REVIEW:

THE OLFACTORY NERVOUS SYSTEM OF TERRESTRIAL AND AQUATIC VERTEBRATES

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INTRODUCTION

Animals in their natural milieu are surrounded by odors. These odors are rich source of information, and are perceived by sophisticated olfactory systems, that have evolved over time. The sense of smell helps species to localize prey, evade predators, explore food and recognize viable mates. In humans, memoirs, thoughts, emotions, and associations are more readily reached through the sense of smell than through any other channel. This suggests that olfactory processing is imperative and may differ fundamentally from processing in other sensory modalities. The molecular age in olfaction initiated in 1991 with the significant discovery of a large, multigene family of olfactory receptors in rat by Linda Buck and Richard Axel (Buck and Axel, 1991). The first cloned olfactory receptors consisted of a diverse repertoire of G-protein coupled receptors (GPCRs) with seven-trans membrane topology, and they were sparsely expressed in the olfactory epithelium. This Nobel Prize worthy pioneering discovery, together with availability of modern techniques and numerous completely sequenced genomes opened the way to characterize the gene families of olfactory receptors through exhaustive computational data mining in different species genome as well as by *in vitro* biology. In this review , I will explain about the two main model organism of olfactory perceptions, zebrafish and mouse.

OLFACTORY SYSTEM

The generalized initial point of olfactory system is the nose that contains the olfactory epithelium (O.E). The O.E contains olfactory sensory neurons (OSNs) that express olfactory receptor molecules (ORs) on their apical surfaces. The number of OR genes varies according to the species e.g. 388 in human, 155 in zebrafish and 1063 in mice (Nei et al., 2008). The olfactory system perceives myriad of odorants and translates the primary input into diverse odor perception. The primary event in olfactory perception is the recognition of odorants by odorant receptors (ORs), this may occur by diffusion or by the binding of the odorant to odorant binding proteins (OBPs) first, that lead to docking at the respective odorant receptor. One odorants receptor (OR) can bind to odorant of same or different chemical structures. Odorant receptors (ORs) that bind to the same types of odorants unite in the olfactory bulb and form glomeruli. The odorant information is then passed through the olfactory bulb (OB) to the olfactory cortex, in due

course reaching the higher cortical areas involved in odour determination, as well as limbic areas supposedly mediating the emotional and physiological effects of odours (Kapur and Haberly, 1998) .Odorants are perceived and encoded by different combinations of olfactory receptors (Malnic et al., 1999). In the nose, neurons expressing the same OR are scattered throughout olfactory epithelium (Vassar et al., 1993), however, in the olfactory bulb their axons converge at a specific glomeruli, where they form synapses with mitral and tufted relay neurons of olfactory bulb (Mombaerts et al., 1996; Ressler et al., 1994). This results in a rather stereotyped spatial map in which inputs from different ORs are targeted to different glomeruli. An odorant's receptor code is represented in the olfactory epithelium by a dispersed ensemble of neurons and in the bulb by a specific combination of glomeruli (Mori et al., 1999).

Mammalian Olfactory System

Contrary to the fish, many terrestrial vertebrates, including rodents, have up to five main discrete and segregated olfactory systems, including a main olfactory system, which detects volatile odorants and a vomeronasal (accessory olfactory) system, which detects pheromones (Buck, 2000; Mombaerts, 2004). Recently, it has become obvious that there is functional overlap between the main olfactory epithelium and the vomeronasal organ. Certain pheromones activate neurons in the main olfactory system, and this activity has been found necessary for pheromone dependent behaviors (Mandiyan et al., 2005; Restrepo et al., 2004; Spehr et al., 2006b). Likewise, some general odorants categorized as non-pheromones activate the accessory olfactory system and modulate behavior in the absence of a functional main olfactory system (Sam et al., 2001; Trinh and Storm, 2003). In mammals, the olfactory information is processed through anatomically separated neural pathways. Volatile odorants are perceived by a large repertoire of olfactory receptors (ORs) expressed on the cilia and dendritic knob of the ciliated olfactory sensory neurons (OSNs) in the olfactory epithelium (OE), that project their axons to the main olfactory bulb (OB). Two additional receptor families (V1R, V2R) appear to detect pheromones and are expressed by microvillous sensory neurons in the vomeronasal organ that induce hormonal and behavioral responses through the accessory olfactory bulb (AOB). The axons from the accessory olfactory bulb project towards the amygdala and hypothalamus that are involved in aggression and mating behavior (Hasen and Gammie, 2009).

Organ	Receptors	Ligands
MOE	ORs, TAARs, GC-D	general odors, MHC class I peptides volatile amines, CO_2 (bicarbonate)
VNO	V1Rs,V2Rs, FPRs	volatile pheromones, MHC class I peptides, formyl peptides
GG	TAARs, V2r83	alarm pheromones
SO	ORs	general odors

Table.1. Mammalian olfactory organs and their respective receptors with possible ligands

A third mammalian organ, the septal organ of Masera (S-O), also contains sensory neurons ((Kaluza et al., 2004; Tian and Ma, 2004) that express odor receptors (Table.1). The S.O was recently shown to perceive multiple volatile odorants that are also detected by the main olfactory epithelium (Grosmaitre et al., 2007; Ma et al., 2003).Interestingly, a subset of OSNs from both the SO and the main olfactory epithelium may respond to mechanical pressure and thus may report changes in air pressure induced by sniffing (Grosmaitre et al., 2007). Recently, another mammalian organ named the Grueneberg ganglion (GG) was found to subserve olfaction (Fleischer et al., 2006; Fleischer et al., 2007). The Grueneberg ganglion (GG) located in the vestibule of the anterior nasal cavity is considered as an olfactory organ based on the presence of the olfactory marker protein (OMP), expression of V2R and TAARs olfactory receptors and olfactory neurons axonal projection to the olfactory bulb (Fleischer et al., 2007). These neurons are activated by volatile alarm pheromones and are required for the freezing behavior in mice, indicating a role in pheromonal signaling (Brechbuhl et al., 2008).

