverse chemostimuli, including both general odors (e.g., food odors) and semiochemicals (e.g., pheromones) (Munger et al. 2009, Spehr et al. 2006). This stimulus diversity suggested that canonical ORs (Buck & Axel 1991) might not be the only members of the GPCR family serving as chemosensory receptors in the main olfactory system. A screen of OSN-enriched cDNA isolated from mice provided evidence of other GPCRs (Liberles & Buck 2006). These included all but one member of the trace amine-associated receptor (TAAR) family, which are related to other aminergic GPCRs such as metabotropic serotonin and dopamine receptors (Borowsky *et al.* 2001) but which had no established physiological function. The expression of TAARs in small subpopulations of mouse OSNs (Liberles & Buck 2006) suggests an olfactory role.

TAARs are expressed in sparse, nonoverlapping subsets of OSNs within the MOE (Liberles & Buck 2006). Furthermore, although TAAR-expressing OSNs appear to use a similar cAMP-mediated odor transduction cascade to that employed by OR-expressing (i.e., canonical) OSNs, it seems that the expression of these two types of olfactory receptor is mutually exclusive: OSNs expressing both an OR and a TAAR have not been identified (Liberles & Buck 2006). Reminiscent of the distribution of OSNs expressing the same OR, OSNs expressing the same TAAR are restricted within epithelial zones but randomly distributed within those zones (Liberles & Buck 2006).

As predicted (Borowsky et al. 2001), many mouse TAARs respond to biogenic amines including isoamylamine, β-phenylethylamine and trimethylamine (ligands for the mouse TAAR3, TAAR4 and TAAR5 proteins, respectively). TAAR5 also responds to adult male mouse urine (Liberles & Buck 2006), consistent with a role in the detection of conspecific olfactory signals. Other chemosensory receptors implicated in the detection of conspecific

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

olfactory signals (e.g., vomeronasal receptors and the receptor guanylyl cyclase GC-D; see below) are largely pseudogenized in humans and other higher primates (Young *et al.* 2005, Young *et al.* 2007). In contrast, numerous intact TAAR genes are found in diverse vertebrate genomes, including the zebrafish, chicken, platypus and human genomes (Liberles & Buck 2006, Gloriam *et al.* 2005, Mueller *et al.* 2008, Grus & Zhang 2008). The maintenance of apparently functional TAAR genes across such diverse species suggests a common olfactory role.

## Vomeronasal type 1 receptors (V1Rs)

After identification of the OR multigene family (Buck & Axel 1991), initial attempts to uncover putative VNO-specific chemoreceptor genes based on homology with ORs were unsuccessful. In 1995, Dulac and Axel (Dulac & Axel 1995) employed a different approach. Based on the hypothesis that individual VSNs express a single or a neuron-specific subset of receptors, single rat VSN cDNA libraries were differentially screened for putative GPCRs. This elegant experimental strategy led to the discovery of the *V1r* multigene family (Dulac & Axel 1995); in mouse, this family contains over 130 members with uninterrupted, full-length open reading frames as well as over 150 likely pseudogenes (Rodriguez *et al.* 2002). *In situ* hybridization studies revealed a punctate, non-overlapping expression pattern restricted to the apical layer of the VNO sensory epithelium (Dulac & Axel 1995). V1R-expressing VSNs also express the G protein subunit $G\alpha_{i2}$ (Halpern *et al.* 1995, Berghard & Buck 1996), which has been hypothesized to play a role in sensory transduction in these cells (Mombaerts 2004a). Although ORs and V1Rs share no significant sequence homology, both are Class-A (i.e., rhodopsin-like) GPCRs. Further common features include an intron-free coding region, a scattered, largely clustered chromosomal organization (Rodriguez et al. 2002), tightly controlled monogenic (Rodriguez *et al.* 1999) and monoallelic (Roppolo *et al.* 2007) expression, and a punctuate tissue expression pattern consistent with the ‘one neuron – one (or a few) receptor(s)’ hypothesis (Mombaerts 2004b).

A hallmark of the V1R superfamily is its unusual diversity: within each of the 12 extremely isolated gene subfamilies/clades (each containing between one and 30 members) sequence identity is at least 40%, but interfamily homology is less than 15%. Conserved sequence motifs are largely missing. This interfamily divergence is in sharp contrast to the smooth continuum in OR sequence variability (Dulac & Wagner 2006). Intact *V1r* genes are found in species ranging from teleost fish to man (Dulac & Wagner 2006, Rodriguez & Mombaerts 2002, Shi & Zhang 2007, Pfister & Rodriguez 2005, Saraiva & Korsching 2007). While *V1r*-like genes in different teleost species appear highly conserved, mammals display high across-species variability in both total *V1r* gene count and primary sequence (Grus et al. 2005, Grus *et al.* 2007). Primates, including humans, appear to have experienced the most substantial degeneration of the functional *V1r* repertoire. For example, among ~200 identified human *V1r*-like sequences, only five retain an intact open reading frame (Rodriguez & Mombaerts 2002). Although one human receptor, V1RL1, is expressed in human olfactory epithelium (Rodriguez *et al.* 2000), it remains unclear whether human V1Rs simply represent non-functional remnants of a massively decayed V1R repertoire (Mombaerts 2004a).

Author Manuscript



Gene targeting of one *V1r* gene, *V1rb2* (previously designated $VR_i2$) (Rodriguez *et al.* 1999), has been useful in understanding the monoallelic expression of V1r genes, the axonal wiring and convergence patterns of V1R-expressing VSNs, and the stimulus tuning of individual V1Rs. For example, $Ca^{2+}$ imaging and patch-clamp recordings from GFP-tagged V1Rb2-expressing VSNs identified 2-heptanone – a urinary constituent shown to extend the length of female estrous cycles (Brennan & Zufall 2006, Novotny 2003) – as an *in-situ* ligand for V1Rb2 (Boschat *et al.* 2002). Recently, the same mouse strain was employed for a comprehensive biophysical characterization of the ionic properties of a defined subpopulation of apical VSNs (Ukhanov *et al.* 2007). All other vertebrate V1Rs remain orphan receptors whose putative chemosensory function can only indirectly be inferred from their expression in VSNs and their influence on stereotyped behaviors. For example, the deletion of 16 V1R genes in a single cluster on mouse chromosome 6 resulted in specific deficits in mating behavior and maternal aggression (Del Punta et al. 2002).

What stimuli activate V1Rs? The role of the VNO in mediating several stereotyped responses in mice (e.g., male aggression, puberty acceleration or estrus synchronization) suggests that semiochemicals are good candidates (Brennan & Zufall 2006). Indeed, studies combining functional single cell imaging and cell-attached patch-clamp recordings observed specific and highly sensitive sensory responses to seven putative mouse pheromones, including compounds isolated from mouse urine or glandular secretions (Novotny 2003), in non-overlapping VSN subpopulations (Leinders-Zufall *et al.* 2000). Although recent experimental results suggest that urine-dependent sensory signals in the VNO are predominantly generated by apical (i.e., $G\alpha_{i2}$/V1R-expressing) VSNs neurons (Holekamp *et al.* 2008) and that sulfated steroids represent a major source of this urinary activity (Nodari *et al.* 2008), other data show robust sensory activation in basal (i.e., $G\alpha_o$-expressing) VSNs as well (Kelliher *et al.* 2006, Leinders-Zufall *et al.* 2004b, He *et al.* 2008).

## Vomeronasal type 2 receptors (V2Rs)

The topographically restricted expression of V1Rs in the apical, $G\alpha_{i2}$-expressing layer of the VNO neuroepithelium (Dulac & Axel 1995) strongly suggested that one or more additional families of vomeronasal receptors are expressed in the VNO, likely in the basal neurons that express $G\alpha_o$. Indeed, three different groups simultaneously reported the identification of a second multigene family of VNO-specific GPCRs (Herrada & Dulac 1997, Matsunami & Buck 1997, Ryba & Tirindelli 1997). Exclusively expressed in $G\alpha_o$-positive VSNs, these receptors were named V2Rs. The Buck and Dulac groups both employed a differential screening approach in which they compared gene expression profiles of single VNO neurons from mouse (Matsunami & Buck 1997) or rat (Herrada & Dulac 1997). Ryba and Tirindelli (Ryba & Tirindelli 1997), on the other hand, used a degenerate rat OR probes to search for novel vomeronasal receptors (a single V2R cDNA was identified serendipitously by this approach even though V2Rs share no significant sequence homology with ORs). Approximately 120 intact *V2r* genes (and an additional ~160 apparent pseudogenes) are present in the mouse genome (Young & Trask 2007).

As with other Class C GPCRs including metabotropic glutamate receptors, $Ca^{2+}$-sensing receptors, and sweet/umami-sensing taste receptors, V2Rs have a large hydrophobic amino

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



(N)-terminal extracellular domain and a seven transmembrane domain, joined by a cysteine-rich linker domain (Mombaerts 2004a, Pin *et al.* 2003). Although this N-terminal domain is the primary site of ligand binding to most Class C GPCRs, allosteric interactions of some ligands with the transmembrane domain have been reported (Lagerstrom & Schioth 2008, Vigues *et al.* 2008).

