

# Chapter 2

## Mechanisms of Olfaction

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**Abstract** Molecular mechanisms of olfaction have been intensively studied in the last quarter century. Receptors by which olfactory stimuli are detected are vastly different between different animal species and even between different olfactory organs of the same species. This chapter includes a description of the anatomy of the mammalian olfactory system and an overview of the receptors. The signaling mechanism and expression pattern of these receptors is discussed along with how the brain decodes olfactory information gathered from the environment and then translates these signals into behaviors. This chapter also contains brief comparison of the fish, insect and nematode olfactory receptors.

### 2.1 Importance of Olfaction

A sense of smell is crucial for the survival of any species, and in our own experience, we detect dangers like fire or spoiled food with our noses. The hedonistic pleasure derived from eating and drinking is also heavily reliant on the sense of smell. Olfaction is an important tool used in so many facets of animal life and is often the deciding factor between surviving an encounter with a predator, passing on genes to offspring and finding a source of food. Although Santiago Cajal described the peripheral olfactory system in 1891, very little was uncovered about the mechanisms of odor detection and differentiation in the next 100 years, and the olfactory system continues to elude our understanding. The method by which sensory inputs are converted to behavioral output is still a mystery.

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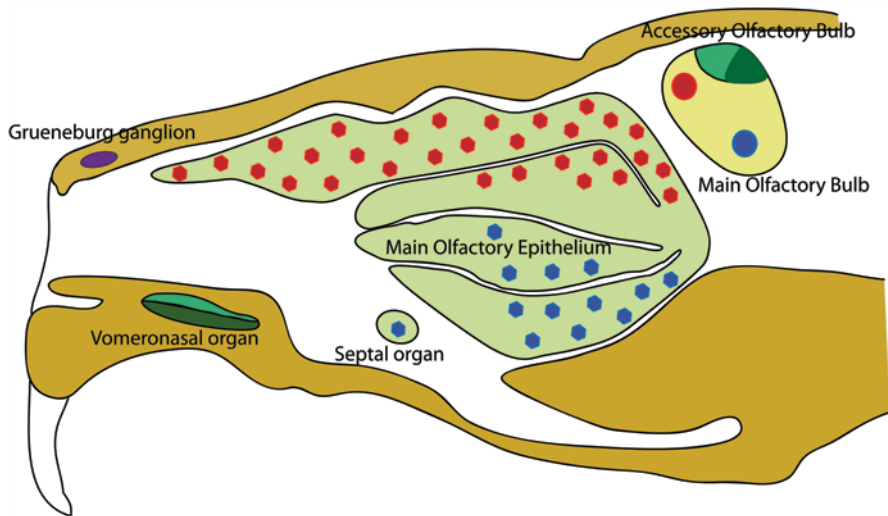
Phylogenetically, chemosensation such as olfaction and gustation are one of the oldest senses and are important for the simplest to the most complex organisms. Our taste system only allows us to detect and distinguish a limited number of taste modalities: sweet, sour, salty, bitter and umami. In contrast, our sense of smell can detect and distinguish among tens of thousands of volatiles, such as flavors offered to our palate from food. The important role of olfaction in our eating experience was most notably demonstrated in a study where a majority of participants who ate either a piece of apple or boiled potato while wearing a blindfold and a nose plug failed to make the correct identification when asked what they had eaten [2].

Certain odorants released by predators evoke innate avoidance behaviors in animals that have never encountered that predator before. One example of such a phenomenon is trimethylthiazoline (TMT), a chemical found in the feces of the red fox (*Vulpes vulpes*), which causes laboratory bred mice, who have never experienced an external environment, to freeze and display other anxious behaviors [3]. Mating and maternal behavior are both heavily reliant on olfaction as manipulating the olfactory system has been shown to change social behaviors in both vertebrates and invertebrates [4–9]. In fruit flies, the male-specific transcription factor *fruitless* is required for the appropriate functioning of the components of the olfactory system, and in its absence male flies have been shown to court other male flies or to abolish all courtship behaviors. Female rats whose olfactory bulbs are surgically removed have been shown to lose their normal nursing behaviors as well as other maternal behaviors like retrieving pups that have been taken out of their nest, and male rats lose their aggression to non-conspecific male intruders [10–12].