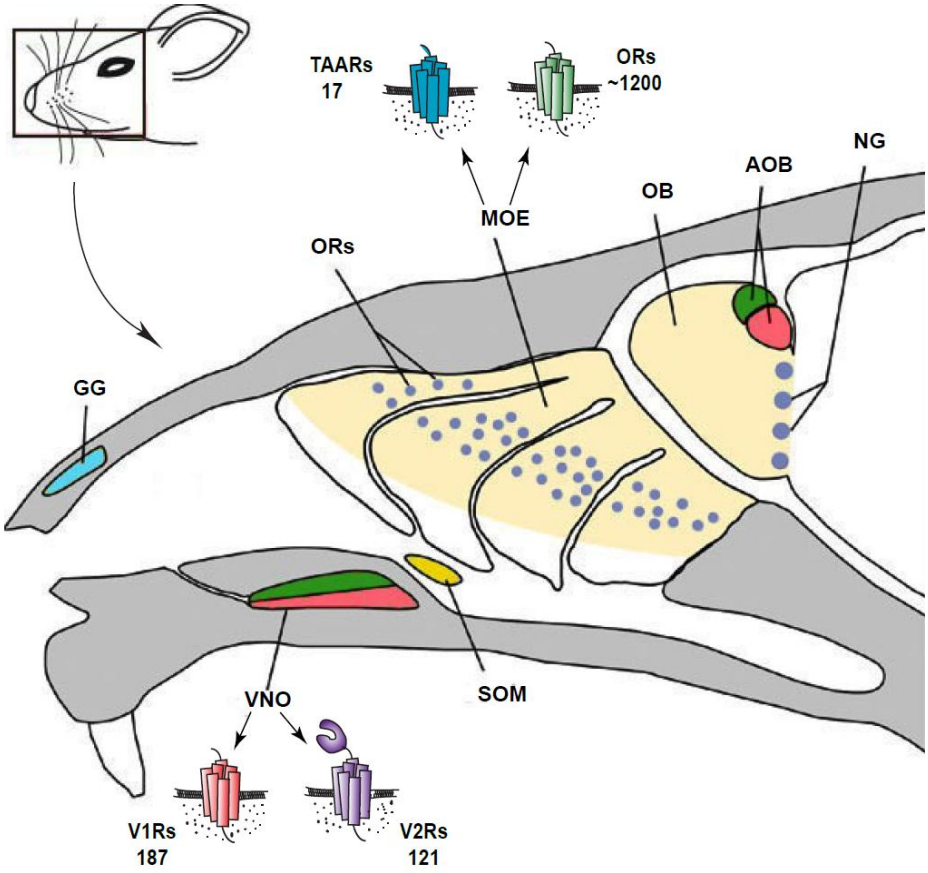

Fig. 1. Schematic representation of mouse olfactory systems. Main olfactory epithelium (MOE), olfactory bulb(OB), accessory olfactory Bulb(AOB),Grueneberg ganglion (GG), Vomeronasal organ (VNO), septal organ of Masera, guanylyl cyclase D (GCD), necklace glomeruli (NG), trace amine associated receptors (TAARs), olfactory receptors (ORs),vomeronasal receptors type1(V1Rs), vomeronasal receptors type2(V2Rs).

Zebrafish Olfactory System

Zebrafish is equipped with only one olfactory system, the main olfactory system that contains a single olfactory epithelium as first site of odor perception. The olfactory epithelium has two distinguished areas: central sensory area and peripheral non-sensory area. The sensory area contains 3 types of olfactory sensory neurons (OSNs) called ciliated, microvillous and crypt OSNs that project their axons to the OB (Hansen and Zielinski, 2005). Ciliated, crypt and microvillous OSNs can be labeled with OMP, S100 and TRPC2 neural markers respectively (Germana et al., 2004; Sato et al., 2005). Ciliated OSNs express odorant receptors (ORs) and trace amine associated receptors (TAARs), crypt OSNs may express a vomeronasal receptor type1 (V1Rs, also called ORAs in zebrafish) (Hansen and Zielinski, 2005; Saraiva and Korsching, 2007) and Microvillous OSNs express vomeronasal receptors type2 (V2Rs, also called OlfCs in zebrafish (Alioto and Ngai, 2006). Mitral and tufted cells of the OB synapse with incoming axons

from OE and transfer the signals to the olfactory cortex. These three types of OSNs show several different properties with respect to their morphology, relative position in the OE, and molecular expression(Yoshihara, 2009).