As with genes encoding the ORs and V1Rs, *V2rs* are found in clusters on most chromosomes and can be grouped into distinct subfamilies [family-A, -B, -D, and -C (synonymously named *V2r2* family)] (Young & Trask 2007, Silvotti *et al.* 2007). With over 100 members, family-A represents approximately 80% of all *V2r* genes (Young & Trask 2007, Yang *et al.* 2005). On the other extreme, family-D includes only 4 members (Silvotti *et al.* 2007). The *V2r* gene repertoire is relatively large in teleosts (Shi & Zhang 2007). The intact *V1r:V2r* ratio, however, is dramatically increased in tetrapods, paralleling the simultaneous evolutionary jump in the class II : class I ratio of vertebrate OR genes. In some mammalian species (including human, chimpanzee, macaque, cow and dog), *V2r* repertoires have completely degenerated (Young & Trask 2007).

Most V2Rs show a punctate expression pattern in the VNO, likely generated by stochastic selection and subsequent negative feedback on the expression of other family members (mutual and allelic exclusion). An exception is the V2R2 (family-C) receptors, which are expressed in most, if not all, $G\alpha_o$-positive VSNs (Martini *et al.* 2001), though individual V2R2 members appear to be expressed in segregated non-overlapping VSN subsets (Silvotti *et al.* 2007). In mammalian species marked by substantial *V2r* degeneration, a single *V2r2* orthologue is commonly found. Few inactivating mutations indicate a relatively recent pseudogenization process and, thus, a likely conserved function across species (Young & Trask 2007). The unusual characteristics of *V2r2* genes are reminiscent of the atypical insect odorant receptor (OR83b in *Drosophila* (Larsson *et al.* 2004)) that acts as a heterodimeric partner to other insect ORs (see discussion of *Drosophila* ORs below). Or83b and its orthologues are required for proper membrane targeting and function of coexpressed ORs, thus suggesting an analogous role for V2R2 proteins in vomeronasal chemoreception. Individual V2R-expressing basal VSNs also express one of nine members of a family of nonclassical class Ib major histocompatibility complex (MHC) genes (Loconto *et al.* 2003, Ishii *et al.* 2003). The proteins encoded by these *H2-Mv* genes, in complex with β2-microglobulin, have been implicated in V2R trafficking and surface expression (Dulac & Torello 2003). However, it appears that a substantial fraction of basal VSNs tolerate absence of MHC Ib escort molecule for proper V2R membrane targeting (Ishii & Mombaerts 2008, Silvotti *et al.* 2007). Moreover, it has recently become apparent that the *H2-Mv* family is restricted to rodents (Shi & Zhang 2007).

As is the case for V1Rs, direct functional information for V2R receptor-ligand interactions is sparse. One popular model hypothesizes that the anatomical and molecular dichotomy of the vomeronasal system is reflected by distinct chemical properties of V1R- and V2R-specific ligands (Dulac & Torello 2003, Brennan & Zufall 2006) such that V1Rs respond to small, relatively volatile molecules while V2Rs are activated by peptides or proteins. Major urinary proteins (MUPs, which are members of the lipocalin family) have long been intriguing candidates as VSN stimuli (Cheetham *et al.* 2007, Sherborne *et al.* 2007, Dulac &

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Torello 2003), but whether they function as carriers of small organic ligands or as receptor ligands themselves has been a longstanding question. A recent functional imaging study revealed that purified MUPs alone are sufficient to activate dissociated $G\alpha_o$-positive VSNs and trigger robust male–male territorial aggression (Chamero *et al.* 2007). Other mammalian species, however, possess only a single intact MUP gene, challenging the general applicability of these findings.

Other potential V2R receptor ligands identified thus far are either genetically encoded or metabolically processed peptides. For example, exocrine gland-secreting peptide 1 (ESP1) stimulates basal V2R-expressing VSNs as measured by c-Fos immunohistochemistry and field potential recordings at the epithelial surface of the VNO (Kimoto *et al.* 2005, Kimchi *et al.* 2007). The product of one of 24 related genes in mouse, this peptide is secreted from the male extraorbital lacrimal gland (Kimoto *et al.* 2005). Intriguingly, some ESPs appear to be expressed in a sexually dimorphic pattern. There are only ten putative ESP genes in rat, possibly one in opossum, and none in human (Kimoto *et al.* 2007), suggesting species-specific functions for these proteins.

It has long been recognized that mice are able to both discriminate the body odors of conspecifics that differ genetically only in their MHC haplotypes and to translate this olfactory individuality code into ethologically relevant information (Beauchamp *et al.* 1985, Yamazaki *et al.* 1983, Penn & Potts 1998, Restrepo *et al.* 2006). These observations suggested that MHC class I peptides, small peptides that are bound to and presented by MHC proteins at the cell surface, could represent one molecular component of this MHC ‘odor’ (Leinders-Zufall et al. 2004a). MHC class I peptides, indeed, activate V2R-expressing VSNs *in-situ* and are also able to generate a robust olfactory memory sufficient to trigger pregnancy failure (the Bruce effect) (Leinders-Zufall et al. 2004a, Brennan & Zufall 2006). In this scenario, however, both volatile compounds and MHC peptides most likely play complementary roles (Restrepo et al. 2006).

## The role of non-GPCRs in the detection of olfactory stimuli

The discovery of the ORs by Buck and Axel fit nicely with the extensive biochemical and molecular biological evidence implicating a G protein-coupled, cAMP-mediated odor-transduction mechanism in mammals and other vertebrates. We have already discussed how diverse olfactory GPCRs confer an exquisite sensitivity on the main and accessory olfactory systems to a broad range of volatile and non-volatile sensory stimuli. However, findings in both rodents and insects now indicate that olfactory systems may also employ a number of non-GPCRs to detect chemosensory stimuli.

### Receptor Guanylyl Cyclases

Both ORs and TAARs seem to transduce olfactory stimuli through a signaling mechanism that employs G proteins and the second messenger cAMP (Munger et al. 2009). Identification of an orphan receptor guanylyl cyclase (GC) expressed in a small number of OSNs (Fulle *et al.* 1995), called GC-D, suggested that the repertoire of olfactory receptors might not be restricted to GPCRs. The receptor GC family includes peptide and orphan receptors expressed in numerous species and tissues (Gibson & Garbers 2000). First isolated

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from sea urchin sperm, receptor GCs have been implicated in diverse functions including invertebrate sperm chemotaxis, the regulation of natriuresis and diuresis, mammalian phototransduction and nematode chemosensation (Gibson & Garbers 2000, Kuhn 2009). These proteins share a common structure, including an extracellular receptor domain, an intracellular regulatory (kinase homology) domain, and an intracellular catalytic domain (Gibson & Garbers 2000, Kuhn 2009). In mammals, deorphaned receptor GCs respond to various natriuretic peptides (Kuhn 2009). Peptide binding to the extracellular domain leads to activation of the intracellular cyclase domain and the elevation of intracellular cGMP. The activity of some receptor GCs may also be regulated by intracellular proteins such as $Ca^{2+}$-inhibited GCAPs (guanylyl cyclase activating proteins) (Kuhn 2009).

Until recently, little was known about the function of the GC-D protein or the olfactory role of the OSNs that express it. The observation that the gene encoding GC-D is intact in rodents, canines and some prosimians (though not in other primates) suggests a conserved function across many mammalian species (Young *et al.* 2007). GC-D-expressing OSNs contain several proteins that could participate in a cGMP-mediated cascade (including GC-D, the cGMP-stimulated phosphodiesterase PDE2 and the cGMP-gated channel subunit CNGA3) but lack many of the proteins required for cAMP-mediated odor transduction by canonical OSNs (Juilfs *et al.* 1997, Meyer *et al.* 2000, Munger *et al.* 2008, Luo 2008). GC-D-expressing OSNs, like those expressing individual OR or TAAR types, comprise a small percentage of the OSNs in the MOE and are restricted to an epithelial zone (Fulle et al. 1995, Juilfs *et al.* 1997, Walz *et al.* 2007). In contrast to these other OSN populations, however, GC-D-expressing OSNs are not randomly distributed throughout this zone: they can be found in clusters within the dorsal recesses of the nasal cavity or more sparsely distributed in other areas of the MOE (Fulle et al. 1995, Juilfs et al. 1997, Walz et al. 2007, Cockerham *et al.* 2009).

Functional studies in gene-targeted mice and in heterologous cells now indicate that GC-D, and the neurons that express it, are essential for the detection and transduction of certain peptide stimuli by the main olfactory system (Leinders-Zufall *et al.* 2007, Duda & Sharma 2008). Field potential recordings from wildtype or *Cnga2* null mice (which lack responses to general odors recognized by ORs) show that the mouse MOE responds to uroguanylin and guanylin (Leinders-Zufall et al. 2007), two natriuretic peptides involved in the regulation of salt and fluid balance in the gut and kidney (Forte 2004). However, they are completely abolished in the MOE of mice in which the *Gucy2d* gene (which encodes GC-D) or *Cnga3* gene has been deleted (Leinders-Zufall et al. 2007). Patch-clamp electrophysiological recordings and $Ca^{2+}$-imaging from identified OSNs expressing GC-D confirmed the requirement of GC-D for uroguanylin and guanylin responses in the MOE (Leinders-Zufall et al. 2007). These responses are highly effective and specific: $K_{1/2}$ values were as low as 66 pM, while some other receptor GC ligands [e.g., urodilatin, heat-stable enterotoxin; (Forte 2004)] did not activate the MOE. GC-D-expressing OSNs also respond to mouse urine (Leinders-Zufall et al. 2007), a rich source of chemostimuli (Brennan & Zufall 2006) that contains uroguanylin (Forte 2004).