In humans, olfactory dysfunction has been linked to a number of neurodegenerative diseases, primary amongst which are idiopathic Parkinson's disease and Alzheimer's [13, 14]. Our sense of smell is also linked to our limbic system. Hence, there is a strong, but as of yet elusive link between memory, emotion and certain smells that invoke them. Further study of this sensory modality is of vital importance for us to better understand many aspects of our own nature and behavior.

## 2.2 The Vertebrate Olfactory System

The peripheral olfactory system is comprised of the main olfactory epithelium and the accessory olfactory system, which contains the vomeronasal organ (VNO), the Grueneberg ganglion and the septal organ of Maser in rodents (Fig. 2.1). The main olfactory epithelium's primary function is the detection of volatile odorants in the air [15] whereas the VNO detects semiochemicals such as pheromones [16–18]. The Grueneberg ganglion is implicated in the detection of stress signals from conspecifics [19–21] and the septal organ contains neurons expressing a subset of odorant receptors (ORs) that are also expressed in the ventral domain of the olfactory epithelium [22, 23].

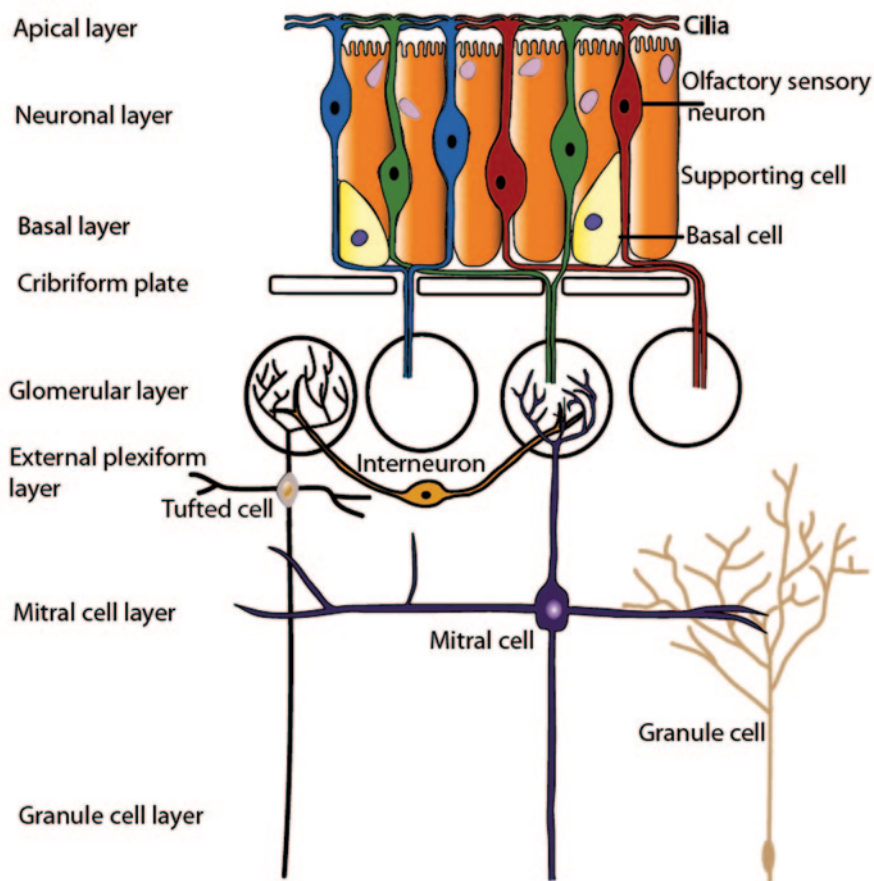


**Fig. 2.1** Sagittal view of a mouse head. The antero-dorsal tip contains the *Gruenberg ganglion*. The VNO is located on the ventral side and is divided into 2 halves, each expressing a unique receptor repertoire, which project to the accessory olfactory bulb located at the distal end of the olfactory bulb. The dorsal receptors in the olfactory epithelium are depicted in *red* and the ventral receptors in *blue*. The areas they project to on the olfactory bulb are labeled with the same *colors*. The septal organ is shown at the ventral base of the nasal septum