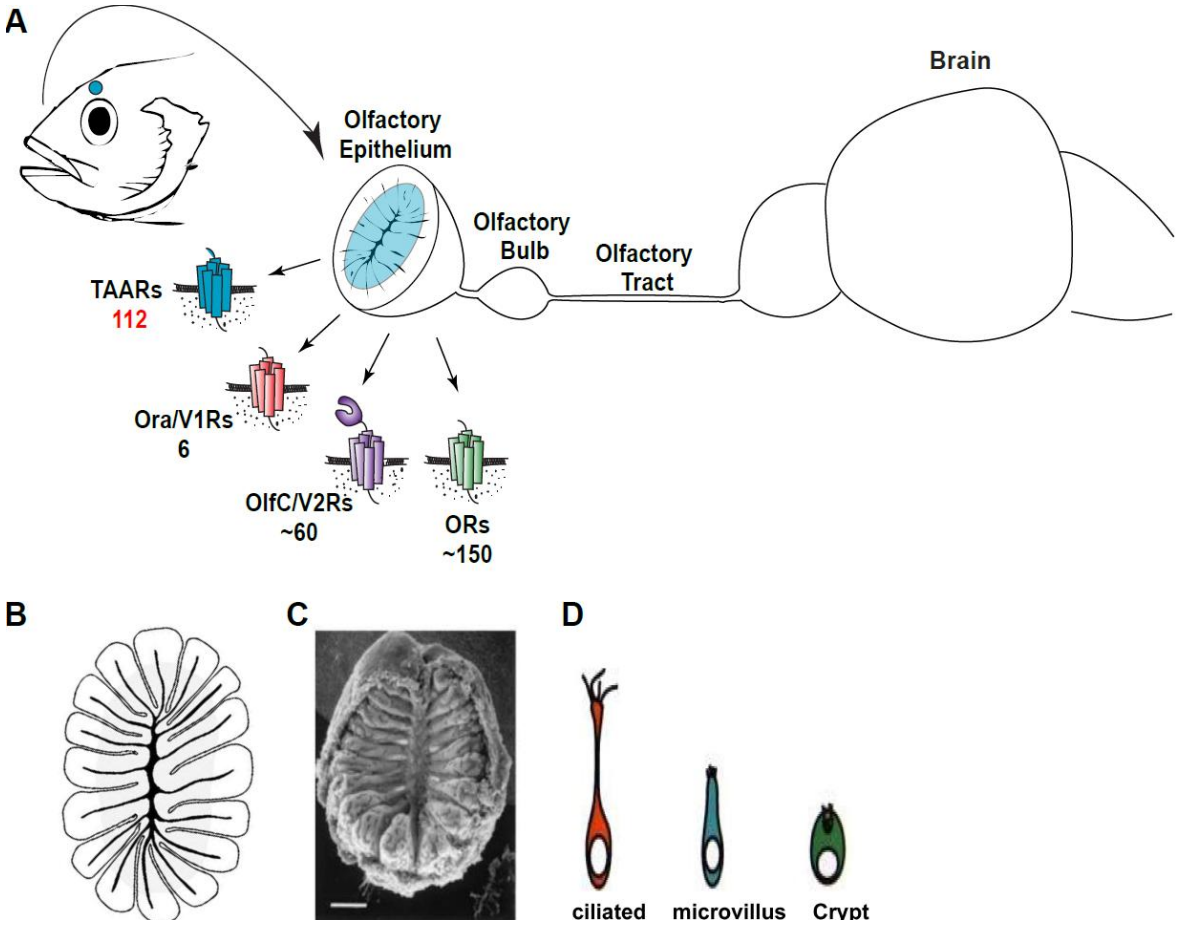

Fig. 2. General Organization of zebrafish fish olfactory system. (A) spatial organization of the olfactory system and four olfactory receptor families expressed in the olfactory epithelium. (*B*) Schematic representation of a horizontal cross-section through an olfactory rosette. (*C*) Scanning electron micrograph of an olfactory rosette of an adult zebrafish. (D) Types of OSNs expressed in olfactory epithelium. Numerous tiny hair-like cilia protrude from the dendrites of ciliated olfactory receptor cells and house the different olfactory G protein-coupled receptors. (SEM courtesy Prof. Sigrun I. Korsching).

Olfactory Sensory Neuron (OSNs)

The olfactory epithelium of fish contains three types of morphologically distinguished and functional olfactory sensory neurons (OSNs): Ciliated, Microvillous and Crypt. The three types of OSNs show different properties with respect to their morphology, relative position in the OE, and molecular expression. Zebrafish is equipped with only one olfactory organ that expresses all three types of olfactory sensory neurons (Korsching, 2009).The relationships among cell morphology, molecular signatures, and axonal terminations of different OSNs suggest that the two segregated neural pathways are responsible

for coding and processing of different types of odor information in the zebrafish olfactory system (Miyasaka et al., 2005).

1- Ciliated sensory neurons

Ciliated sensory neurons with their somata rooted in the deep layer of the olfactory epithelium, have long dendrites (Fig. 2d) and express ORs and possibly TAARs in the zebrafish olfactory epithelium, the main sensory organ in teleosts (Hansen et al., 2004; Speca et al., 1999). Volatile odorants are perceived by a large repertoire of odorant receptors (ORs) sparsely expressed in the OE and the information is transmitted to the main olfactory bulb (OB).The signal transduction of ciliated OSN uses cyclic nucleotide-gated channel A2 subunit, and olfactory marker protein (Friedrich and Korsching, 1998; Hansen et al., 2003; Sato et al., 2005). Ciliated OSNs project their axons mostly to the dorsal and medial regions of the OB, whereas the microvillous OSNs project their axons to the lateral region of the olfactory bulb (Sato et al., 2005). The LOT is involved in the perception of amino acids (von Rekowski and Zippel, 1993) that induce feeding behavior (Hamdani et al., 2001), whereas the mMOT is involved in the perception of alarm reaction (Hamdani et al., 2000). The axons of ciliated OSN, which bind the same odors synapse with mitral cells, to form glomeruli in the medial and ventral regions of olfactory bulb. Transgenic fish labeled with molecular cell markers, OMP for ciliated OSN have been generated in recent years (Sato et al., 2005).

2-Microvillous sensory neurons

Microvillous OSNs are located in the apical layer of olfactory epithelium of teleosts and express OlfCs (mammalian V2R-type receptors homologue) and transient receptor potential channel C2 (TRPC2) (Hansen et al., 2004; Morita and Finger, 1998). Microvillous OSNs have short dendrites that possess microvilli for stimulus detection (Fig. 2d). In mammals Microvillous OSNs express vomeronasal receptors2 (V2R) in the vomeronasal organ. Pheromones (olfactory cues capable of inducing stereotypical social and sexual behaviors among conspecifics) are perceived mostly by V2R receptors expressed by microvillous OSN that project their axons to the accessory OB. The lateral region of the OB is innervated by the microvillous OSNs (Hamdani et al., 2002; Hansen et al., 2003). In zebrafish, Microvillous neurons are also involved in perception of amino acids and nucleotides (Friedrich and Korsching, 1998; Hansen et al., 2003) and probably project through the LOT that elicits feeding behavior (Sato et al., 2005).

3-Crypt sensory neurons

Crypt cells (CCs), a third type of OSN located in the OE of actinopterigians (ray-finned fishes) and some other vertebrates (Hansen and Finger, 2000), were described in teleosts in 1998 (Hansen and Finger, 2000; Morita and Finger, 1998). Crypt cells are absent in both sarcopterigians (lobe-finned fishes), tetrapods and in American alligator (*A. mississippiensis)* (Hansen, 2007; Hansen and Finger, 2000). Crypt cells have a typical morphology, clearly distinguished from that of common olfactory receptor neurons (ORNs). Crypt cells are ovoid cells and with a crypt-like apical invagination where cilia protrude, as their exceptional characteristic (Fig. 2d). Crypt cells are located in the upper third of the OE and scattered along the olfactory lamellae (Catania et al., 2003; Ferrando et al., 2006; Hansen et al., 2003). Their presence and distribution in fishes seem to vary from specimen to specimen and from season to season, suggesting a certain variability and feedback control of the expression of the CN population (Hamdani el and Doving, 2006; Hansen and Finger, 2000). Although the precise function of crypt ORNs in olfactory pathways is still tentative, it has been shown in crucian carp (*Carassius carassius*), that their axons project through the lateral bundle of the medial olfactory tract (lMOT), which mediates reproductive behavior (Weltzien et al., 2003), to a central region in the ventral olfactory bulb (Hamdani el and Doving, 2006), whose neurons are triggered by pheromones (Lastein et al., 2006).