But is GC-D itself a chemosensory receptor in this subpopulation of OSNs? Several lines of evidence suggest that it is. First, other receptor GCs function as peptide receptors (Gibson &

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Garbers 2000, Kuhn 2009). Second, all GC-D-expressing OSNs respond to a mixture of uroguanylin and guanylin, but OSNs that don't express GC-D (either normally or through genetic deletion) are unresponsive to these peptides (Leinders-Zufall et al. 2007). Third, guanylyl cyclase activity of the rat GC-D isoform is stimulated by uroguanylin (but not guanylin) when expressed in heterologous cells (Duda & Sharma 2008). The specificity of rat GC-D for uroguanylin alone was not wholly surprising, as GC-D-expressing OSNs in mice are differentially tuned to the two peptide stimuli: though some are activated by both peptides, others are activated by only uroguanylin or guanylin (Leinders-Zufall et al. 2007). Together, these findings suggest that stimulus tuning of the GC-D protein can be regulated (e.g., by differential splicing, posttranslational modifications, or the presence of "co-receptor" proteins). Other mammalian receptor GCs function as homodimers (Gibson & Garbers 2000, Kuhn 2009), but heterodimerization remains a possibility.

Evidence for intracellular modulation of GC-D activity is limited, but growing. Studies in heterologous cells and in rat MOE extracts from Sharma and colleagues (Duda *et al.* 2001, Duda *et al.* 2004) suggest that the $Ca^{2+}$-binding protein neurocalcin-δ mediates a $Ca^{2+}$-dependent stimulation of GC-D cyclase activity. These authors suggest that elevation of intracellular $Ca^{2+}$ upon activation of OSNs by general odors will stimulate GC-D activity as part of a general transduction pathway. However, such a model assumes that GC-D is expressed in many, if not most, OSNs, and is in conflict with numerous immunohistochemical, *in situ* hybridization, gene-targeting and functional studies in mouse and rat that support the restricted expression of GC-D in just a small subpopulation of OSNs (Fulle et al. 1995, Juilfs et al. 1997, Meyer et al. 2000, Leinders-Zufall et al. 2007, Hu *et al.* 2007, Walz et al. 2007). A more likely source of intracellular $Ca^{2+}$ is the cGMP-dependent opening of cyclic nucleotide-gated channels present in these cells (Meyer et al. 2000). Indeed, specific stimulation of GC-D neurons promotes an increase in $Ca^{2+}$ within the dendritic knobs of these cells that is sensitive to the cyclic nucleotide-gated channel blocker l-cis-diltiazem (Leinders-Zufall et al. 2007, Hu et al. 2007).

A second mechanism for intracellular regulation of GC-D is likely. GC-D expressing neurons are also sensitive $CO_2$ sensors (Hu et al. 2007). $CO_2$ responses in GC-D-expressing OSNs are mediated by $CO_2$ catalyzing enzyme carbonic anhydrase type II (CAII), which is specifically expressed in this group of OSNs: $CO_2$ responses in GC-D-expressing neurons are blocked by a $CO_2$ inhibitor, while behavioral responses are perturbed in *Car2* null mice, which lack CAII (Hu et al. 2007). Sensitivity of $CO_2$–dependent $Ca^{2+}$ responses in GC-D-expressing neurons to l-cis-diltiazem suggests that both $CO_2$ and the natriuretic peptides uroguanylin and guanylin are transduced through a final common pathway, perhaps both employing GC-D (Leinders-Zufall et al. 2007, Hu et al. 2007). Recent findings that bicarbonate, a product of $CO_2$ metabolism by carbonic anhydrase, can stimulate GC-D cyclase activity are consistent with this model (Sun *et al.* 2009). Together, these studies indicate that GC-D acts both as a chemosensory receptor and an effector of sensory transduction. Furthermore, the CAII enzyme could also be considered a specialized olfactory receptor.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## *Drosophila* olfactory receptors: the ORs

*Drosophila* and other insects possess multiple olfactory sensory structures that could help define olfactory subsystems in these species, as well. Chemosensory sensilla containing OSNs are found on both the distal segment of the antenna and on the maxillary palp (Laissue & Vosshall 2008). Each sensillar type (basiconic, trichoid or coeloconic on the antenna; basiconic only on the maxillary palp) contains unique complements of OSNs that differ in the receptors they express, the stimuli to which they respond, and their axonal targets in the antennal lobe. For example, OSNs in basiconic sensilla are largely responsive to food odors, while OSNs in trichoid sensilla appear to be pheromone-responsive (Laissue & Vosshall 2008). However, while the concept of olfactory subsystems may be reminiscent of the mammalian olfactory system, insects exhibit one key difference: the receptors used to recognize olfactory stimuli appear to be ion channels, not (or not only) GPCRs.

After numerous attempts to identify insect olfactory receptors based on homology to mammalian ORs or nematode olfactory receptors (both of which are GPCRs), several groups took advantage of the publication of the *Drosophila* genome to identify a family of 62 putative olfactory receptors in this species with a combination of *in silico* analysis (Vosshall *et al.* 1999, Clyne *et al.* 1999, Gao & Chess 1999) and differential screening (Vosshall *et al.* 1999). Thirteen of these ORs are specifically expressed in larval OSNs (Fishilevich *et al.* 2005, Kreher *et al.* 2005, Stocker 2008). Orthologous receptors were subsequently identified in numerous other insect species (Sato & Touhara 2008, Bohbot *et al.* 2007, Engsontia *et al.* 2008, Hill *et al.* 2002, Krieger *et al.* 2002, Nakagawa *et al.* 2005, Robertson & Wanner 2006, Wanner *et al.* 2007). Even though the *Drosophila* ORs showed no obvious homology to GPCRs other than a predicted seven-transmembrane structure, the precedent for GPCR-type olfactory receptors in both vertebrate and invertebrate species was strong (Mombaerts 2004a).

Indeed, there are obvious similarities between *Drosophila* and mammalian ORs. The expression of most *Drosophila* ORs is reminiscent of the punctate arrangement seen in canonical OSNs and VSNs of the mouse: of those ORs expressed in adult OSNs, each is expressed in a subset in either the antenna or maxillary palp, with all but OR35a expressed in basiconic and/or trichoid OSNs (Couto *et al.* 2005, Fishilevich & Vosshall 2005, Sato & Touhara 2008, Spletter & Luo 2009). However, unlike most mammalian OSNs, some *Drosophila* OSNs simultaneously express up to four distinct OR genes (Couto et al. 2005, Fishilevich & Vosshall 2005, Goldman *et al.* 2005). Therefore, the control of OR expression is unlikely to be conserved between insects and mammals.

One *Drosophila* OR family member, OR83b, is expressed in all OSNs that express at least one other OR (Vosshall et al. 1999, Larsson et al. 2004). OR83b orthologues are found in other insect species as well (Sato & Touhara 2008). OR83b, which heterodimerizes with other ORs (Neuhaus *et al.* 2005, Benton *et al.* 2006), is required for normal functioning of ORs and OR-expressing OSNs (Larsson et al. 2004, Laissue & Vosshall 2008, Sato & Touhara 2008, Benton et al. 2006). Two subpopulations of *Drosophila* OSNs do not express OR83b: the majority of coeloconic OSNs (see next section), and a small group of $CO_2$-sensitive basiconic OSNs. Surprisingly, the $CO_2$-sensitive receptors expressed in this latter

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

subpopulation, GR21a and GR63a, are members of the gustatory receptor family (Jones *et al.* 2007, Kwon *et al.* 2007).

It was noted that insect OSNs do not contain typical GPCR motifs. Epitope mapping of OR83b indicated that this protein (and likely other insect ORs) had a flipped topology in comparison with GPCRs, with an intracellular N-terminus and extracellular C-terminus (Benton et al. 2006). This intriguing observation suggested that insect ORs might not be GPCRs at all. Two studies, which were published side-by-side, recently provided strong support for this hypothesis. The first (Sato *et al.* 2008) concluded that insect ORs are heteromeric ligand-gated ion channels in which the unique subunit (i.e., that which is not OR83b) provides for ligand selectivity of the complex. Several lines of evidence using ORs from *Drosophila* and other insect species support this model. First, stimulation of heteromeric ORs (e.g., *Drosophila* OR47a/OR83b) expressed in heterologous cells resulted in $Ca^{2+}$ influx via a non-selective cation conductance dependent on OR83b. Pharmacological treatments showed that odor responses were independent of G protein signaling, cyclic nucleotide or phosphoinositide signaling cascades, and both ATP and GTP. Analyses of single channel currents in excised patches confirmed that heteromeric insect ORs act as ligand-gated ion channels.