## 2.3 Main Olfactory Epithelium

### 2.3.1 Anatomy

Neurons responsible for the detection of odors from the environment are found in the olfactory epithelium (OE) located in the dorso-caudal nasal vault along the upper portion of the nasal septum, cribriform plate and the medial wall of the superior turbinate from where they project their dendrites into the nasal passage. These olfactory sensory neurons (OSNs) lie in a pseudostratified columnar epithelium along with supporting cells (sustentacular cells), microvillar cells and basal cells [24]. The epithelium lies on top of a highly vascular lamina propria, which contains the Bowman's gland. Basal cells are stem cells responsible for the replacement of the OSNs and the Bowman's gland is responsible for secreting the serous component of the mucous layer covering the OE [25]. OSNs form a dendritic knob at the junction between the tissue and the nasal passage from which 5 to 20 cilia emerge; these cilia are bathed in the mucus in which odor molecules dissolve and then come in contact with the OR [26]. These cilia lack dynein and therefore do not exhibit any motility. Their main advantage is to increase the surface area of the neuron so as to increase the probability of a molecule encountering its receptor [27]. Mature OSNs are identified by the expression of olfactory marker protein (OMP) [28, 29] and are bipolar cells that project their un-myelinated axons through the cribriform



**Fig. 2.2** A representation of the cellular organization of the olfactory system. OSNs expressing the same receptors (represented here in the same *color*) project to the same glomeruli, which are innervated by afferent mitral and tufted cells. Interneurons connect neighboring glomeruli and coordinate their response. Supporting cells flank the OSNs while the underlying basal cell layer contains the stem cells from which OSNs are regularly replaced

plate to the olfactory bulb (OB) where they converge with axons from other neurons expressing the same OR to form a single anatomical unit called the glomerulus [30]. (Fig. 2.2) Glomeruli are spheroid structures composed of a cellular shell composed of periglomerular cells surrounding a core of neuropil [31].

## 2.4 Odorant Receptors

ORs are members of the seven transmembrane domain super family of G protein coupled receptors (GPCR). The number of ORs in different species is highly variable. Most mammals have a very large number of ORs (humans ~400 and mice

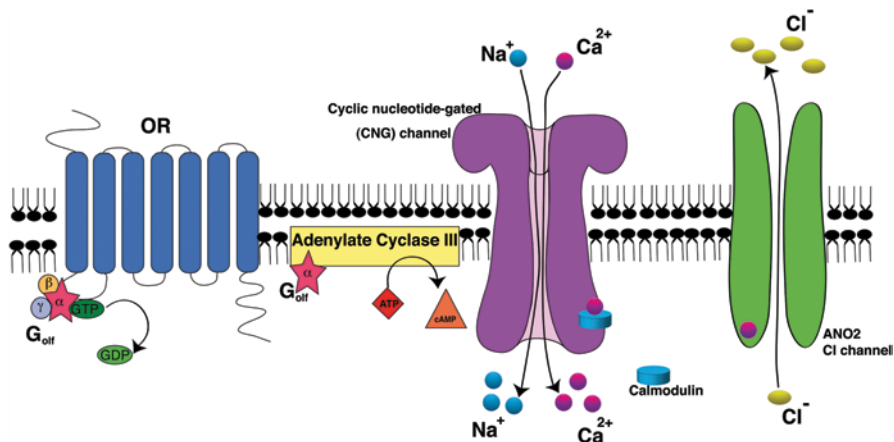
~1,000 ORs) while others such as dolphins may only have a handful of ORs, which likely reflects the relative importance of olfaction in a given species [15, 32–34]. Though the ORs contain several conserved motifs, some of which may be important for G-protein coupling, there are many variable sequences in transmembrane and extracellular domains of the protein that come together in the tertiary structure to form a ligand-binding site. The high degree of variability is thought to be important for activation by a structurally diverse set of volatile odor molecules [35].

### 2.4.1 Receptor Signaling and Termination

The binding of a cognate ligand to its OR releases a specialized stimulatory G protein  $\alpha$  subunit called  $G_{\text{olf}}$  into the membrane from the  $\beta\gamma$  subunits [36].  $G_{\text{olf}}$  activates adenylate cyclase III (ACIII), and causes the conversion of ATP into cAMP [37, 38]. This was demonstrated by a rapid increase in cAMP levels when cilia from OSNs were exposed to odorants [39, 40]. A surge in cAMP levels in the neurons leads to the activation of calcium permeable, tetrameric cyclic nucleotide gated (CNG) [41] channels and an influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  [42], which in turn leads to an efflux of chloride resulting in the depolarization of the membrane [43]. (Fig. 2.3) Once an action potential has been generated, the cell extrudes  $\text{Ca}^{2+}$  by  $\text{Na}^+/\text{Ca}^{2+}$  exchangers in order to return to its resting membrane potential [44, 45]. The major subunit of the  $\text{Ca}^{2+}$  channel was identified as CNGA2 [46] and the  $\text{Cl}^-$  channel as Ano2/Tmem16b [47–49]. The deletion of CNGA2 causes mice to become anosmic [50] but the deletion of ANO2 does not seem to abolish the sense of smell, although electrophysiological and cell culture experiments show that the OSNs maintain a high baseline of  $\text{Cl}^-$  concentration and the opening of these channels leads to a low noise, nonlinear amplification of the signal [51–53]. An increase in inositol-1,4,5-trisphosphate (IP3) and cGMP levels is also commensurate with depolarization in some cases [54], although these are produced on a different time scale [46, 55] and may have more to do with desensitization [56].