Olfactory Receptor Gene Family Repertoire

The discovery of olfactory receptors (Buck and Axel, 1991) opened a new age for molecular study of GPCRs. So for, five olfactory receptor gene families, all of them G protein-coupled receptors, have been identified and characterized in mammals(Liberles et al., 2009; Riviere et al., 2009), while for teleost have four olfactory receptor gene families have been described up to now (Korsching, 2009). They include the odorant receptors (OR), vomeronasal receptor (V1R/ORA and V2R/OlfC), formyl peptide receptor (FPRs, found only in mammals) and trace amine-associated receptors (TAARs). The number of identified olfactory receptors expanded rapidly by data-mining due to the availability of complete genome of several model organisms, not only in rodents but also in other mammals, amphibians, fish and birds. Olfactory GPCRs families involved in perception of pheromones were identified (Belluscio et al., 1999; Dulac and Axel, 1995) . Recently a new class of GPCRs named trace amine-associated receptors (TAARs) was recognized in rodents (Liberles and Buck, 2006), zebrafish and other species (Berghard and Dryer, 1998; Gloriam et al., 2005; Hussain et al., 2009). Olfactory receptor gene families vary between species considering that each species have their own characteristic set of chemical signals that are important for survival and reproduction. The remarkable species-specific and ambiance related discriminatory capacity of the chemosensory system is directly linked to the diversity of the olfactory receptor gene families (Dryer, 2000). ORs, FPRs and TAARs belong to the classA (rhodopsin-like) GPCRs, with short

extracellular N- terminal ligand binding domain and short cytosolic C-terminal domain. V1Rs are also considered closed to classA. Although ORs and V1Rs do not share considerable sequence homology, both are Class-(rhodopsin-like) GPCRs. Widespread features among ORs and V1Rs include an intronless coding region, exclusively monogenic (Rodriguez et al., 1999) and monoallelic (Roppolo et al., 2007) expression, a scattered and mainly clustered chromosomal organization (Del Punta et al., 2002), and a sparsely distributed tissue expression pattern consistent with the 'one neuron – one (or a few) receptor(s)' hypothesis (Feinstein et al., 2004).V2Rs belong to classC, which is structurally close to the metabotropic glutamate receptor, with an additional large N-terminal extracellular domain (Feinstein and Mombaerts, 2004).

Human can perceive a vast number of volatile chemicals yet human are considered to have a poor sense of smell compared to the other animals like rodents, dogs and snake. Humans have about 350 functional odorant receptors (Niimura and Nei, 2003) much less than the ~1000-1200 in the mouse and rat genomes, respectively (Young et al., 2003; Zhang et al., 2004b). In fish the numbers are several fold smaller, ranging from 86 to 155 putatively functional OR genes in fugu and zebrafish, respectively (Nei et al., 2008). There are more ORs than all other known GPCRs combined that make ORs one of the largest gene families known so far (Dryer, 2000). In rats, OR comprise about 6% of their total functional genes, emphasizing the importance of olfaction to the species. The olfactory repertoire of teleost fish is smaller in size (OR, ORA), comparable (olfC), or even larger (TAAR) than the corresponding mammalian gene repertoires (Dryer, 2000; Hussain et al., 2009; Nei et al., 2008). Despite smaller repertoire size, teleost OR and ORA families show higher divergence than their mammalian counterpart (Korsching, 2009). Olfactory receptors families are evolutionary dynamic that is evident with positive selection in teleost ORs. However, it is still not evident whether the putatively selected amino acid changes are correlated with a novel gain of function. The *ora* genes are subject to strong negative selection, and in fact are being conserved among all teleost species investigated. A small subset of "olfactory" genes may have other non-olfactory functions, in addition to or instead of a primary olfactory role. The highly conserved TAAR1 (shark, mammalian, and teleost orthologs) is not expressed in the olfactory epithelium of zebrafish and mouse and may represent the sole remnant of a primordial, non-olfactory function of this family (Hussain et al., 2009; Liberles and Buck, 2006). Human OR, hOR17-4, is expressed in the nose as well as in the testis, responding to the chemical bourgeonal, thus allowing sperm to undergo chemotaxis to find the egg cell (Spehr et al., 2006a).

Evolution history of olfactory gene families in several species revealed that gene gain and loss is fundamental and had major significance in defining the current total number of genes in these families (Young and Trask, 2002). High species specificity and rapid evolution are characteristics of olfactory receptor gene families. Local gene duplication is the most probable cause of gene birth. The duplicate

genes can follow many evolutionary trajectories. If the new gene is functionally redundant, one of the copies may be removed from the functional repertoire by inactivating mutation. In contrast, if the new copy acquires mutations that allow it to recognize a novel, useful odorant molecule, then it is likely that natural selection will favor the retention of the new, modified sequence. Species-specific expansion and loss of genes and even whole subfamilies is a persistent phenomenon in the mammalian receptor families (Grus et al., 2005; Lane et al., 2004; Zhang et al., 2004a). The rate of nucleotide substitution (dN/dS) induces diverse selective pressure. Nucleotide substitutions in genes, coding for proteins, can be either synonymous (no change in the amino acid or non-synonymous (changes in the amino acid), and this ratio of the rate of non-synonymous substitutions (dN) to the rate of synonymous substitutions (dS), can be used as an indicator of selective pressure acting on a protein-coding gene (Bielawski et al., 2000; Yang and Bielawski, 2000). Higher rates of non-synonymous to synonymous substitutions are a signature of positive selection. Usually, most non-synonymous changes are expected to be eliminated by purifying selection, but under certain conditions Darwinian selection may lead to their preservation. Conversely, if changes in the sequence eliminate useful ligand-recognition patterns, they would be subject to ‘‘negative or purifying selection’’, i.e. the numbers of synonymous substitutions would be more frequent than the non-synonymous ones, as is observed for genes in general. The incidence of positive selection in the genome is generally associated with transcription factors and some receptor families, including olfactory receptors (Bustamante et al., 2005), although the frequency of positive selection is conflict-ridden (Studer et al., 2008). Ratio of synonymous and non-synonymous substitutions may provide information about the degree of selective pressure. Numerous studies have found support for amino acid signatures of positive selection on the olfactory receptors in mammal and fish species (Hughes and Hughes, 1993). However, it remains unclear whether the putatively selected amino acid changes are linked with a novel gain of function.

1-ODORANT RECEPTOR FAMILY (ORS)

Olfactory receptors are members of a large family of seven-transmembrane (TM)-domain G-protein coupled receptors (GPCRs), comprising about 6% of their total functional genes in rat, emphasizing the importance of olfaction to the species. ORs are small (~1 kb), intronless and are expressed in the ciliated neurons, in a monogenic pattern i.e. a particular olfactory sensory neuron expresses only one OR (Buck and Axel, 1991; Mombaerts, 2004; Sato et al., 2007). The TM regions are connected by three extracellular and intracellular loops, with an extracellular amino-terminus and an intracellular carboxy-terminus. Olfactory receptors possess highly conserved motifs, hyper variable protein regions are also found in the third, fourth and fifth TM region (Trabanino et al., 2004). MAYDRYVAIC is the highly conserved amino acid motifs within and across species located at TM3 end (Liu et al., 2003). OR genes occur in clusters in vertebrate genomes (Niimura and Nei, 2003). Despite this fact, the evolutionary

dynamic nature of this family is characterized by rapid expansion, gene duplication, extensive gene loss via pseudogenization, and diversifying selection (Alioto and Ngai, 2005; Young and Trask, 2002). Since the cloning of the first rodent OR genes in 1991, ORs have been isolated from C. elegans, drosophila, lamprey, teleosts, amphibian, avian and humans (Nei et al., 2008). Vertebrate ORs contain introns and sequence identity between vertebrates and invertebrates are very low (Dahanukar et al., 2005). ORs of *C.elegans* share only ~10% sequence identity with vertebrate OR genes. This leads to the question whether non-vertebrate and vertebrate OR genes derive from a common ancestor (Gaillard et al., 2004). Vertebrates can detect and discriminate higher number of different volatile chemicals than the number of ORs encoded in the genome. This perception is achieved through a mechanism known as the 'combinatorial receptor code' i.e. one odour molecule can be recognized by several ORs, and one olfactory receptor can recognize several odour molecules (Malnic et al., 1999).