However, a contemporaneous study (Wicher *et al.* 2008) came to a somewhat different conclusion. Patch clamp recordings in whole cell and excised patch configurations from heterologous cells expressing OR22a and OR83b revealed a fast odor-dependent response independent of ATP and GTP (a likely ionotropic response) as well as a slower ATP- and GTP-dependent component. In contrast to the study by Sato and colleagues (Sato *et al.* 2008), pharmacological analyses performed by Wicher and colleagues (Wicher *et al.* 2008) indicate that the later component of the odor response is mediated by a G protein-dependent signaling cascade that includes $G\alpha_s$, adenylyl cyclase and cAMP. For example, odor stimulation activated co-expressed, cAMP-sensitive ion channels. Furthermore, the G protein inhibitor GDP-β-S increased the $EC_{50}$ for odor stimulation. Interestingly, odor induced cAMP signaling required OR22a (or OR47a, which was also tested) but was independent of OR83b. In contrast, cAMP (but not odors) induced a current in cells expressing only OR83b. Mutation of predicted channel pore residues in OR83b reduced the cAMP-dependent current. Together, the results of this study suggest a model in which the unique OR subunit contributes ligand selectivity and G protein coupling, and can mediate fast activation of the OR83b-dependent conductance; OR83b is also stimulated by cAMP, but on a slower time course.

How do we reconcile these two studies? It has been noted that the two studies focused on different parts of the odor response, and could thus each be seeing only part of the picture (Chesler & Firestein 2008). Indeed, Sato and colleagues (Sato *et al.* 2008) do report a small, ligand-independent sensitivity of some receptor complexes to cyclic nucleotides. Clearly, resolution of this potential conflict requires further investigation.

### *Drosophila* olfactory receptors: the IRs

Though the mechanisms of odorant detection by insect ORs had become slightly clearer, a major gap remained: olfactory receptors utilized by most coeloconic OSNs remained

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



unidentified (the exception being the coeloconic OSN population expressing OR35a/OR83b). Thus, the recent report of a third major class of insect chemosensory receptors (Benton *et al.* 2009) expressed in coeloconic, but not trichoid or basiconic OSNs, was not surprising. However, the protein family to which these receptors belong most certainly was. Building on results from an earlier bioinformatic screen for genes expressed in the olfactory system (Benton *et al.* 2007), Benton, Vosshall and colleagues identified a group of six genes encoding proteins related to ionotropic glutamate receptors (iGluRs) such as AMPA and NMDA receptors (Benton *et al.* 2009). Further bioinformatic analyses revealed these genes to be part of a large family (61 predicted intact genes and two pseudogenes) dubbed the IRs (ionotropic receptors) (Benton *et al.* 2009).

Several factors establish the IRs as a novel class of odorant receptors (Benton et al. 2009, Spletter & Luo 2009). Most obviously, they are not members of the GPCR superfamily. But furthermore, unlike the insect ORs and most mammalian olfactory receptors (ORs, TAARs, V1Rs and V2Rs), the IRs are not members of the even larger class of seven transmembrane domain proteins. Instead, they appear to have three membrane-spanning segments and a pore-forming domain; this topology is an obvious similarity to iGluRs. An apparent structural similarity between IRs and iGluRs is also suggested in the bipartite ligand-binding domain (Benton *et al.* 2009). However, it appears that glutamate is not the cognate ligand for most or any *Drosophila* IRs. The putative ligand-binding domains of individual IRs are highly variable, with little evidence for conservation of key residues important for glutamate binding in iGluRs (Benton *et al.* 2009). This hypervariability in areas likely to interact with olfactory stimuli is reminiscent of the hypervariable regions seen in putative ligand-binding pockets of the mammalian ORs (Buck & Axel 1991, Mombaerts 2004a), and suggests that the IR family responds to a number of distinct stimuli.

Coeloconic odorant receptors should meet several criteria, including: expression in the dendrites of coeloconic OSNs; an expression pattern consistent with coeloconic OSN functional subtypes (Yao *et al.* 2005); and the ability to confer odor sensitivity in a predictable way upon ectopic expression in novel coeloconic OSNs. Indeed, the IRs meet these criteria. *In situ* hybridization experiments in *Drosophila* antenna using probes to any of several IR genes as well as OR83b revealed that IRs are expressed in coeloconic OSNs or in neurons of the arista and sacculus (two specialized antennal structures); they are not expressed in basiconic or trichoid OSNs (Benton *et al.* 2009). IRs are not coexpressed with ORs, with the exception of one single coeloconic OSN that expresses IR76b, OR83b and OR35a. Antibodies recognizing IR25a, which is broadly expressed in several coeloconic OSNs as well as in the arista and sacculus, revealed immunoreactivity enriched in the outer dendrites, the site of olfactory transduction (Benton *et al.* 2009).

Interestingly, systematic double-label *in situ* hybridizations revealed stereotypical patterns of IR expression. Certain IRs are always expressed in adjacent coeloconic OSNs, establishing four distinct clusters of IR-expressing OSNs (Benton *et al.* 2009). This expression pattern was reminiscent of four coeloconic OSN subtypes previously defined by their distinct, though overlapping, stimulus tuning profiles (Yao et al. 2005). Indeed, the maps of OSN clusters, defined by unique IR expression profiles, and functional maps, established by recording electrophysiological responses to odors diagnostic of OSN

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

functional subtypes, were highly correlated (Benton *et al.* 2009). To what extent individual IRs contribute to odor recognition remains unclear. Coeloconic OSNs express up to three IR genes, suggesting that IRs, like iGluRs, could function as heteromeric receptors. Individual IRs may also serve distinct functions, including ligand recognition, stimulus transduction or receptor trafficking. For example, experiments in which three IRs normally expressed in phenylacetaldehyde-sensitive OSNs, IR75d, IR76a and IR84a, were ectopically expressed in phenylacetaldehyde-insensitive OSNs showed that only IR84a expression conferred phenylacetaldehyde sensitivity on the targeted OSNs (Benton *et al.* 2009). However, the specific chemosensory functions of most IRs remain to be determined.

## Conclusions

The structural and functional diversity of olfactory receptors is far greater than what was anticipated even a decade ago. Do any other olfactory receptor families remain unidentified? In mammals, at least, this seems likely. The rodent olfactory system contains several subsystems for which chemosensory receptors have not been established. Some of these subsystems may utilize recognized olfactory receptor families. For example, ORs (Fleischer et al. 2006), TAARs (Fleischer *et al.* 2007) and GC-G (a paralogue of GC-D) (Fleischer *et al.* 2009) are expressed in neurons of the Grueneberg ganglia, which mediates the response to alarm pheromone (Brechbuhl *et al.* 2008) and is responsive to cool temperatures (Mamasuew *et al.* 2008). However, distinct sensory neuron subpopulations in the MOE that appear to use cyclic nucleotide-independent transduction mechanisms could employ novel olfactory receptors. These putative OSNs for which no olfactory receptor has been identified include one population expressing the ion channel TRPM5 and effector enzyme phospholipase C β2 (Lin *et al.* 2007), and expressing olfactory marker protein but not the cAMP-specific phosphodiesterase 4A that innervates necklace glomeruli (the MOB target of GC-D-expressing OSNs) (Cockerham et al. 2009, Juilfs et al. 1997). The possibility that the VNO epithelium displays an unrecognized cellular (and receptor) diversity on par with that of the MOE remains to be seen.

Why do animals require so many different olfactory receptor families? A remote possibility is that the diversity of receptor families is simply a byproduct of evolutionary chance. This seems unlikely, as there is strong evidence for adaptive evolution of vertebrate ORs, TAARs, V1Rs and V2Rs (Kambere & Lane 2007) as well as for insect ORs [e.g., (McBride *et al.* 2007, Nozawa & Nei 2007)]. Instead, multiple olfactory receptor families may help animals derive critical information from a complex chemosensory world. Most odors are complex mixtures of chemicals. In biologically important sources of chemosensory cues such as urine, feces and glandular secretions, the classes of compounds that can serve as olfactory stimuli can be quite diverse (e.g., gases, volatile compounds, peptides and proteins). As different types of receptors seem to be preferentially tuned to certain types of stimuli (e.g., TAARs respond to trace amines, GC-D is stimulated by peptides), a large repertoire of diverse olfactory receptor families can increase the range of chemical sensitivity in the olfactory system. However, this is not to say that stimulus selectivity is not tightly controlled. The restricted expression of specific olfactory receptor families to one or a few olfactory subsystems (Munger et al, 2009; Spletter and Luo, 2009) affords the opportunity to extract different kinds of information from the same olfactory stimulus. For

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

example, mice in which the dorsal aspects of the main olfactory system have been genetically-disrupted fail to avoid, but can still detect, odorants associated with predators or with spoiled food (Kobayakawa *et al.* 2007). Results such as these indicate the kind of information derived from the detection of a particular chemostimulus depends on the olfactory subsystem detecting it. Therefore, the use of diverse olfactory receptor families can increase both stimulus sensitivity and selectivity of the olfactory system.

## Acknowledgments

Work in the authors' laboratories is supported by the National Institute on Deafness and Other Communication Disorders (DC005633, DC005786; SDM) and by the Emmy Noether-Program of the Deutsche Forschungsgemeinschaft (SP724/2-1; MS) and grants from the Mercator Foundation (MS) and the Volkswagen Foundation (MS).