### 2.4.2 Desensitization and Adaptation

Constant exposure to a stimulus makes OSNs lose their responsiveness in a process called desensitization or adaptation, which can take place via various negative feedback pathways. CNG channels, ACIII and cAMP hydrolysis by phosphodiesterase are mediated by calcium through a calcium binding protein calmodulin [57–59]. (Fig. 2.3) ORs themselves may be phosphorylated or internalized to desensitize the cell. [60–62]. GRK3 and  $\beta$  arrestin 2 mediate the uncoupling of the OR from its G protein. [62–64]. Incubating OSNs with antibodies to  $\beta$  arrestin 2 and GRK3 leads to elevation of cAMP response in the presence of an odorant [60] and  $\beta$  arrestin 2 has also been shown to be responsible for receptor internalization [61].



**Fig. 2.3** The Olfactory signaling cascade. When activated, the OR catalyzes the release of the  $G_{off}$  subunit, which in turn activates ACIII, leading to an increase in the cAMP levels that activate CNGA2, the calcium channel responsible for the depolarization, and the chloride channel Tmem16b that opens after the surge of  $Ca^{2+}$  into the cell

### 2.4.3 Receptor Surface Expression

The vast majority of ORs are still “orphan” receptors, i.e. the ligands that activate those ORs remain unknown. One of the most straightforward ways to study OR-odor interaction would be to first express the OR in cell culture and screen a number of odorous ligands to see which ligands activate the receptor, and then to study common motifs in ORs activated by the same ligand. The main obstacle in this type of study is that ORs in cell culture accumulate in the ER and are not transported to the cell surface [65, 66]. In 2004, studies showed that co-expression of ORs with a family of proteins called RTP (Receptor Transporting Protein) increased the efficiency of the trafficking of receptors to the cell surface [67]. Based on these findings, a heterologous cell assay system was developed for large scale screening with ligands in order to identify active ligands for many ORs [68]. It is now routine to study a single OR and find out which ligands activate it. However, whether the current system allows functional expression of all ORs is unclear.

### 2.4.4 Receptor Gene Regulation

A single OSN expresses a single allele of one OR [32, 69, 70]. Experiments have shown that OSNs do not express the endogenous OR if a transgene carrying an OR has been forcibly expressed. Additionally, in the case of multiple integrations of the same transgene in tandem, only one of these transgenes is expressed, suggesting

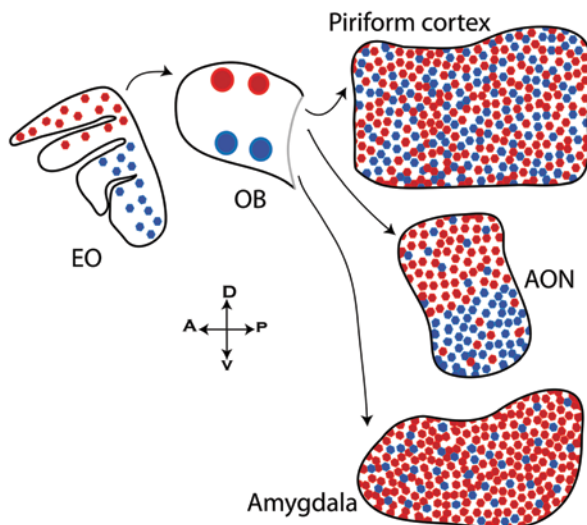
that there are cellular mechanisms limiting expression to only one OR per OSN [71]. The mechanisms used by cells to make this choice remain largely unknown but studies indicate that the epigenetic modification of OR genes as well as the unfolded protein response pathway (UPR) [195] inactivate all but one OR in a given OSN [72–80].