The evolutionary origin of Zebrafish dates back to the most common ancestor of teleost and tetrapods as evident by the comparison of teleost fish, amphibian, and mammalian OR repertoires (Alioto and Ngai, 2005; Niimura and Nei, 2005). Some OR genes even go back to the common ancestor of jawed and jawless fish (Freitag et al., 1999). The zebrafish OR repertoire is several folds larger than that of two pufferfish species, which have less than 50 OR genes (Alioto and Ngai, 2005; Niimura and Nei, 2005). Interestingly, teleost OR genes do show signs of positive selection, although the evolutionary rate of teleost is slow compared to tetrapods (Alioto and Ngai, 2005). Many Teleost ORs are located in clusters in the genome although some genes are sparsely present (Alioto and Ngai, 2005). Within the gene clusters, subfamilies are largely contiguous and subfamily members usually exhibit the same transcriptional orientation, suggesting tandem duplication as a mechanism of gene expansion.

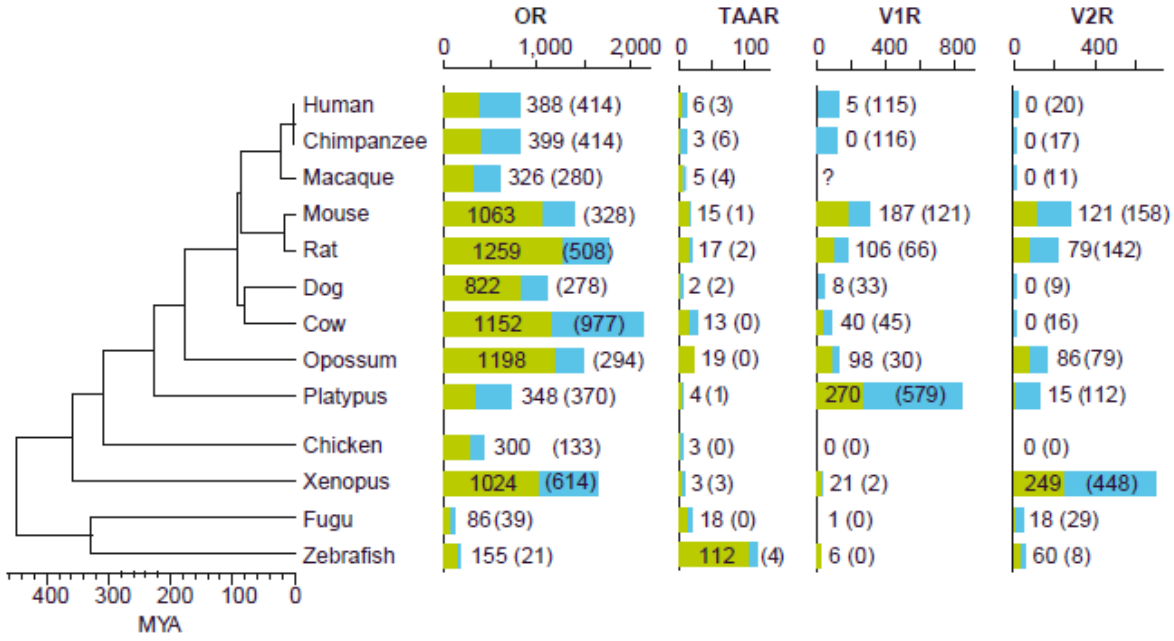

Fig. 3. Numbers of olfactory receptor genes in different species. The green and blue bars represent the numbers of functional (intact) genes and pseudogenes (disrupted genes), respectively. The numbers next to each bar represent the number of functional genes and the number of pseudogenes, which is shown in parentheses. A question mark indicates that data are unavailable. ORs, odorant receptors; TAARs, trace amine associated receptors; V1R, Vomeronasal receptors type1; V2R, Vomeronasal receptors type2.

2-Trace Amine-Associated Receptor Family (TAARs)

In addition to ORs, olfactory system also contains other chemosensory receptors to detect chemical stimuli. TAARs were identified in 2001 (Borowsky et al., 2001).Trace amine associated receptors (TAARs) are close relatives of G protein-coupled aminergic neurotransmitter receptors as dopamine and serotonine receptors and recognize derivatives of the classical monoamines such as ß-phenylethylamine, octopamine, tryptamine, and tyramine (Lindemann and Hoener, 2005). Initially, TAARs have been considered neurotransmitter receptors as well, based on the expression and effects of some family members in the central nervous system (Lindemann and Hoener, 2005). However, recently, Liberles and Buck (Liberles and Buck, 2006) reported for several mammalian *taar* genes, some of whom they could deorphanize, the expression in olfactory sensory neurons. Thus, the *taar* genes joined a growing number of GPCR families that serve as olfactory receptors (Liberles and Buck, 2006). Surprisingly, the fish *taar* gene repertoire appeared to be much larger than the mammalian repertoire (Gloriam et al., 2005), whereas the opposite holds true for the other olfactory receptor families. After the cloning of the first TAAR receptors in mammals (Borowsky et al., 2001), TAAR genes have been found in genomes from lower vertebrate species (Gloriam et al., 2005; Hussain et al., 2009). The first study evaluating teleost

taar genes (Gloriam et al., 2005) made use of very incomplete databases, and thus many of its conclusions, including the size of the family, the phylogenetic reconstruction, the genomic location, the frequency of pseudogenes, the absence of introns, and the suggested nomenclature are now outdated. Still valid are its observations that the *taar* gene family exhibits rapid evolution and correspondingly remarkably species-specific repertoires. A follow-up study confirmed these observations using a more complete data set (Hashiguchi and Nishida 2007), double the number of *taar genes* found in stickleback (Hashiguchi and Nishida, 2007). The selective pressure acting on teleost *taar genes* takes the form of positive selection, of which incidences have been observed in the OR, V1R, and V2R families. Currently, *taar gene* repertoires have been established for fugu, stickleback, medaka, and zebrafish. Fugu has the smallest repertoire, less than 20 genes, followed by medaka with 25 genes, stickleback with 49 genes, and zebrafish with 109 genes (Hashiguchi and Nishida, 2007).