## Abbreviations used

| | |
|---|---|
| **GPCR** | G protein-coupled receptor |
| **OR** | odorant receptor |
| **OSN** | olfactory sensory neuron |
| **MOE** | main olfactory epithelium |
| **VNO** | vomeronasal organ |
| **VSN** | vomeronasal sensory neuron |
| **TAAR** | trace amine-associated receptor |
| **V1R** | vomeronasal type 1 receptor |
| **V2R** | vomeronasal type 2 receptor |
| **MHC** | major histocompatibility complex |
| **MUP** | major urinary protein |
| **ESP** | exocrine gland-secreting peptide |
| **GC** | guanylyl cyclase |
| **GC-D** | receptor guanylyl cyclase type D |
| **CAII** | carbonic anyhydrase type II |
| **IR** | ionotropic receptor |

## References

Abaffy T, Matsunami H, Luetje CW. Functional analysis of a mammalian odorant receptor subfamily. J Neurochem. 2006; 97:1506–1518. [PubMed: 16606354]

Ache BW, Young JM. Olfaction: diverse species, conserved principles. Neuron. 2005; 48:417–430. [PubMed: 16269360]

Anholt RR, Mumby SM, Stoffers DA, Girard PR, Kuo JF, Snyder SH. Transduction proteins of olfactory receptor cells: identification of guanine nucleotide binding proteins and protein kinase C. Biochemistry. 1987; 26:788–795. [PubMed: 3105575]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bargmann CI. Comparative chemosensation from receptors to ecology. Nature. 2006; 444:295–301. [PubMed: 17108953]

Beauchamp GK, Yamazaki K, Wysocki CJ, Slotnick BM, Thomas L, Boyse EA. Chemosensory recognition of mouse major histocompatibility types by another species. Proc Natl Acad Sci U S A. 1985; 82:4186–4188. [PubMed: 3858876]

Benton R, Sachse S, Michnick SW, Vosshall LB. Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo. PLoS Biology. 2006; 4:e20. [PubMed: 16402857]

Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell. 2009; 136:149–162. [PubMed: 19135896]

Benton R, Vannice KS, Vosshall LB. An essential role for a CD36-related receptor in pheromone detection in Drosophila. Nature. 2007; 450:289–293. [PubMed: 17943085]

Berghard A, Buck LB. 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. 1996; 16:909–918. [PubMed: 8558259]

Bohbot J, Pitts RJ, Kwon HW, Rutzler M, Robertson HM, Zwiebel LJ. Molecular characterization of the Aedes aegypti odorant receptor gene family. Insect Mol Biol. 2007; 16:525–537. [PubMed: 17635615]

Borowsky B, Adham N, Jones KA, et al. Trace amines: identification of a family of mammalian G protein-coupled receptors. Proc Natl Acad Sci U S A. 2001; 98:8966–8971. [PubMed: 11459929]

Boschat C, Pelofi C, Randin O, Roppolo D, Luscher C, Broillet MC, Rodriguez I. Pheromone detection mediated by a V1r vomeronasal receptor. Nat Neurosci. 2002; 5:1261–1262. [PubMed: 12436115]

Bozza T, Feinstein P, Zheng C, Mombaerts P. Odorant receptor expression defines functional units in the mouse olfactory system. J Neurosci. 2002; 22:3033–3043. [PubMed: 11943806]

Brechbuhl J, Klaey M, Broillet MC. Grueneberg ganglion cells mediate alarm pheromone detection in mice. Science. 2008; 321:1092–1095. [PubMed: 18719286]

Breer H, Fleischer J, Strotmann J. The sense of smell: multiple olfactory subsystems. Cell Mol Life Sci. 2006; 63:1465–1475. [PubMed: 16732429]

Brennan PA, Zufall F. Pheromonal communication in vertebrates. Nature. 2006; 444:308–315. [PubMed: 17108955]

Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 1991; 65:175–187. [PubMed: 1840504]

Buck LB. The molecular architecture of odor and pheromone sensing in mammals. Cell. 2000; 100:611–618. [PubMed: 10761927]

Chamero P, Marton TF, Logan DW, Flanagan K, Cruz JR, Saghatelian A, Cravatt BF, Stowers L. Identification of protein pheromones that promote aggressive behaviour. Nature. 2007; 450:899–902. [PubMed: 18064011]

Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. Nature. 2006; 444:288–294. [PubMed: 17108952]

Cheetham SA, Thom MD, Jury F, Ollier WE, Beynon RJ, Hurst JL. The genetic basis of individual-recognition signals in the mouse. Curr Biol. 2007; 17:1771–1777. [PubMed: 17949982]

Chesler A, Firestein S. Neuroscience: current views on odour receptors. Nature. 2008; 452:944. [PubMed: 18432233]

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron. 1999; 22:327–338. [PubMed: 10069338]

Cockerham RE, Puche AC, Munger SD. Heterogeneous sensory innervation and extensive intrabulbar connections of olfactory necklace glomeruli. PLoS ONE. 2009; 4:e4657. [PubMed: 19247478]

Couto A, Alenius M, Dickson BJ. Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr Biol. 2005; 15:1535–1547. [PubMed: 16139208]

De la Cruz O, Blekhman R, Zhang X, Nicolae D, Firestein S, Gilad Y. A signature of evolutionary constraint on a subset of ectopically expressed olfactory receptor genes. Mol Biol Evol. 2008; 26:491–494. [PubMed: 19103638]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript



Del Punta K, Leinders-Zufall T, Rodriguez I, Jukam D, Wysocki CJ, Ogawa S, Zufall F, Mombaerts P. Deficient pheromone responses in mice lacking a cluster of vomeronasal receptor genes. Nature. 2002; 419:70–74. [PubMed: 12214233]

Duda T, Fik-Rymarkiewicz E, Venkataraman V, Krishnan A, Sharma RK. Calcium-modulated ciliary membrane guanylate cyclase transduction machinery: constitution and operational principles. Mol Cell Biochem. 2004; 267:107–122. [PubMed: 15663192]

Duda T, Jankowska A, Venkataraman V, Nagele RG, Sharma RK. A novel calcium-regulated membrane guanylate cyclase transduction system in the olfactory neuroepithelium. Biochemistry. 2001; 40:12067–12077. [PubMed: 11580282]

Duda T, Sharma RK. ONE-GC membrane guanylate cyclase, a trimodal odorant signal transducer. Biochem Biophys Res Commun. 2008; 367:440–445. [PubMed: 18178149]

Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell. 1995; 83:195–206. [PubMed: 7585937]

Dulac C, Torello AT. Molecular detection of pheromone signals in mammals: from genes to behaviour. Nat Rev Neurosci. 2003; 4:551–562. [PubMed: 12838330]

Dulac C, Wagner S. Genetic analysis of brain circuits underlying pheromone signaling. Annu Rev Genet. 2006; 40:449–467. [PubMed: 16953793]

Engsontia P, Sanderson AP, Cobb M, Walden KK, Robertson HM, Brown S. The red flour beetle's large nose: an expanded odorant receptor gene family in Tribolium castaneum. Insect Biochem Mol Biol. 2008; 38:387–397. [PubMed: 18342245]

Feldmesser E, Olender T, Khen M, Yanai I, Ophir R, Lancet D. Widespread ectopic expression of olfactory receptor genes. BMC Genomics. 2006; 7:121. [PubMed: 16716209]

Firestein S. How the olfactory system makes sense of scents. Nature. 2001; 413:211–218. [PubMed: 11557990]

Fishilevich E, Domingos AI, Asahina K, Naef F, Vosshall LB, Louis M. Chemotaxis behavior mediated by single larval olfactory neurons in Drosophila. Curr Biol. 2005; 15:2086–2096. [PubMed: 16332533]

Fishilevich E, Vosshall LB. Genetic and functional subdivision of the Drosophila antennal lobe. Curr Biol. 2005; 15:1548–1553. [PubMed: 16139209]

Fleischer J, Mamasuew K, Breer H. Expression of cGMP signaling elements in the Grueneberg ganglion. Histochem Cell Biol. 2009; 131:75–88. [PubMed: 18830617]

Fleischer J, Schwarzenbacher K, Besser S, Hass N, Breer H. Olfactory receptors and signalling elements in the Grueneberg ganglion. J Neurochem. 2006; 98:543–554. [PubMed: 16805845]

Fleischer J, Schwarzenbacher K, Breer H. Expression of trace amine-associated receptors in the Grueneberg ganglion. Chem Senses. 2007; 32:623–631. [PubMed: 17556730]

Forte LR Jr. Uroguanylin and guanylin peptides: pharmacology and experimental therapeutics. Pharmacol Ther. 2004; 104:137–162. [PubMed: 15518884]

Freitag J, Beck A, Ludwig G, von Buchholtz L, Breer H. On the origin of the olfactory receptor family: receptor genes of the jawless fish (Lampetra fluviatilis). Gene. 1999; 226:165–174. [PubMed: 10084886]

Fulle HJ, Vassar R, Foster DC, Yang RB, Axel R, Garbers DL. A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. Proc Natl Acad Sci U S A. 1995; 92:3571–3575. [PubMed: 7724600]

Gao Q, Chess A. Identification of candidate Drosophila olfactory receptors from genomic DNA sequence. Genomics. 1999; 60:31–39. [PubMed: 10458908]