#### ***2.4.5 Receptor Expression Zones and Projection of OSN Axons***

Examination of OR mRNAs in the OE showed topographically distinct expression patterns that could be broadly divided into two zones along the dorso—ventral axis [70, 81, 82]. Within a zone, it seems that OR expression is largely stochastic, although in the ventral zone there are further subdivisions into several overlapping zones [83]. OR genes are divided into class I and class II receptors based on their phylogeny with class I being expressed in the dorsal zone [84, 85]. Microdissection followed by microarray experiments showed about 300 class II receptors expressed in the dorsal epithelium while the remaining are expressed in the ventral zone [83]. Axons from OSNs expressing the same receptors converge and project to a few glomeruli in the OB. Axon targeting is primarily controlled by gradients of molecules divided into 2 major classes. Class I molecules include Neuropilin-1 and Plexin-A1, which establish the anterior-posterior axis, while class II molecules like Kirrel2 and Kirrel3 aid in activity-dependent refined sorting. More recently the deletion of BIG-2, which is only expressed in a subset of OSNs, lead to the erroneous innervation of glomeruli by those axons [86]. ORs play a pivotal role in axon targeting because the expression of some axon targeting molecules depends on the cAMP levels, which are in turn modulated by a functioning OR [87–91]. (Fig. 2.3) In the case of a non-functioning OR, the cAMP levels are low and the axon is often unable to converge and find its position; such OSNs undergo apoptosis [91, 92]. Compromised cilia in the OE lead to aberrant axon targeting in the OB, suggesting proper OSN activation is also important in this process [93]. Studies have shown that the OR itself does not have a unique role in axon targeting as replacing an OR with a functional G protein-like  $\beta$  adrenergic receptor leads to an ectopic but seemingly functional glomerulus [90].

The organization of the glomeruli follows the logic of the epithelium where all the dorsal receptors project to the dorsal portion of the OB, while the class II ventral receptors project to the ventral portion of the bulb. The glomeruli in the OB are connected to one another by the dendro-dendritic connections of local inhibitory interneurons found in the glomerular layer [94–96]. It seems that these neurons are capable of silencing neighboring glomeruli responding with lower intensity to the same odor in order to reduce redundancy [97]. Mitral and tufted cells are long distance projection neurons that sample information from glomeruli with their dendrites and project their axons in the olfactory tract to the primary olfactory cortex in the brain [98]. (Fig. 2.2) The primary olfactory cortex is defined by the accessory

**Fig. 2.4** OSNs expressing dorsal receptors project to the dorsal portion of the *OB*, and the same logic applies to the ventral receptors. The projections from the *OB* to the accessory olfactory nucleus (*AON*) also have the same pattern with the dorsal glomeruli projecting to the dorsal regions in the *AON*. On the other hand, the projections to the piriform seem to be organized randomly, and the projections to the *amygdala* are heavily biased towards the dorsal glomeruli







olfactory nucleus, piriform cortex, lateral enterorhinal cortex, olfactory tubercle and the amygdala [99]. Projections from the primary cortex are diffused over the brain and project to a large number of regions like the limbic system and the neocortex [27]. The dorso-ventral projection pattern is conserved in the anterior olfactory nucleus, but for the amygdala, projections are mostly traced back to the dorsal portion of the bulb, and no discernable pattern can be traced from the projections to the piriform cortex [100]. (Fig. 2.4)

## 2.5 Odor Coding

Precise odor detection and discrimination has its basis in deciphering a combinatorial code of activated ORs. A given OR may be activated by a number of molecules and one odorant is capable of activating a number of ORs, enabling the olfactory system to detect and discriminate among tens of thousands of odorants [101]. (Fig. 2.5) The range of unique odorants able to elicit a response from the OR defines how broadly or narrowly it is tuned. There are ORs that are excited by a wide range of molecules and ORs that respond to only very specific cues. One example of a narrowly tuned OR is the human OR7D4, which is activated very selectively by androsteneone and androstadienone. The receptor has common variants that differ the function and alter the perception of these volatile steroids, showing an essential role of a single OR in odor perception [102–104]. The first step towards the identification of an odorant is the specific set of ORs it can activate to cause the OSN to depolarize, and hence the pattern of glomeruli it excites. It has been found that the glomeruli excited by a single odorant are consistent

**Fig. 2.5** Combinatorial code for odor recognition. One odorant can activate many receptors (indicated by the green +) and a single receptor can get activated by a number of odorants. The second receptor is narrowly tuned, responding to only 1 odorant whereas one and three are broadly tuned, responding to many odorants

Receptor \ Ligand	1	2	3	4
	+	-	-	+
	+	+	+	-
	+	-	+	-
	-	-	+	+

across different individuals and that the higher centers of the brain are capable of identifying odors based on these patterns of activation [105]. The olfactory system may also utilize temporally coded odor information because when different odorants stimulate the same OSN, the OSN depolarizes at different frequencies [106]. The OB could also be generating complex temporal patterns encoding information about odors [107, 108].