Taar genes occur in a single cluster in tetrapods, evidence of a genesis from local gene duplications, possibly via illegitimate crossover during meiotic recombination. In teleosts, *taar genes* form two large clusters (Hashiguchi and Nishida, 2007), presumably resulting from the whole genome duplication occurring early in the teleost lineage (Nakatani et al., 2007) . Additionally, several isolated genes and small groups are found; however, due to the still unfinished genome build in zebrafish, this may not be the final distribution. The most recent common ancestor of tetrapods and teleosts (of lobe-finned and ray-finned fishes) presumably already had a small cluster of *taar genes*. Whereas all mammalian and all zebrafish *taar genes* are monoexonic, an intron was found in many medaka, fugu, and stickleback genes (Hashiguchi and Nishida, 2007), consistent with an intron gain early in the evolution of neoteleosts, i.e., relatively late in vertebrate evolution. This is rather remarkable since several whole genome scanning studies found very little evidence for any intron gains during all of vertebrate evolution (Coulombe-Huntington and Majewski, 2007) and may be related to the apparently low selective pressure in the *taar* gene family. TAAR genes were shown to co-express GαOlf, suggesting that they are expressed at least in ciliated neurons (Liberles and Buck, 2006). In this thesis I have analyzed both the scope and the evolutionary history of the TAAR gene family in fish. Natural ligands identified for mouse TAARs have been detected in mouse urine which is known to be a major source of social cues (Liberles and Buck, 2006). Therefore, it has been suggested that TAARs may be highly relevant for social communication and individual recognition.

3-Vomeronasal Receptors Family Type1 (V1R)

Vomeronasal receptor family is expressed in the accessory olfactory organs named Vomeronasal organ. The vomeronasal organ is a tubular crescent shape paired structure located separately from the nasal cavity. The vomeronasal sensory neurons are formed in the olfactory placode along with other sensory

olfaction neurons. Vomeronasal receptors in vomeronasal sensory epithelium are lining an elongated cavity (lumen) inside the bone capsule which encloses the organ. The only way of access for stimulus in VNO is a thin duct that opens onto the floor of the nasal cavity inside the nostril ((Dulac, 2000). The vomeronasal receptors are GPCRs and are often referred to as pheromone receptors since vomeronasal receptors have been tied to detecting pheromones. The axons of vomeronasal receptors transducer signals through accessory olfactory bulbs (AOB) to olfactory Amygdala. There have been two types of Vomeronasal receptors, each found in distinct regions: V1R, located on the apical compartment; V2R located on the basal compartment of the VNO (Buck, 2000; Dulac, 2000).

Mammalian V1Rs are homologues of teleost ORA family. Telesost ORA family belongs to classA GPCRs, hence named odorant receptors A (ORA). ORA in teleost are expressed in the main olfactory epithelium as teleost lack vomeronasal organ. ORA receptors have short N-terminal and high sequence diversity sequence diversity in transmembrane domains. V1R display a 1 kilobase, intronless genomic structure (Buck and Axel, 1991), while teleost homolog ora genes have introns in two of six genes (Saraiva and Korsching, 2007). Ora genes have been the most recent of the four teleost olfactory receptor families (ORs, TAAR, ORA, OlfC) .The first member of this family was uncovered in 2005 (Pfister and Rodriguez, 2005). The teleost ORA receptor gene family is relatively small with only 6 members compared to over 100 genes in the corresponding rodent V1R gene family. Ora genes form a monophyletic clade, supporting their identification as a single family separate from the other chemosensory receptor families. Ora genes have been identified already in the lamprey (Saraiva and Korsching, 2007). Orthologues (closest homologs between species) are more closely related to each other than any paralog Ora genes (closest homologs within species), indicating that all six family members are evolutionarily much older than the speciation events in the teleost lineage. Noticeably, ora genes are highly conserved among all teleost species analyzed so far, such that individual orthologs for all six genes can be detected in all five teleost species analyzed so far (bar a single gene loss in the pufferfish genus) (Saraiva and Korsching, 2007). ora genes show no evidence for positive selection, in contrast to the other olfactory receptor families including the mammalian V1R family ((Saraiva and Korsching, 2007). Contrary to the other olfactory receptors families, ORA genes do not occur in cluster in teleost genome, four of the six ora genes are arranged in closely linked gene pairs across all fish species studied. 2-heptanone, a putative pheromone, was identified as a ligand for one member of the V1R family (V1Rb2) (Boschat et al., 2002), but no follow-up studies have been done with this ligand. V1R genes are linked to reproductive behavior (Del Punta et al., 2002). All six ora genes are expressed specifically in the olfactory organ of zebrafish, in sparse cells within the sensory surface (Saraiva and Korsching, 2007), consistent with the expectation for olfactory receptors and similar to the expression of the tetrapod subclade V1R.Taken together, the high conservation of the ora gene repertoire across teleosts, in striking contrast to the frequent species-specific expansions observed in tetrapods, especially

mammalian V1Rs, possibly reflects a major shift in gene regulation as well as gene function upon the transition to tetrapods. Humans have five intact V1R genes. It has been argued that although these five V1R genes have an open reading frame, they are not functional because a calcium channel gene (*TRPC2*) that is essential in the signal transduction pathway of the mouse VNO has become a pseudo gene in the lineage that leads to hominoids and Old world monkeys (Liman and Innan, 2003) However, at least one of the five V1R genes is expressed in the human olfactory mucosa ((Rodriguez et al., 2000). A recent study suggests that that these five genes can activate an OR-like signal transduction pathway in a heterologous expression system. It is therefore possible that the products of these genes function as pheromone or olfactory receptors. Adult humans do not have a VNO but seem to be sensitive to pheromones (Shepherd, 2006). Another interesting observation is that chicken (Gallus gallus) have no functional or non-functional V1R and V2R genes(Grus and Zhang, 2008), while dog (Canis familiaris) have no functional V2R genes(Grus and Zhang, 2008), although birds use pheromones for mate choice and other behaviors (Bonadonna et al., 2009; Caro and Balthazart; Hirao et al., 2009; Zhang et al.). It is possible that some OR genes in the MOE are able to detect pheromones, as in humans (Keller et al., 2007).