Gibson AD, Garbers DL. Guanylyl cyclases as a family of putative odorant receptors. Annu Rev Neurosci. 2000; 23:417–439. [PubMed: 10845070]

Gloriam DE, Bjarnadottir TK, Yan YL, Postlethwait JH, Schioth HB, Fredriksson R. The repertoire of trace amine G-protein-coupled receptors: large expansion in zebrafish. Mol Phylogenet Evol. 2005; 35:470–482. [PubMed: 15804416]

Glusman G, Yanai I, Rubin I, Lancet D. The complete human olfactory subgenome. Genome Res. 2001; 11:685–702. [PubMed: 11337468]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gold GH. Controversial issues in vertebrate olfactory transduction. Annu Rev Physiol. 1999; 61:857–871. [PubMed: 10099713]

Goldman AL, Van der Goes van Naters W, Lessing D, Warr CG, Carlson JR. Coexpression of two functional odor receptors in one neuron. Neuron. 2005; 45:661–666. [PubMed: 15748842]

Grosmaitre X, Vassalli A, Mombaerts P, Shepherd GM, Ma M. 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. 2006; 103:1970–1975. [PubMed: 16446455]

Grus WE, Shi P, Zhang J. Largest vertebrate vomeronasal type 1 receptor gene repertoire in the semiaquatic platypus. Mol Biol Evol. 2007; 24:2153–2157. [PubMed: 17666439]

Grus WE, Shi P, Zhang YP, Zhang J. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. Proc Natl Acad Sci U S A. 2005; 102:5767–5772. [PubMed: 15790682]

Grus WE, Zhang J. Distinct evolutionary patterns between chemoreceptors of 2 vertebrate olfactory systems and the differential tuning hypothesis. Mol Biol Evol. 2008; 25:1593–1601. [PubMed: 18460446]

Halpern M, Shapiro LS, Jia C. Differential localization of G proteins in the opossum vomeronasal system. Brain Res. 1995; 677:157–161. [PubMed: 7606461]

He J, Ma L, Kim S, Nakai J, Yu CR. Encoding gender and individual information in the mouse vomeronasal organ. Science. 2008; 320:535–538. [PubMed: 18436787]

Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell. 1997; 90:763–773. [PubMed: 9288755]

Hill CA, Fox AN, Pitts RJ, et al. G protein-coupled receptors in Anopheles gambiae. Science. 2002; 298:176–178. [PubMed: 12364795]

Holekamp TF, Turaga D, Holy TE. Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy. Neuron. 2008; 57:661–672. [PubMed: 18341987]

Hu J, Zhong C, Ding C, Chi Q, Walz A, Mombaerts P, Matsunami H, Luo M. Detection of near-atmospheric concentrations of CO2 by an olfactory subsystem in the mouse. Science. 2007; 317:953–957. [PubMed: 17702944]

Imai T, Sakano H. Odorant receptor-mediated signaling in the mouse. Curr Opin Neurobiol. 2008; 18:251–260. [PubMed: 18721880]

Ishii T, Hirota J, Mombaerts P. Combinatorial coexpression of neural and immune multigene families in mouse vomeronasal sensory neurons. Curr Biol. 2003; 13:394–400. [PubMed: 12620187]

Ishii T, Mombaerts P. Expression of nonclassical class I major histocompatibility genes defines a tripartite organization of the mouse vomeronasal system. J Neurosci. 2008; 28:2332–2341. [PubMed: 18322080]

Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB. Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature. 2007; 445:86–90. [PubMed: 17167414]

Jordt SE, McKemy DD, Julius D. Lessons from peppers and peppermint: the molecular logic of thermosensation. Curr Opin Neurobiol. 2003; 13:487–492. [PubMed: 12965298]

Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, Beavo JA. 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. 1997; 94:3388–3395. [PubMed: 9096404]

Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H, Touhara K. Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. J Neurosci. 2001; 21:6018–6025. [PubMed: 11487625]

Kambere MB, Lane RP. Co-regulation of a large and rapidly evolving repertoire of odorant receptor genes. BMC Neurosci. 2007; 8(Suppl 3):S2. [PubMed: 17903278]

Katada S, Hirokawa T, Oka Y, Suwa M, Touhara K. Structural basis for a broad but selective ligand spectrum of a mouse olfactory receptor: mapping the odorant-binding site. J Neurosci. 2005; 25:1806–1815. [PubMed: 15716417]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Keller A, Vosshall LB. Better smelling through genetics: mammalian odor perception. Curr Opin Neurobiol. 2008; 18:364–369. [PubMed: 18938244]

Kelliher KR, Spehr M, Li XH, Zufall F, Leinders-Zufall T. Pheromonal recognition memory induced by TRPC2-independent vomeronasal sensing. Eur J Neurosci. 2006; 23:3385–3390. [PubMed: 16820028]

Kimchi T, Xu J, Dulac C. A functional circuit underlying male sexual behaviour in the female mouse brain. Nature. 2007; 448:1009–1014. [PubMed: 17676034]

Kimoto H, Haga S, Sato K, Touhara K. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. Nature. 2005; 437:898–901. [PubMed: 16208374]

Kimoto H, Sato K, Nodari F, Haga S, Holy TE, Touhara K. Sex- and strain-specific expression and vomeronasal activity of mouse ESP family peptides. Curr Biol. 2007; 17:1879–1884. [PubMed: 17935991]

Krautwurst D, Yau KW, Reed RR. Identification of ligands for olfactory receptors by functional expression of a receptor library. Cell. 1998; 95:917–926. [PubMed: 9875846]

Kreher SA, Kwon JY, Carlson JR. The molecular basis of odor coding in the Drosophila larva. Neuron. 2005; 46:445–456. [PubMed: 15882644]

Krieger J, Raming K, Dewer YM, Bette S, Conzelmann S, Breer H. A divergent gene family encoding candidate olfactory receptors of the moth Heliothis virescens. The Eur J Neurosci. 2002; 16:619–628.

Kobayakawa K, Kobayakawa R, Matsumoto H, et al. Innate versus learned odour processing in the mouse olfactory bulb. Nature. 2007; 450:503–508. [PubMed: 17989651]

Kuhn M. Function and dysfunction of Mammalian membrane guanylyl cyclase receptors: lessons from genetic mouse models and implications for human diseases. Handb Exp Pharmacol. 2009; 191:47–69. [PubMed: 19089325]

Kwon JY, Dahanukar A, Weiss LA, Carlson JR. The molecular basis of CO2 reception in Drosophila. Proc Natl Acad Sci U S A. 2007; 104:3574–3578. [PubMed: 17360684]

Lagerstrom MC, Schioth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat Rev Drug Discov. 2008; 7:339–357. [PubMed: 18382464]

Laissue PP, Vosshall LB. The olfactory sensory map in Drosophila. Adv Exp Mol Biol. 2008; 628:102–114.

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron. 2004; 43:703–714. [PubMed: 15339651]

Leinders-Zufall T, Brennan P, Widmayer P, et al. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science. 2004a; 306:1033–1037. [PubMed: 15528444]

Leinders-Zufall T, Brennan P, Widmayer P, et al. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science. 2004b; 306:1033–1037. [PubMed: 15528444]

Leinders-Zufall T, Cockerham RE, Michalakis S, Biel M, Garbers DL, Reed RR, Zufall F, Munger SD. Contribution of the receptor guanylyl cyclase GC-D to chemosensory function in the olfactory epithelium. Proc Natl Acad Sci U S A. 2007; 104:14507–14512. [PubMed: 17724338]

Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, Zufall F. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature. 2000; 405:792–796. [PubMed: 10866200]

Levai O, Feistel T, Breer H, Strotmann J. Cells in the vomeronasal organ express odorant receptors but project to the accessory olfactory bulb. J Comp Neurol. 2006; 498:476–490. [PubMed: 16874801]

Lewcock JW, Reed RR. A feedback mechanism regulates monoallelic odorant receptor expression. Proc Natl Acad Sci U S A. 2004; 101:1069–1074. [PubMed: 14732684]

Liberles SD, Buck LB. A second class of chemosensory receptors in the olfactory epithelium. Nature. 2006; 442:645–650. [PubMed: 16878137]

Lin W, Margolskee R, Donnert G, Hell SW, Restrepo D. Olfactory neurons expressing transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals. Proc Natl Acad Sci U S A. 2007; 104:2471–2476. [PubMed: 17267604]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Loconto J, Papes F, Chang E, Stowers L, Jones EP, Takada T, Kumanovics A, Fischer Lindahl K, Dulac C. Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. Cell. 2003; 112:607–618. [PubMed: 12628182]

Luo M. The Necklace Olfactory System in Mammals. J Neurogenet. 2008; 15:1–10.