2.6 Minority Receptors

ORs are the predominant receptors found in the olfactory epithelium but they are not the only sensory receptors expressed in the OE. A small number of OSNs express trace amine-associated receptors (TAARs) of which there are 15 members in mice [109–111]. These receptors, which detect volatile amines found in urine, are implicated in playing a role in stress response, gender recognition and predator avoidance [109, 112–114]. 0.1 % of OSNs in the OE express guanyl cyclase D (GC-D) and do not have the signaling elements associated with OR signal transduction [115–117]. They instead express a cGMP-gated  $\text{Ca}^{2+}$  channel CNGA3 [115] and project to very specific glomeruli known as the necklace glomeruli [116]. These neurons all express carbonic anhydrase II and show a concentration dependent  $\text{Ca}^{2+}$  response to  $\text{CO}_2$ , indicating that they might be responsible for its detection. They also respond to certain natriuretic peptide hormones and seem to be responsible for the detection of carbon disulphide, which is a signal associated with food related social learning [6, 118, 119].

## 2.7 Odorant Binding Proteins (OBPs)

OBPs are extracellular proteins localized to chemosensory systems of most terrestrial species. Non-neuronal support cells secrete a small number of vertebrate odorant binding protein (vOBP) into the mucus [120]. vOBPs are members of the lipocalin family of molecules, like the retinol binding proteins, and bind to odorants. For example, a vOBP binds to an odorant pyrazine with dissociation constants in the micromolar range [121]. Insect binding proteins (iOBPs) are a family unrelated to vOBPs in sequence and x-ray crystallography reveals that there are no structural analogs between the two. vOBPs are active as dimers whereas iOBPs are active as monomers [122].

Lush is a iOBP mutation in *Drosophila* that causes their repulsion to high concentrations of alcohol [123]. Studies have shown that in addition to alcohol, lush also binds to the insect pheromone 11-cis-vaccenyl acetate (VA), and that non-functional mutants do not display aggregation behavior usually displayed in response to its release [124]. Other OBPs have still not been assigned well defined roles.

## 2.8 Vomeronasal Organ (VNO)

The VNO in mammals, a tube-like organ also called Jacobson's organ after the scientist who first described it in 1811, is found separated from the main OE in a bony cartilaginous cavity opening into the anterior portion of the nasal cavity [125]. It is split by the nasal septum, forming two crescent-shaped lumens lined by a pseudostratified epithelium consisting of supporting cells, neurons and basal cells that act like stem cells for regeneration, similar to the organization of the OE [126]. The VNO neurons do not possess cilia, but rather have apical microvilli [27] and project their axons to the accessory olfactory bulb (AOB) [127], which may be sexually dimorphic with males having a slightly larger bulb than females [128]. The VNO contains neurons expressing receptors belonging to a 7 transmembrane domain GPCR family, unrelated to the ORs. They are divided into 2 subgroups: V1R and V2R [16, 129–132], each expressed in a distinct region of the VNO. V1Rs are expressed in the apical region of the VNO, linked to  $G_{\alpha_{i2}}$  [133], have short amino terminals and have great sequence diversity in their transmembrane domains [134] consistent with the idea that the transmembrane domains are important for responding to structurally diverse ligands. The V2Rs are linked to  $G_{\alpha_{o}}$ , have long amino terminal chains and are located in the basal region of the VNO. The amino terminal domains of V2Rs are diverse, suggesting that these domains may be responsible for binding to ligands. TRPC2 channels, a diacylglycerol activated transient receptor potential  $Ca^{2+}$  ion channel, mediates the signaling cascade in VNO neurons. These channels are found exclusively in the VNO [135, 136]. TRPC2<sup>-/-</sup> mice were shown to be deficient in pheromone sensing using neurophysiology and behavior.

Bioelectronic Nose

Integration of Biotechnology and Nanotechnology

Park, T.H. (Ed.)

2014, XI, 290 p. 70 illus. in color., Hardcover

ISBN: 978-94-017-8612-6