4-Vomeronasal Receptors Family Type2 (V2R)

Mammalian V2Rs are homologues of teleost OlfC. Teleost OlfC receptors belong to the class C metobotropic glutamate GPCRs, like the mammalian V2Rs. Humans do not have any functional V2R genes. OlfC are distinguished by their long extracellular NH2 terminals which are thought to be the binding domain for pheromones. The V2R genes in mammals are species specific and meticulous specificity has led to the loss of this family in several mammalian species (Young and Trask, 2007). Number of V2R genes varies from 0 (human, chimpanzee, macaque, dog and cow) to 121 (mouse) (Nei et al., 2008). All olfC subfamilies are present in zebrafish, but not in neoteleosts, and many indicate the species-specific gene expansions in zebrafish. OlfC repertoire size varies several folds between teleost species but stays in parallels range of mammalian homologue V2R. Zebrafish has the largest repertoire of all teleost OlfC repertoires (Alioto and Ngai, 2006; Hashiguchi and Nishida, 2006). Local gene duplication has also played a large role in the evolution of the OlfC family, as suggested by the arrangement of most OlfC genes in clusters of phylogenetically related genes (Alioto and Ngai, 2006; Hashiguchi and Nishida, 2006) .OlfC, unlike the other three olfactory receptor gene families, are not monophyletic. The three distinct clades fall together under the olfC heading (Alioto and Ngai, 2006). OlfC genes exhibit five conserved intron/exon borders that result in six exons in a characteristic short-short-long-short-short-long arrangement (Alioto and Ngai, 2006). Metabotropic glutamate receptors do not show these intron/exon borders. Negative selection is observed at distal ligand binding sites in OlfC and there is no evidence of positive selection (Alioto and Ngai, 2006). Although currently no ligands are

known for any member of the largest group of OlfC genes (group 1), modeling suggests that many of them have amino acids as ligands like the one well investigated OlfC member from one of the small groups, OlfC a1 (Luu et al., 2004). Thus, OlfC receptors may constitute the molecular basis to explain odor response studies, which predict many independent receptors for amino acids (Fuss and Korsching, 2001). V2R gene family has undergone an even more marked decline than the V1R gene family, with no functional genes remaining in the cow, dog, human, and chimpanzee or macaque genomes. Such decline demonstrates that V2Rs are no longer important for these species, either because other receptor families now detect pheromones or because pheromone-mediated signaling is now of lesser importance (Liman, 2006). By contrast, the large number of functional V2R genes and species-specific V2R gene family expansions in the mouse, rat and opossum genomes probably contribute to the ability of these species to detect large repertoires of pheromones (Young and Trask, 2007).

5-Formyl Peptide Receptor Family (FPR)

FPRs are a new family of olfactory GPCRs in the vomeronasal organ, so for found in the mammalian species. FPRs are also expressed in the immune system, where they are believed to stimulate chemotaxis to sites of infection upon recognition of their ligands, such as formylated peptides from bacteria or mitochondria (Yang et al., 2002). FPRs are characterized by monogenic transcription and their expression patterns are remarkably similar to those of V1Rs and V2Rs. FPRs were reported to be expressed in diverse tissues (Migeotte et al., 2006; Panaro et al., 2006). Most recently, it has been shown that out of the seven murine FPR subtypes, some are predominantly expressed in a highly dispersed, small subset of neurons that bind with $G\alpha_{i2}$ or $G\alpha_{o,}$ in the VNO. Most recently FPRs have been identified as olfactory receptors expressed in the vomeronasal organ of mouse (Liberles et al., 2009; Riviere et al., 2009). Phylogenetic analyses indicate that genes encoding vomeronasal organ FPRs evolved recently in the rodent lineage, raising the possibility that these receptors impart a novel chemosensory function to rodents.

Olfactory Signaling Transduction

Olfactory perception is mediated by large, diverse family of G-protein-coupled receptors in both vertebrates and invertebrates. In the vertebrate zebrafish, 328 olfactory receptors have been discovered that are involved in olfaction (the detection of volatile compounds). At the most basic level, the olfactory system in any animal must allow the brain to discern which olfactory receptors have encountered odorant at any given time. In mammals, olfaction is accomplished by approximately 1,000 diverse olfactory receptor genes (Mombaerts et al., 1996). Brain can determine which set of olfactory receptors are activated by identifying excited neurons, as each neuron expresses only one receptor. Mammalian

olfactory neurons appear to use the same machinery for transducing signals from its odorant receptor molecules. The cell bodies of the set of neurons expressing a given olfactory receptor are distributed in specific zones of olfactory epithelium and intermingle with neurons expressing different receptors, but their projections converge to discrete loci in the olfactory bulb called glomeruli (Mombaerts et al., 1996). Thus, the brain could in principle determine which receptors have been activated by examining the spatial pattern of activity in the olfactory bulb; individual odorants are associated with specific spatial patterns. The *adaptation* of odorants is thought to derive from at least two different physiological mechanisms. First, the interaction of an odorant receptor with its ligand may be followed by inactivation, or *desensitization*, of the receptor due to phosphorylation of the receptor by a protein kinase. Second, the olfactory neuron may adapt to different concentrations of an odorant by adjusting the sensitivity of its cyclic nucleotide gated ion channels to cAMP, an effect conceptually analogous to light adaptation in the visual system, where light sensitivity is adjusted to match the intensity of light in the environment.

Olfactory signaling transduction is GTP-dependent, suggesting that olfactory transduction, like visual transduction, proceeds via a G protein-coupled mechanism. Olfactory receptors activate Golfa, Gsα-like G protein (Jones and Reed, 1989) upon perception of ligand. Golf-mediated activation of adenylate cyclase III then raises intracellular cAMP levels, causing a cyclic-nucleotide-gated channel to open (Fig. 4). The influx of cations through this channel ultimately leads to the formation of an action potential, which allows the primary neuron to signal to the brain. The axonal projections of the olfactory sensory neurons converge on defined glomeruli in the olfactory bulb. Olfactory receptors themselves play an instructive role in axon guidance and same olfactory receptor- initiated signal transduction machinery is used to mediate both olfactory perception and axon targeting (Belluscio et al., 1998; Wang et al., 1998).