Ma M. Encoding olfactory signals via multiple chemosensory systems. Crit Rev Biochem Mol Biol. 2007; 42:463–480. [PubMed: 18066954]

Malnic B, Hirono J, Sato T, Buck LB. Combinatorial receptor codes for odors. Cell. 1999; 96:713–723. [PubMed: 10089886]

Mamasuew K, Breer H, Fleischer J. Grueneberg ganglion neurons respond to cool ambient temperatures. Eur J Neurosci. 2008; 28:1775–1785. [PubMed: 18973593]

Martini S, Silvotti L, Shirazi A, Ryba NJ, Tirindelli R. Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ. J Neurosci. 2001; 21:843–848. [PubMed: 11157070]

Matsunami H, Buck LB. A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell. 1997; 90:775–784. [PubMed: 9288756]

McBride CS, Arguello JR, O'Meara BC. Five Drosophila genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. Genetics. 2007; 177:1395–1416. [PubMed: 18039874]

Meyer MR, Angele A, Kremmer E, Kaupp UB, Muller F. A cGMP-signaling pathway in a subset of olfactory sensory neurons. Proc Natl Acad Sci U S A. 2000; 97:10595–10600. [PubMed: 10984544]

Mombaerts P. Genes and ligands for odorant, vomeronasal and taste receptors. Nat Rev Neurosci. 2004a; 5:263–278. [PubMed: 15034552]

Mombaerts P. Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. Curr Opin Neurobiol. 2004b; 14:31–36. [PubMed: 15018935]

Mueller JC, Steiger S, Fidler AE, Kempenaers B. Biogenic Trace Amine-Associated Receptors (TAARS) are encoded in avian genomes: evidence and possible implications. J Hered. 2008; 99:174–176. [PubMed: 18296390]

Munger SD, Leinders-Zufall T, Zufall F. Subsystem Organization of the Mammalian Sense of Smell. Annu Rev Physiol. 2009; 71:115–140. [PubMed: 18808328]

Nakagawa T, Sakurai T, Nishioka T, Touhara K. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science. 2005; 307:1638–1642. [PubMed: 15692016]

Nakamura T, Gold GH. A cyclic nucleotide-gated conductance in olfactory receptor cilia. Nature. 1987; 325:442–444. [PubMed: 3027574]

Neuhaus EM, Gisselmann G, Zhang W, Dooley R, Stortkuhl K, Hatt H. Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. Nat Neurosci. 2005; 8:15–17. [PubMed: 15592462]

Niimura Y, Nei M. Evolution of olfactory receptor genes in the human genome. Proc Natl Acad Sci U S A. 2003; 100:12235–12240. [PubMed: 14507991]

Nodari F, Hsu FF, Fu X, Holekamp TF, Kao LF, Turk J, Holy TE. Sulfated steroids as natural ligands of mouse pheromone-sensing neurons. J Neurosci. 2008; 28:6407–6418. [PubMed: 18562612]

Novotny MV. Pheromones, binding proteins and receptor responses in rodents. Biochem Soc Trans. 2003; 31:117–122. [PubMed: 12546667]

Nozawa M, Nei M. Evolutionary dynamics of olfactory receptor genes in Drosophila species. Proc Natl Acad Sci U S A. 2007; 104:7122–7127. [PubMed: 17438280]

Oka Y, Nakamura A, Watanabe H, Touhara K. An odorant derivative as an antagonist for an olfactory receptor. Chem Senses. 2004; 29:815–822. [PubMed: 15574817]

Pace U, Hanski E, Salomon Y, Lancet D. Odorant-sensitive adenylate cyclase may mediate olfactory reception. Nature. 1985; 316:255–258. [PubMed: 3927168]

Pace U, Lancet D. Olfactory GTP-binding protein: signal-transducing polypeptide of vertebrate chemosensory neurons. Proc Natl Acad Sci U S A. 1986; 83:4947–4951. [PubMed: 3088569]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Penn D, Potts WK. Untrained mice discriminate MHC-determined odors. Physiol Behav. 1998; 64:235–243. [PubMed: 9748088]

Pfister P, Rodriguez I. Olfactory expression of a single and highly variable V1r pheromone receptor-like gene in fish species. Proc Natl Acad Sci U S A. 2005; 102:5489–5494. [PubMed: 15809442]

Pin JP, Galvez T, Prezeau L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther. 2003; 98:325–354. [PubMed: 12782243]

Restrepo D, Lin W, Salcedo E, Yamazaki K, Beauchamp G. Odortypes and MHC peptides: Complementary chemosignals of MHC haplotype? Trends Neurosci. 2006; 29:604–609. [PubMed: 16904761]

Robertson HM, Wanner KW. The chemoreceptor superfamily in the honey bee, Apis mellifera: expansion of the odorant, but not gustatory, receptor family. Genome Res. 2006; 16:1395–1403. [PubMed: 17065611]

Rodriguez I, Del Punta K, Rothman A, Ishii T, Mombaerts P. Multiple new and isolated families within the mouse superfamily of V1r vomeronasal receptors. Nat Neurosci. 2002; 5:134–140. [PubMed: 11802169]

Rodriguez I, Feinstein P, Mombaerts P. Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. Cell. 1999; 97:199–208. [PubMed: 10219241]

Rodriguez I, Greer CA, Mok MY, Mombaerts P. A putative pheromone receptor gene expressed in human olfactory mucosa. Nat Genet. 2000; 26:18–19. [PubMed: 10973240]

Rodriguez I, Mombaerts P. Novel human vomeronasal receptor-like genes reveal species-specific families. Curr Biol. 2002; 12:R409–411. [PubMed: 12123587]

Roppolo D, Vollery S, Kan CD, Luscher C, Broillet MC, Rodriguez I. Gene cluster lock after pheromone receptor gene choice. EMBO J. 2007; 26:3423–3430. [PubMed: 17611603]

Ryba NJ, Tirindelli R. A new multigene family of putative pheromone receptors. Neuron. 1997; 19:371–379. [PubMed: 9292726]

Saito H, Chi Q, Zhuang H, Matsunami H, Mainland JD. Odor coding by a Mammalian receptor repertoire. Sci Signal. 2009; 2:ra9. [PubMed: 19261596]

Saraiva LR, Korsching SI. A novel olfactory receptor gene family in teleost fish. Genome Res. 2007; 17:1448–1457. [PubMed: 17717047]

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature. 2008; 452:1002–1006. [PubMed: 18408712]

Sato K, Touhara K. Insect Olfaction: Receptors, Signal Transduction, and Behavior. Results Probl Cell Differ. 200810.1007/400_2008_10

Serizawa S, Miyamichi K, Nakatani H, Suzuki M, Saito M, Yoshihara Y, Sakano H. Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. Science. 2003; 302:2088–2094. [PubMed: 14593185]

Sherborne AL, Thom MD, Paterson S, Jury F, Ollier WE, Stockley P, Beynon RJ, Hurst JL. The genetic basis of inbreeding avoidance in house mice. Curr Biol. 2007; 17:2061–2066. [PubMed: 17997307]

Shi P, Zhang J. Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Res. 2007; 17:166–174. [PubMed: 17210926]

Shirokova E, Schmiedeberg K, Bedner P, Niessen H, Willecke K, Raguse JD, Meyerhof W, Krautwurst D. Identification of specific ligands for orphan olfactory receptors. G protein-dependent agonism and antagonism of odorants. J Biol Chem. 2005; 280:11807–11815. [PubMed: 15598656]

Silvotti L, Moiani A, Gatti R, Tirindelli R. Combinatorial co-expression of pheromone receptors, V2Rs. J Neurochem. 2007; 103:1753–1763. [PubMed: 17854397]

Sklar PB, Anholt RR, Snyder SH. The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. J Biol Chem. 1986; 261:15538–15543. [PubMed: 3536906]

Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK, Hatt H. Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science. 2003; 299:2054–2058. [PubMed: 12663925]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Spehr M, Schwane K, Heilmann S, Gisselmann G, Hummel T, Hatt H. Dual capacity of a human olfactory receptor. Curr Biol. 2004; 14:R832–833. [PubMed: 15458659]

Spehr M, Spehr J, Ukhanov K, Kelliher KR, Leinders-Zufall T, Zufall F. Parallel processing of social signals by the mammalian main and accessory olfactory systems. Cell Mol Life Sci. 2006; 63:1476–1484. [PubMed: 16732428]

Spletter ML, Luo L. A new family of odorant receptors in Drosophila. Cell. 2009; 136:23–25. [PubMed: 19135885]

Stocker RF. Design of the larval chemosensory system. Adv Exp Med Biol. 2008; 628:69–81. [PubMed: 18683639]

Sun L, Wang H, Hu J, Han J, Matsunami H, Luo M. Guanylyl cyclase-D in the olfactory CO2 neurons is activated by bicarbonate. Proc Natl Acad Sci U S A. 2009; 106:2041–2046. [PubMed: 19181845]

Tian H, Ma M. Molecular organization of the olfactory septal organ. J Neurosci. 2004; 24:8383–8390. [PubMed: 15385621]

Tirindelli R, Mucignat-Caretta C, Ryba NJ. Molecular aspects of pheromonal communication via the vomeronasal organ of mammals. Trends Neurosci. 1998; 21:482–486. [PubMed: 9829690]

Touhara K, Sengoku S, Inaki K, Tsuboi A, Hirono J, Sato T, Sakano H, Haga T. Functional identification and reconstitution of an odorant receptor in single olfactory neurons. Proc Natl Acad Sci U S A. 1999; 96:4040–4045. [PubMed: 10097159]

Touhara K, Vosshall LB. Sensing odorants and pheromones with chemosensory receptors. Annu Rev Physiol. 2009; 71:307–332. [PubMed: 19575682]

Ukhanov K, Leinders-Zufall T, Zufall F. Patch-clamp analysis of gene-targeted vomeronasal neurons expressing a defined V1r or V2r receptor: ionic mechanisms underlying persistent firing. J Neurophysiol. 2007; 98:2357–2369. [PubMed: 17715188]

Vigues S, Dotson CD, Munger SD. The Receptor Basis of Sweet Taste in Mammals. Res Probl Cell Differ. 200810.1007/400_2008_2

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. A spatial map of olfactory receptor expression in the Drosophila antenna. Cell. 1999; 96:725–736. [PubMed: 10089887]

Walz A, Feinstein P, Khan M, Mombaerts P. Axonal wiring of guanylate cyclase-D-expressing olfactory neurons is dependent on neuropilin 2 and semaphorin 3F. Development. 2007; 134:4063–4072. [PubMed: 17942483]

Wanner KW, Nichols AS, Walden KK, Brockmann A, Luetje CW, Robertson HM. A honey bee odorant receptor for the queen substance 9-oxo-2-decenoic acid. Proc Natl Acad Sci U S A. 2007; 104:14383–14388. [PubMed: 17761794]

Wetzel CH, Oles M, Wellerdieck C, Kuczkowiak M, Gisselmann G, Hatt H. Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and Xenopus Laevis oocytes. J Neurosci. 1999; 19:7426–7433. [PubMed: 10460249]

Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS. Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature. 2008; 452:1007–1011. [PubMed: 18408711]

Yamazaki K, Beauchamp GK, Egorov IK, Bard J, Thomas L, Boyse EA. Sensory distinction between H-2b and H-2bm1 mutant mice. Proc Natl Acad Sci U S A. 1983; 80:5685–5688. [PubMed: 6577450]

Yang H, Shi P, Zhang YP, Zhang J. Composition and evolution of the V2r vomeronasal receptor gene repertoire in mice and rats. Genomics. 2005; 86:306–315. [PubMed: 16024217]

Yao CA, Ignell R, Carlson JR. Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna. J Neurosci. 2005; 25:8359–8367. [PubMed: 16162917]

Young JM, Friedman C, Williams EM, Ross JA, Tonnes-Priddy L, Trask BJ. Different evolutionary processes shaped the mouse and human olfactory receptor gene families. Hum Mol Genet. 2002; 11:535–546. [PubMed: 11875048]

Young JM, Kambere M, Trask BJ, Lane RP. Divergent V1R repertoires in five species: Amplification in rodents, decimation in primates, and a surprisingly small repertoire in dogs. Genome Res. 2005; 15:231–240. [PubMed: 15653832]

Young JM, Trask BJ. V2R gene families degenerated in primates, dog and cow, but expanded in opossum. Trends Genet. 2007; 23:212–215. [PubMed: 17382427]

Young JM, Waters H, Dong C, Fulle HJ, Liman ER. Degeneration of the olfactory guanylyl cyclase D gene during primate evolution. PLoS ONE. 2007; 2:e884. [PubMed: 17849013]

Zhang X, De la Cruz O, Pinto JM, Nicolae D, Firestein S, Gilad Y. Characterizing the expression of the human olfactory receptor gene family using a novel DNA microarray. Genome Biol. 2007; 8:R86. [PubMed: 17509148]

Zhang X, Firestein S. The olfactory receptor gene superfamily of the mouse. Nat Neurosci. 2002; 5:124–133. [PubMed: 11802173]

Zhang X, Rogers M, Tian H, Zhang X, Zou DJ, Liu J, Ma M, Shepherd GM, Firestein SJ. High-throughput microarray detection of olfactory receptor gene expression in the mouse. Proc Natl Acad Sci U S A. 2004; 101:14168–14173. [PubMed: 15377787]

Zhao H, Ivic L, Otaki JM, Hashimoto M, Mikoshiba K, Firestein S. Functional expression of a mammalian odorant receptor. Science. 1998; 279:237–242. [PubMed: 9422698]

Zozulya S, Echeverri F, Nguyen T. The human olfactory receptor repertoire. Genome Biol. 2001; 2 RESEARCH0018.

Zufall F, Leinders-Zufall T. Mammalian pheromone sensing. Curr Opin Neurobiol. 2007; 17:483–489. [PubMed: 17709238]

Zufall F, Munger SD. From odor and pheromone transduction to the organization of the sense of smell. Trends Neurosci. 2001; 24:191–193. [PubMed: 11249988]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



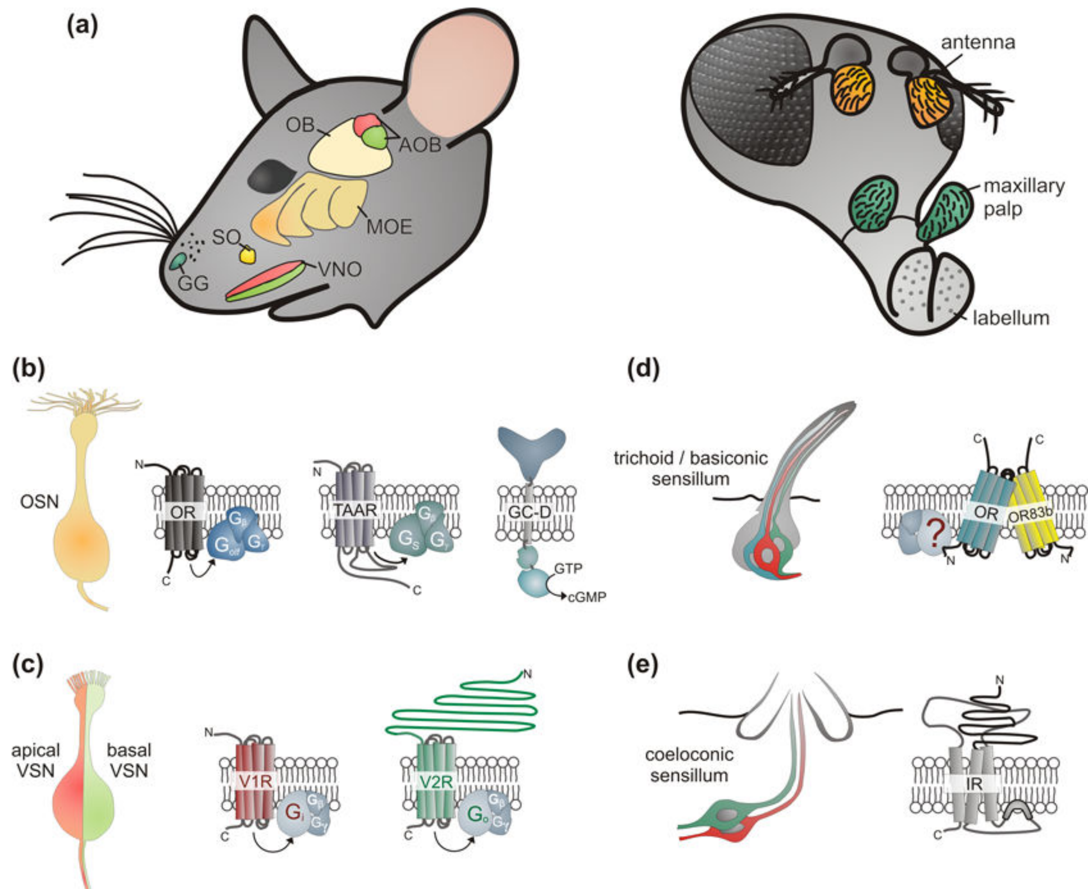


**Figure 1.**

Olfactory receptors of mouse and fruitfly. **(a)** *Left panel:* In the mouse nasal cavity, chemosensory neurons are found within several sensory organs, including the Grueneberg ganglion (GG), septal organ of Masera (SO), vomeronasal organ (VNO), and main olfactory epithelium (MOE). The VNO neuroepithelium is divided into apical (red, contains V1R-expressing VSNs) and basal (green, contains V2R-expressing VSNs); VSNs in these two regions innervate the rostral and caudal divisions of the accessory olfactory bulb (AOB), respectively. OR-, TAAR-, and GC-D-expressing OSNs are found in the MOE; their axons project to the main olfactory bulb (MOB). *Right panel*, The fruitfly *Drosophila melanogaster* has two olfactory organs: the distal segment of the antenna (orange) and the maxillary palp (green). The labellum contains gustatory receptors. **(b)** Receptors expressed in ciliary OSNs of the mouse MOE include two groups of GPCRs, the ORs and the TAARs, as well as the receptor guanylyl cyclase GC-D. Both ORs and TAARs appear to couple to the G protein $G\alpha_{olf}$. **(c)** V1Rs are expressed in apical VSNs, while V2Rs are expressed in basal VSNs. Both may couple to layer-specific G proteins ($G\alpha_{i2}$ and $G\alpha_o$, respectively). **(d)** *Drosophila* ORs, almost exclusively expressed in basiconic and trichoid OSNs, are heteromeric receptors. The common subunit OR83b appears to be an ion channel, while the partner OR (isoforms vary between OSN subtypes) dictates ligand selectivity and may also couple to a G protein. **(e)** IRs are found in coeloconic OSNs and are ligand-gated ion channels. In some cases they may form homomeric or heteromeric complexes.