Additional signal transduction cascades activated by odor binding include inositol 1,4,5-trisphosphate (IP3), cyclic GMP, and carbon monoxide, but their roles in transduction is not considered primary and is not currently understood completely. IP3 is also known as a second messenger and is involved in transmission of chemical signal (hormone, neurotransmitters, growth factors , Beta-adrenergic receptor agonists) received by the cell, to various signaling networks within the cell. IP3 is known to play a crucial role in initiating and broadcasting of chemical messages; however, the exact mechanism of how IP3 relates to the subsequent element in its signaling pathway, the calcium wave, remains highly controversial. Two essential signaling pathways have been identified that involve the intracellular signaling generation of IP3. The first signaling pathway is commenced by cytosolic soluble proteins PLC (Phospholipase-C). Neurotransmitters and hormones bind to GPCR and both the heterotrimeric G-AlphaQ/11, and G-Beta Gamma subunits regulate the function of PLC-Beta (Szlufcik et al., 2006). Release of second messengers DAG (1, 2-Diacylglycerol) and IP3 activation takes place as a results of the hydrolysis of PIP2 (Phosphatidylinositol-4, 5-Bisphosphate). ERK1/2 (Extracellular Signal Regulated

Kinase-1/2) signaling pathway resulting in transcription factor activation and cell survival are activated by DAG, a physiological activator of PKC (Protein Kinase-C). The second IP3 signaling pathway is initiated by an enzyme PI3K (Phosphoinositide 3-Kinase) involved in phosphorylation of inositol lipids. The enzyme PI3K is also involved in generation of two signaling molecules, PIP2 (Phosphatidylinositol 3, 4-Bisphosphate) and PIP3 (Phosphatidylinositol 3, 4, 5-Trisphosphate). PI3K is activated by CD19, a co-receptor complex in B-cells. IP3, generated by PIP2 plays a vital role in the organization of cellular and physiological processes including fertilization, apoptosis, cell-division, cell proliferation, development, learning, memory and behavior (Futatsugi et al., 2005).

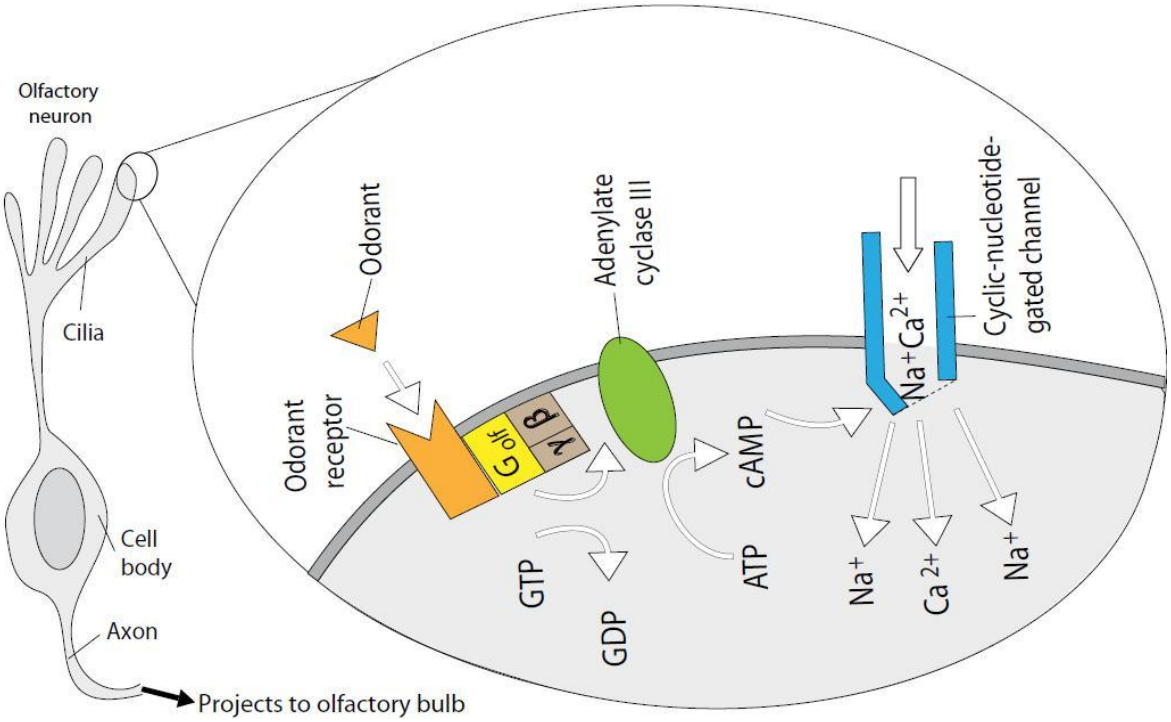

Fig. 4. the mouse olfactory signal transduction cascade. Odorant binding to the olfactory receptor is thought to activate G protein's GTP-coupled α-subunit, Gαolf. Activated Gαolf then dissociates from Gβɤ and activates adenylate cyclase III, leading to an increase in the intracellular cAMP concentration. The increased cAMP leads to the opening of cyclic nucleotide gated cations channels, causing a depolarization that leads to the influx of cations and generation of action potentials in the sensory axon and the transmission of signals to the olfactory bulb.

Ligands for Olfactory Receptors

Olfactory receptor gene families vary between species. This lead to the hypothesis that olfactory receptor within the species may have their own characteristic set of chemical signals that are important for their survival and reproduction in a specific environment. Odorants/ligands for olfactory receptors are typically small organic molecules of less than 400 Da and can vary in size, shape, functional groups and charge (Malnic et al., 1999). Odorants include a set of various aliphatic acids, aldehydes, alcohols, ketones, esters and amines; chemicals with aromatic, alicyclic, polycyclic or heterocyclic ring structures; and numerous substituted and combinations of these chemicals. Odorants generally bind to several receptors with diverse affinities and individual receptors generally bind more than one odorant (Buck, 2000; Kajiya et al., 2001), except some highly specific and unique receptors i.e. pheromones receptors (Friedrich and Korsching, 1998; Kajiya et al., 2001). The olfactory receptor genes are regard as the first centre of olfactory information processing. However, only few olfactory receptors genes are deorphanized in mammals ((Luu et al., 2004). The identification of ligand is a complex task due to the inefficient heterologous expression system for many olfactory receptors. Mammalians and to some extent teleost olfactory receptors GPCR including OR, TAAR, V1R, and V2R genes are expressed in a monogenic fashion (a particular receptor neuron expresses only a single gene from a single receptor family (Liberles and Buck, 2006; Mombaerts, 2004; Sato et al., 2007). The neurons expressing the same olfactory receptor converge into a single glomerulus in the olfactory bulb. Both genetic and imaging studies confers that each receptor gene designate a separate input channel of the olfactory system and the olfactory bulb comprises a receptotopic map of odor sensitivities, an odor map ((Friedrich and Korsching, 1998; Fuss and Korsching, 2001; Sato et al., 2005; Sato et al., 2007). In teleost, the only olfactory receptor with identified ligands is a member of the OlfC family, OlfCa1 (Alioto and Ngai, 2006). Interestingly, the optimal ligands for the goldfish receptor are basic amino acids, whereas the zebrafish receptor reacts most strongly to acidic amino acids. Odorant receptors expressed in heterologous cells couple to Gαolf that leads to odorant-induced increases in cAMP. The increases in cAMP can be monitored using a reporter gene assay (Liberles and Buck, 2006).

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